



AMERICAN SOCIETY OF TROPICAL MEDICINE & HYGIENE  
ADVANCING GLOBAL HEALTH SINCE 1903

VOLUME 97

NOVEMBER 2017

NUMBER 5 SUPPLEMENT

SIXTY-SIXTH  
ANNUAL MEETING  
November 5–9, 2017

The Baltimore Convention Center | Baltimore, Maryland USA

# Abstract Book



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Supplement to  
The American Journal of Tropical Medicine and Hygiene

## 1

## CHIKUNGUNYA INFECTION DURING GESTATION: IMPACT ON PREGNANCY AND NEONATAL OUTCOMES

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In July 2014, an outbreak of Chikungunya virus (CHIKV) occurred in Grenada, West Indies with an estimation of 65% of the population infected. During this outbreak, approximately 710 women gave birth with limited information available regarding the consequences of CHIKV infection on their pregnancies. Studies in La Reunion Island have identified neonatal complications resulting from mother-to-child CHIKV transmission at birth; however, no observable effect on pregnancy outcomes has been shown. Our objective was to study CHIKV impacts on pregnancy outcomes during the outbreak in Grenada. Mothers who gave birth during the 2014 CHIKV outbreak and up to 1 year after the outbreak were recruited. Questionnaire data was collected on the timing and symptoms of their CHIKV infection and pregnancy, delivery and newborn outcomes. To date 156 participants have been recruited and classified into 2 groups by reported history and confirmed exposure to CHIKV by IgG ELISA: those infected with CHIKV during pregnancy and those not infected during pregnancy. Demographic and symptom data, pregnancy and neonatal outcomes were compared. Of the 156 participants, 110 (71%) reported CHIKV during pregnancy and were CHIKV IgG positive. Infection occurred during the first trimester for 24 (22%) of women, second trimester for 40 (36%) of women and third trimester for 45 (41%) and during delivery for 1 (1%) woman. The most frequent maternal symptoms reported were, arthralgia (86%), fever (65%), muscle pains (39%), headache (38%), rash (38%), itchiness (37%) and generalized body aches (27%). There was no significant difference in duration or types of symptoms reported between those infected during pregnancy compared to those who experienced CHIKV infection prior to pregnancy. Pregnancy outcomes were similar between the two groups; however, neonatal complications were significantly higher in the CHIKV during pregnancy group (26% vs. 7%;  $p = 0.006$ ). This ongoing study adds to the limited data on the effects of CHIKV infection during pregnancy on birth outcomes and will be useful to inform physicians caring for pregnant women with CHIKV infection and their newborns.

## 2

## SAFETY AND IMMUNOGENICITY OF A LIVE RECOMBINANT MEASLES VECTOR BASED CHIKUNGUNYA VACCINE IN HEALTHY ADULTS: A RANDOMIZED, PLACEBO CONTROLLED PHASE 2 STUDY

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Themis is developing a safe, effective and affordable preventive vaccine platform against priority pathogen diseases that have the potential to cause epidemics such as Chikungunya or Zika virus infection by using a "plug-and-play" vaccine technology. This technology is based on a measles vaccine vector (MV) that can be easily genetically modified to express immunoprotective proteins for designated emerging infectious pathogens. This delivery platform technology has already demonstrated proof of principle in humans through a Phase 1 clinical trial in 42 healthy volunteers with a recombinant measles vaccine against Chikungunya virus (MV-CHIK). We showed that the vaccine was well tolerated. One immunization

induced functional, neutralizing antibodies in up to 90% of immunized subjects, a second immunization induced 100% seroconversion. Importantly, immunogenicity was independent of pre-existing anti-vector (measles) immunity. The vaccine efficacy was demonstrated in non human primates that were challenged with wildtype Chikungunya virus. All animals were protected against CHIKV viremia and disease symptoms. A Phase 2 clinical trial in 320 healthy subjects is currently ongoing in Austria and Germany with the goal to demonstrate the vaccine vector safety and immunogenicity in up to 300 subjects. Preliminary data will be presented here for the first time.

## 3

## AN INDEX CLUSTER STUDY OF CHIKUNGUNYA IN NICARAGUA WITH SPATIAL AND RISK FACTOR ANALYSES

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Chikungunya virus (CHIKV) is a mosquito-borne pathogen that has recently emerged in the Americas. Three main lineages of CHIKV exist, and the Asian lineage is responsible for the American epidemic. In light of this recent introduction, an urgent need exists to understand the transmission and characteristics of CHIKV in this new ecology. Therefore, we carried out an index cluster study of 60 RT-PCR-confirmed symptomatic chikungunya index cases presenting to the study health center and 236 household family members (contacts). Baseline (day 1) and follow-up RT-PCR and serological testing via IgM ELISA and Inhibition ELISA (day 3 and day 14 or 35), combined with questionnaires of clinical manifestations at all time-points, enabled identification of incident symptomatic and asymptomatic infections among the at-risk household contacts. We identified 90 contacts with current infections at baseline and 21 contacts who were RT-PCR-negative at baseline but who seroconverted over the study period. We estimated a symptomatic-to-asymptomatic (S:A) ratio of 1:1.91 (95% bootstrapped-based CI: 1.06, 4.40). A variable importance analysis, combined with GEE logistic regression to account for clustered observations, identified a high frequency of trash collection (OR = 0.38, 95% CI: 0.21, 0.67), having an outside water faucet (OR = 0.41, 95% CI: 0.22, 0.76), ownership of a car or motorcycle (OR = 1.99, 95% CI: 1.11, 3.58), and age (OR = 1.02, 95% CI: 1.00, 1.03) as significant predictors of CHIKV infection over the study period and across households. Spatial analysis identified two households that were hotspots for seroconversions. Our analyses suggest that the Asian clade of CHIKV present in the Americas causes fewer symptomatic cases than the more widespread East/Central/South African CHIKV lineage, which has a reported S:A ratio of 1:0.03 to 1:0.39. Overall, these results help inform regionally specific public health planning for future CHIKV outbreaks as well as modeling of CHIKV transmission dynamics.

## 4

## IN THE SEARCH OF THE HIDDEN PATH: THE SOURCE AND SPREAD OF CHIKUNGUNYA AND ZIKA VIRUSES

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Recent outbreaks of taxonomically diverse arboviruses around the world have raised concern in the global health community about how to abate the spread of these viruses. A strategy to avoid future outbreaks is not only respond to current endemic events, but also isolate the regions

around the world that serve as hubs for the spread of the viruses. Using phylogenetic analysis of whole genomic sequences as the starting point, we analyze changes of place of isolation in transmission graphs to demonstrate not only the source of outbreaks but also key geographic regions that facilitate the spread. This work uses betweenness centrality of transmission networks to identify geographic hubs for the spread of viral lineages. The identification of hubs for the spread of viruses can show recurrent events that can help to design abatement strategies. Of interest in this comparison is the similarity of the transit across the world of these taxonomically diverse viruses that have similar vector biology. Thus, in addition to understanding hubs for the spread of these viruses, we underscore the importance of vector control. In our results, we observe a major hub for Chikungunya in SE Asia and Oceania as sources for the current outbreaks. In contrast, the outbreak of Zika, which had its origin in Asia, has a hub in South America. Increased viral surveillance, as well rapid sequencing of pathogens from infected patients worldwide is the current path that will allow more granular studies required to investigate emerging diseases as they evolve and spread.

## 5

### ADAPTED LONGITUDINAL MOSQUITO SALIVA COLLECTION METHOD FOR DETERMINING ARBOVIRUS VECTOR COMPETENCE INCREASES BIOSAFETY AND CAPACITY

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The recent emergences of Zika, chikungunya and yellow-fever viruses emphasize the need for rapid, reliable, and safe methods for assaying arbovirus transmission from various species of mosquitoes. For transmission to occur, arboviruses must replicate in the mosquito salivary glands and transmit in the saliva upon feeding on a vertebrate host. Hence, assaying titers of virus in mosquito saliva is an essential aspect of evaluating the transmission potential of the vector-virus system. Current collection methods are laborious and typically require sacrificing the mosquito, limiting researchers to a single saliva collection time point per insect. To optimize the collection of mosquito saliva, we adapted the field-oriented honey-trap method to the laboratory setting. The adaptation of this technique allows us to collect multiple individual saliva samples over time by providing filter paper soaked with a sucrose solution, into which the mosquitoes deposit their saliva. To detect chikungunya virus from experimentally infected mosquitoes, quantitative reverse-transcription PCR, normalized to a standard curve, was performed on RNA purified from the filter paper. We found that 12-well plates sealed with a specially designed lid (made by 3-D printing) and organoids were suitable habitats for both *Anopheles gambiae* and *Aedes aegypti*, as they provided a compact living space and improved biosafety. We optimized several aspects of the feeding, including filter paper size and type, food amount, and dye concentration for visually identifying fed mosquitoes. Our filter paper saliva collection technique is a promising method for assaying arbovirus transmission rates over time from experimentally infected mosquitoes as it facilitates rapid longitudinal collection of infected saliva from individual mosquitoes. Also, by eliminating the need for glass capillaries and reducing manipulation of mosquitoes, this technique improves biosafety, saves time and allows for increased numbers of mosquitoes per experiment.

## 6

### EMERGENCE OF RECOMBINANT MAYARO VIRUS STRAINS FROM THE AMAZON BASIN, THE DAWN OF A NEW EPIDEMIC?

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Mayaro virus (MAYV), the causative agent of Mayaro fever, is an arbovirus transmitted by the *Haemagogus* species of mosquito endemic to the Amazonian forest in South America. Despite its role in a highly debilitating disease and recent evidence of spread areas outside of the Amazonian regions of Central and South America, limited information about the evolution and the epidemiology of MAYV represent an important barrier to prevention of further spread. We analyzed host adaptability, evolutionary and epidemiological history of MAYV strains collected within the Amazon basin, São Paulo State, and Haiti. Bayesian phylogeography based on molecular clock calibrated genealogies was used to investigate the spatiotemporal spread of MAYV lineages in South American and the Caribbean areas, as well as to infer the origin of detected recombination events. Our analysis revealed specific adaptations to a broad host and vector range, including humans and the *Aedes* mosquito species, and assessed recombination events. The first recombinant strain appeared between 2002 and 2013 in Pará State (Brazil), and moved to São Paulo State, giving rise to a second recombinant that was eventually isolated in Haiti in 2015. We hypothesize that human mobility and adaptability to a broad range of host and vector species played a central role in the emergence of recombinant strains, which are usually rare among arboviruses. Moreover, the potential urbanization of this virus might be the beginning of a much larger, more global epidemic and deserves, therefore, close monitoring in the immediate future.

## 7

### RE-EMERGING OF MAYARO VIRUS IN AREAS WITH CIRCULATION OF DENGUE VIRUS IN THE PERUVIAN AMAZON

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Mayaro virus is an Alphavirus (family *Togaviridae*). The circulation of Mayaro virus is frequently a cause of enzootic disease. Climate change is altering its ecological niche and can affect populations of immunologically “virgin” humans. Infection with Mayaro virus presents symptoms similar to dengue, so it is important to consider the differential diagnosis with other arboviruses. The circulation of Mayaro virus in samples with a presumptive diagnosis of Dengue within the surveillance of Dengue in 4 regions of the Amazon region of Peru was investigated. A descriptive study was carried out between January and March 2017, including the analysis of 1,983 serum samples from patients presenting with acute febrile syndrome compatible with dengue, which were referred to the Viral Metaxenic Laboratory of the National Health Institute from dengue surveillance in Peru. Samples were inoculated into C6/36 HT (ATCC 1660, *Aedes albopictus*) and Vero (ATCC, CCL-81, *Cercopithecus aethiops*) cells. Cultures in which the cytopathic effect was evidenced, were evaluated for Dengue, Zika, EEV, Chikungunya, Mayaro and Oropuche virus, by Immunofluorescence Assay (IFA) using monoclonal and polyclonal antibodies (CDC, Puerto Rico). The results show the isolation of 186 viral strains: 155 cases of DENV2 (83.3%), 13 cases DENV3 (6.9%), 5 cases DENV4 (2.7%), 2 cases ZIKAV (1.1%) and 11 cases with MAYV (5.9%). The results obtained were corroborated by RT-PCR, confirming the circulation of Mayaro virus, in addition to Dengue virus in the Peruvian Amazon. It was evidenced the co-circulation of MAYV and DENV in the Peruvian

Amazon. These results highlight the importance to carry out surveillance of the febrile syndrome and the development of tests for a simultaneous and timely diagnostic of other arboviruses with febrile syndrome according to the different ecological niches.

## 8

### NON-INFERIORITY COMPARISON OF TAFENOQUINE SIX-MONTH RELAPSE-FREE EFFICACY VERSUS PRIMAQUINE IN *PLASMODIUM VIVAX* INFECTION: AN INDIVIDUAL PATIENT DATA META-ANALYSIS

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Tafenoquine (TQ; SB-252263; WR238605) is an 8-aminoquinoline drug for the radical cure of *Plasmodium vivax* malaria being co-developed as a single dose treatment by GlaxoSmithKline (GSK) and Medicines for Malaria Venture (MMV). The phase 3 programme for tafenoquine consists of an efficacy study (DETECTIVE part 2, NCT01376167) and a safety study (GATHER, NCT02216123). DETECTIVE part 2 is designed to demonstrate superior efficacy of single-dose TQ 300mg over placebo. An arm taking primaquine (PQ 15mg for 14 days) is included for benchmarking, but the study is not powered to make a direct comparison of TQ versus PQ. GATHER is a safety study comparing the TQ versus PQ, the primary endpoint being clinically-relevant haemoglobin (Hb) decline (defined as a Hb decline >30g/L or ≥30% from baseline, or an absolute fall to <60g/L) and the need for blood transfusion. Recruitment was completed for both trials at the end of 2016. Patients were aged ≥16 years, with glucose-6-phosphate dehydrogenase activity levels ≥70% of the median in normal males for that site (or ≥40% for females in GATHER only). All patients received chloroquine at baseline and were followed up for six months to monitor for *P. vivax* relapse and for safety. Patients were recruited from Brazil, Cambodia, Colombia, Ethiopia, the Philippines, Peru, Thailand, and Vietnam. The studies were approved by the respective ethics committees governing each site. Together, these studies will contain data from ~420 patients treated with TQ and ~210 treated with PQ (exact numbers not known prior to unblinding). We will present a meta-analysis of six-month relapse-free efficacy using individual patient-level data to test the hypothesis that TQ is non-inferior to PQ. The TQ versus PQ hazard ratio and 95% confidence interval (from multivariable Cox regression) will be assessed for non-inferiority based on a pre-determined non-inferiority margin of 1.21 (translated from a 75% preserved effect of PQ versus placebo). Superiority of TQ over PQ will be claimed, if it exists. We will also present summaries for each study of adverse events by frequency and severity, including Hb decline and blood transfusions.

## 9

### CONFIRMATION OF THE BLOOD STAGE SCHIZONTICIDAL ACTIVITY OF TAFENOQUINE IN A RANDOMIZED, DOUBLE-BLINDED, PLACEBO-CONTROLLED *PLASMODIUM FALCIPARUM* INDUCED BLOOD STAGE MALARIA CHALLENGE STUDY

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Tafenoquine (TQ), a primaquine analog, with a half-life of ~14 days, has appealing properties as a drug for malaria chemoprophylaxis. Previous studies have suggested that TQ is effective against both liver and asexual blood stages of *Plasmodium falciparum*. However, it was difficult to determine the contribution of the blood stage activity to its overall

prophylactic effect. To investigate its blood stage schizonticidal activity distinct from liver stage activity, a randomized, double-blinded, placebo-controlled blood stage challenge study with *P. falciparum* was undertaken in 16 healthy, malaria-naïve adults. The study was conducted in 2 cohorts of 8 (6 active/2 placebo), with subjects in each cohort receiving 200 mg of TQ or placebo on days 1, 2, 3 and 10, followed by an IV inoculation with 2,800 *P. falciparum* blood stage parasites on day 13. The primary endpoint was the number of participants requiring rescue treatment with artemether/lumefantrine due to PCR-determined parasitemia >5,000/mL before end of study (Day 32). Secondary endpoints include adverse effects, and TQ concentration if drug failure occurred. All 12 subjects in the TQ arm remained PCR-negative, while all 4 subjects allocated to placebo reached target parasitemia in the expected timeframe. Two subjects had a mild asymptomatic drop in Hb: A female subject allocated to the TQ arm had a 28g/L (20.3% from baseline of 138g/L) drop despite normal G6PD levels. G6pd Genotyping is underway; a male subject, also allocated to TQ had a transient 20g/L (13.7% from baseline of 146g/L) drop in Hb. In conclusion, the findings of this study support the hypothesis that TQ has sufficient blood stage schizonticidal activity to prevent clinical malaria if there is a small subpopulation of liver stage *P. falciparum* parasites that escape its causal prophylactic effect of TQ.

## 10

### BUZZOFF - A PHASE 1A, FIRST-IN-HUMAN STUDY OF (+)-SJ000557733 (SJ733), AN ORAL, NOVEL INHIBITOR OF *PLASMODIUM FALCIPARUM* PLASMA MEMBRANE PROTEIN PFATP4

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SJ773 is a novel, orally available inhibitor of *P. falciparum* ATP4 (PfATP4; a critical sodium-proton antiporter in the parasite), and the second in class to enter clinical development. Preclinical data indicating potent activity against both standard and drug resistant *P. falciparum* and no predicted significant toxicity prompted the conduct of a Phase 1a, First-In-Human Safety and Pharmacokinetic (PK) in adult healthy volunteer study (ClinicalTrials.gov: NCT02661373). The study employed a single ascending dose (SAD) escalation design with an adaptive component, allowing for modifications to the dose increments and number of subjects per cohort based on PK results. In the SAD, six dose increments were administered in a fasting state (75, 150, 300, 600, 900, 1200 mg) using a leap-frog study design. All 23 subjects (22 male, one female) administered 42 single doses of SJ733 tolerated the study drug well, with no serious adverse events, dose limiting toxicities, Grade 3 or 4 adverse events (AE), or clinically significant ECG/safety laboratory tests findings. A majority of the reported AEs were Grade 1 or less and considered unlikely or un-related to SJ733. Plasma exposure of SJ733 and its major N-oxide metabolite SJ506 following single oral doses of SJ733 were dose-proportional across the 75 - 600 mg dose range, but plateaued at 900 mg. The median CL/F for doses ≥ 900 mg was higher than the median value at 75 - 600 mg (56 vs 32 L/h for SJ733 and 27 vs 17 L/h for SJ506). The median Tmax for SJ733 was 1.2 h (range 0.7 - 3.0 h). The median t1/2 was dose-independent around 19 h for both SJ733 and SJ506. 14 of 15 subjects receiving ≥ 600 mg SJ733 exceeded the target efficacious exposure of 13,000 hr-ng/mL defined from a relevant preclinical murine malaria model. A standard FDA high-fat meal at 600 mg did not affect exposure of SJ733, but appeared to slow the absorption. A phase 1a multiple ascending dose study is currently

ongoing. In summary, SJ733 demonstrated favorable PK and safety profiles to date in healthy adults. These results prompted the interposed start of a Phase 1b human challenge study where SJ733 is tested against induced blood-stage malaria.

## 11

### PROPHYLACTIC ACTIVITY OF DSM265 AGAINST PRE-ERYTHROCYTIC *PLASMODIUM FALCIPARUM* CONTROLLED HUMAN MALARIA INFECTION BY MOSQUITO BITES AND DIRECT VENOUS INJECTION

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New anti-malarial drugs are needed for prophylaxis and radical cure. DSM265 is a triazolopyrimidine-based inhibitor of dihydroorotate dehydrogenase (DHODH), a key enzyme in pyrimidine biosynthesis. DSM265 demonstrated promising preclinical activities against liver and blood stages. The drug also showed causal prophylactic activity when given one day prior to controlled human malarial infection (CHMI) by direct venous inoculation (DVI) with *Plasmodium falciparum* (Pf) sporozoites (PfSPZ) but was less efficacious when administered one week before CHMI. This trial was performed to assess the safety, tolerability, pharmacokinetic profile and prophylactic activity of a single 400mg dose of DSM265 3 or 7 days prior to CHMI initiated by either DVI of PfSPZ or by five Pf-infected mosquito bites. The goal was to refine the dosing interval for prophylaxis. Subjects were treated with atovaquone-proguanil upon reaching a qRT-PCR treatment threshold of  $\geq 250$  estimated parasites/mL. Three cohorts (6 treated + 2 placebo control subjects) were completed with DSM265 dosing 3 (Cohort 1) or 7 (Cohort 2a) days prior to PfSPZ DVI CHMI or 7 days prior to mosquito bite CHMI (Cohort 2b). The most common AE related to DSM265/placebo dosing was Grade 1-2 headache. There were no Grade 3 AEs related to DSM265. DSM265 pharmacokinetics were similar for all cohorts (AUC[0-240hr] 1241  $\mu\text{g/mL} \times 240$  hrs). Placebo controls rapidly became treatment eligible a median of 8.3 days post-CHMI. For DSM265-treated subjects, 2/6 in each cohort were sterilely protected while 4/6 showed considerable delay in time to treatment compared to placebo controls [median time to treatment Cohort 1: 21.9 d; Cohort 2a: 15.3 d; Cohort 2b: 16.9 d;  $p = 0.004$ , log-rank test comparing time to treatment DSM265 vs. placebo for each cohort]. One dose of DSM265 (400 mg) given 3 or 7 days prior to CHMI was safe, well tolerated, sterilely protected 33% of subjects and delayed time to treatment in the rest. Future studies should evaluate weekly DSM265 dosing. This is the first drug study to directly compare DVI vs. mosquito bite CHMI, and times to positivity and treatment as well as parasite densities were comparable for both routes.

## 12

### A PHASE 1 EVALUATION OF THE PHARMACOKINETIC-PHARMACODYNAMIC INTERACTION OF THE ANTIMALARIAL AGENTS KAF156 AND PIPERAQUINE

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KAF156, an imidazolopiperazine, represents a new antimalarial class with activity against asexual and sexual blood stages and the preerythrocytic liver stages. Based on *in vitro* data, a two way interaction was hypothesized for KAF156 use in combination with piperavaquine (PPQ), a long acting antimalarial, as both drugs are CYP3A4 substrates and inhibitors. PPQ is known to prolong the QT interval and therefore potential combination effects on the QT interval were also assessed. This was an open-label, parallel-group, single-dose study in healthy volunteers randomized to three parallel dosing arms (1:1:1) of 800 mg KAF156 + 1280 mg PPQ, 800 mg KAF156 alone and 1280 mg PPQ alone. Triplicate ECGs were done over the first 48 hours after dosing. Routine safety and pharmacokinetic assessments were carried out up to 61 days. Of the 72 healthy male subjects recruited, 68 completed the study. Co-administration of PPQ and KAF156 had no overall effect on AUC of both the drugs. However, C<sub>max</sub> values of both KAF156 (~23%) and piperavaquine (~70%) increased. Statistically it could not be concluded or ruled out an at least 30% increase in the C<sub>max</sub>. Both drugs given alone or in combination were well tolerated with no deaths and serious adverse events (SAEs). AEs were observed at the frequency of 87.5%, 79.2% and 58.3% respectively for the KAF156+PPQ, PPQ arm and KAF156 arm. The most common AEs were nausea and headache. There were no Grade 3 or 4 events. There were no ECG related AEs, no QTcF interval >480 msec and no QTcF interval increase from Baseline >60 ms. Mean maximum increase was observed at 24 hours post-dose for both KAF156+PPQ and KAF156 monotherapy arm; the mean increase from pre-dose was 7.9 msec and 6.6 msec, respectively. For PPQ monotherapy arm, the maximum increase from pre-dose was found to be 1.8 msec (mean) at 4 hour post-dose. There was no significant difference between the combination arm and other arms in maximum change QTcF ( $p > 0.05$ ). Overall, no safety/cardiac risk or drug-drug interaction was identified which would preclude use of KAF156 and PPQ combination in future studies.

## 13

### A RANDOMIZED TRIAL OF THE SAFETY AND EFFICACY OF LOW DOSE PRIMAQUINE IN THE TREATMENT OF ADULT PATIENTS WITH *PLASMODIUM FALCIPARUM* MALARIA IN SENEGAL

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The addition of a single dose of primaquine (0.25 mg base/kg) to artemisinin combination treatments (ACTs) is a potentially useful component of malaria pre-elimination or elimination programmes. But before it can be widely used in Africa more information about its safety in populations where G6PD deficiency (G6PDd) is common is required. This study assessed the safety of adding low-dose primaquine to the normal ACT regimen in adult patients treated for malaria in Senegal. Adults with *Plasmodium falciparum* malaria were randomized to receive one of three

ACT with or without primaquine (PQ) (0.25mg/kg). G6PD status was determined using a rapid test. Patients were followed for 28 days to record haemoglobin concentration, adverse events, and gametocyte carriage. The primary end point was the change in Hb at day 7. Other end points included: occurrence of severe anaemia (Hb<8), gametocyte carriage; incidence of clinical adverse events. A mixed model was used to estimate the effect of PQ on haemoglobin concentration at each time point, and to assess the interaction with G6PD status. 274 patients were randomized, 139 received an ACT alone, and 135 received an ACT+PQ. The mean reduction in Hb at day 7 was similar in each group with a difference in the ACT+PQ versus the ACT alone group of -0.04g/dL (95%CI -0.23,0.31) but the effect of primaquine differed according to G6PD status. In G6PDd patients the drop in Hb was 0.63g/dL (95%CI 0.03;1.24) greater in those who received PQ than in those who received an ACT alone. In G6PD-normal patients, the reduction in Hb was 0.22g/dL (95%CI -0.08;0.52) less in those who received PQ (interaction  $p=0.01$ ). Dark urine was more frequent in patients who received PQ. PQ was associated with a 73% (95%CI:24 - 90) reduction in gametocyte carriage ( $p=0.013$ ). In conclusion, Primaquine substantially reduced gametocyte carriage. However, the fall in Hb concentration at day 7 was greater in G6PD-deficient patients who received primaquine than in those who did not.

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### THE PLASMODIAL ACYL CO-A SYNTHETASE 10 AND 11 ARE INVOLVED IN DRUG RESISTANCE TO TWO DISTINCT ANTIMALARIAL COMPOUNDS

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The emergence and spread of drug resistance to current antimalarial therapies remains a pressing concern, escalating the need for compounds that demonstrate novel modes of action and prevent the development of drug resistance. The Malaria Drug Target Identification Project has adopted a chemogenomic approach to identify targets of the most promising compounds from chemically diverse libraries. Study compounds were selected based on availability, purity, and potency in a multi-drug resistant isolate, and lack of known mechanism of action. To generate resistant parasite lines 3D7 parasites were cultured in the presence of these novel compounds until they showed at least a 2-fold shift in EC50 at which time they were cloned and analyzed by whole genome sequencing. Two structurally unrelated compounds analyzed (MMV665924 and MMV019719) selected for parasites with mutations in the acyl Co-A synthetase 11 (ACS 11, PF3D7\_1238800) gene. Selection with MMV665924 also resulted in a single nucleotide polymorphism (SNP) in the ACS10 (PF3D7\_0525100) locus. ACS11 and ACS10 are both members of the ACS gene family that activate fatty acids (FA) scavenged from the host. To confirm the role of these mutations in resistance to the two compounds we used the CRISPR/CAS9 system to introduce the SNPs into a 3D7 line. Introduction of the SNPs increased the level of resistance to the same level as the drug selected lines confirming the role of these SNPs in drug resistance. These are the first functional mutations in this gene family. However, the ACS family is heterogeneous both with regards to gene number and diversity within natural populations. ACS10 and 11 alone have 65 different nonsynonymous SNPs reported. We discovered natural mutations at the same site at high prevalence in the Malawi parasite population. Indeed, an isolate containing the M3001 mutation in ACS10 was fourfold more resistant to MMV665924 than a matched wild-type Malawi isolate. These findings indicate that mutations in *loci* related to FA activation can confer drug resistance, and that these mutations are identified among natural *P. falciparum* isolates from Malawi implicating their importance.

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### GENOMES OF TROMBIDIID MITES UNCOVER ADAPTATIONS TO PARASITISM IN THE SCRUB TYPHUS VECTOR, LEPTOTROMBIDIUM DELIENSE

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The mite superfamily Trombidoidea, in which only the larvae are parasitic, contains two major families, the Trombidiidae and the Trombiculidae. Trombidiids (velvet mites) include species in which the larvae parasitize arthropods of agricultural, veterinary and/or medical importance, and some adult forms are predators of pest arthropods. Larval trombiculids, commonly known as "chiggers," parasitize a wide range of vertebrates, including humans, and are vectors of scrub typhus, a tropical disease of increasing importance worldwide. Here we present the genomes of the giant red velvet mite, *Dinotrumbium tinctorium*, and the primary South-East Asian scrub typhus vector, *Leptotrombidium deliense*. In support of the classical taxonomic scheme, these species form a distinct branch of the order Trombidiformes, with an estimated divergence time from the spider mites (Tetranychidae) of 250 million years. Relative to the phytophagous *Tetranychus urticae*, trombidioid mites exhibit a small expansion in ionotropic receptors involved in olfaction and temperature sensing. Approximately 25 orthologous clusters of putative salivary proteins in the trombidioid mites are shared with ticks but not the more closely related *T. urticae*, and include proteins with potential roles in folate metabolism, countering inflammation, and vasoconstriction. In *L. deliense*, several predicted allergens are present that were not found in other mite genomes, including *Sarcoptes scabiei*. Moreover, analysis of bacterial sequences revealed that *Borrelia* and *Francisella* are among the more abundant components of the *L. deliense* microbiome. These findings highlight adaptations to parasitism of the trombidioid mites and will facilitate further high-throughput and functional analyses for exploitation and control of this group.

## 16

### COMMUNITY DIRECTED VECTOR CONTROL FOR ONCHOCERCIASIS

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Onchocerciasis elimination programs rely primarily upon mass distribution of ivermectin (MDA). However, supplementing MDA with vector control will accelerate, and in some cases may be necessary, to achieve elimination. Insecticide treatment of vector (*Simulium* spp) breeding sites is the accepted method of vector control for onchocerciasis. However, this is both expensive and potentially harmful to the environment. We have evaluated a novel vector control method based upon removal of vegetative substrates for black fly larvae by community members (Slash and Clear). *S. damnosum* s.l. breeding sites in Northern Uganda were identified using a previously developed remote sensing model. A total of eight communities located within 1km of a breeding site were identified and organized into pairs. Biting rates were monitored in all communities for 8 days. Volunteers from one community in each pair were then taught to remove the trailing vegetation at the breeding sites, while no interventions were carried out in the paired control community. Biting rates were then monitored for a period of 21 days post intervention. A significant reduction in the biting rates (averaging 90%) was observed at days 16-21 post intervention the intervention communities, while biting rates in the control averaged 100%

of those seen in the first seven days at days 16-21. Long-term followup in two villages indicated that the effect was long lasting, with fly numbers in two intervention communities reaching just 31% of pre-intervention levels at 130 days post-intervention. These data suggest that slash and clear may represent a viable alternative to insecticide treatment of breeding sites for reduction of *S. damnosum* s.l. biting in onchocerciasis endemic communities in Africa. Combining slash and clear with ivermectin MDA may accelerate elimination of onchocerciasis in many communities where this technique is feasible.

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### ATTRACTION AND OVIPOSITION PREFERENCES OF *PHLEBOTOMUS PAPTASI*, VECTOR OF OLD-WORLD CUTANEOUS LEISHMANIASIS, TO LARVAL REARING MEDIA AND SAPROPHYTIC BACTERIA

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We report the preliminary results of a systematic assessment of media from various developmental stages of the sand fly using oviposition and olfactometer behavioral assays. We conducted multiple-choice oviposition assays in 500 mL Nalgene jars. Six treatments were placed on separate filter paper discs at the bottom of the jar: 2nd/3rd larval instar medium, 4th larval instar/pupae medium, frass from expired colonies, larval food (aged rabbit chow and rabbit feces mix), rabbit feces, and a solvent (water) control. Fifty gravid females were introduced into each jar. Cumulative number of eggs laid on each filter paper per jar was counted at different time intervals from digital images. Attraction of gravid sand flies to these six treatments was assayed with a 3-chamber linear olfactometer. Twenty gravid females were transferred to the middle chamber of the olfactometer and their distribution in treatment and control chambers was recorded after 3 h. Comparing mean cumulative egg deposition among the six treatments, we found that significantly more eggs were oviposited on 2nd/3rd larval rearing medium followed by 4th instar/pupae rearing medium. The olfactometer results were consistent with the oviposition assays. Twelve bacterial species were cultured from this most-attractive substrate and evaluated for their attractiveness to gravid females. Our finding indicates that sand fly-digested host food and feces is attractive to gravid females and suggests that the larvae and larval gut microbiome may be involved in conditioning the oviposition substrate and possibly the production of oviposition attractants and stimulants. Chemical structures of GC-EAD bioactive compounds isolated from attractive bacterial strains were elucidated by gas-chromatography-mass spectrometry (GC-MS). Analysis of volatile compounds using a capillary column of different polarity revealed that the antennal active compound was a mixture of two compounds.

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### A LESS DIVERSE TICK MICROBIOME IS ASSOCIATED WITH *RICKETTSIA* INFECTED TICKS

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Spotted Fever Group Rickettsiosis agents have been identified in more than ten ixodid tick species. Specifically, in southwestern Tennessee *Rickettsia amblyommatis* was recovered from *Amblyomma americanum*, *R. parkeri* from *A. maculatum*, and *R. montanensis* from *Dermacentor variabilis*. Knowing vector microbiomes modulate infection, our objectives were to define and compare the tick microbiome by describing the microbial communities within three different tick species and comparing those communities with the presence and absence of *Rickettsia* species. Field-collected ticks were screened for *Rickettsia* using *ompA* PCR to determine initial infection, then the V3-V4 region of the 16S rRNA segment of

bacterial genomes was amplified using the Illumina MiSeq platform. The Mothur pipeline was used to investigate the structure of the microbiome of each species and determine differences based on tick species, diversity, richness, and presence of *Rickettsia*. We identified unique microbial communities within each tick species, and discovered bacteria common in all three species. We also observed a significant difference in the diversity and richness of *Rickettsia* infected ticks, and that the microbiome of *Rickettsia*-positive ticks were enriched with specific bacteria and less diverse. We also noted that the traditional *ompA*-PCR had lower sensitivity and higher specificity than 16S MiSeq libraries for amplifying *Rickettsia*. These foundational data summarize the microbial communities of three tick species associated with *Rickettsia* infection, and demonstrate the potential use of Illumina sequencing for diagnostics. Identifying the microbiota within Ixodid ticks of different species and pathogen presence-absence can provide an additional option for vector-borne zoonotic disease management to improve human and animal health.

## 19

### SARCONESIN: A NEW ANTIBACTERIAL PEPTIDE FROM BLOWFLY *SARCONESIOPSIS MAGELLANICA* (DIPTERA:CALLIPHORIDAE) LARVAL EXCRETIONS AND SECRETIONS

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Larval therapy (LT) is an alternative treatment for healing chronic wounds; its action is based on debridement, the removal of bacteria and stimulating granulation tissue. The most important mechanism when using LT for combating infection depends on larval excretions and secretions (ES). Larvae are protected against infection by a spectrum of antimicrobial peptides (AMPs); the goal of this study was to evaluate the presence of AMPs in the ES of a medically-important necrophagous fly previously used in LT: *Sarconesiopsis magellanica* (Diptera: Calliphoridae). The study of this specie becomes significant in wound healing, since they degrade necrotic tissue and kill different bacteria during LT. This study shows for the first time a small AMP being isolated from *S. magellanica* ES products obtained from third-instar larvae. For the first analysis, ES were fractionated by RP-HPLC using C18 columns; the antimicrobial activity of the fractions was evaluated by incubation with different pathogens. The primary sequences of the fractions were determined by mass spectrometry and *de novo sequencing*; five fractions with antimicrobial activity were obtained, the Sarconesin fraction was characterized and the minimum inhibitory concentrations (MIC) were tested in serial dilution starting at 1.2 µM. Potent inhibitory activity was shown against Gram-negative (*Escherichia coli* D31, *Pseudomonas aeruginosa* 27853) and Gram-positive (*Staphylococcus aureus* ATCC 29213, *Micrococcus luteus* A270) bacteria. Sarconesin shows similarity with Rho-family GTPases which are important in organelle development, cytoskeletal dynamics, cell movement and wound repair. The data reported here indicated that Sarconesin, a small 13 residues AMP, could be a promissory alternative candidate for use in therapeutics against Gram-negative and Gram-positive bacterial infection. Our study describes one peptide responsible for antibacterial activity when LT is being used. The results shown here support carrying out further experiments aimed at validating *S. magellanica* AMPs as novel resources for combating antibacterial resistance.

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## MATHEMATICAL MODELLING OF FOCAL VECTOR CONTROL AS A COMPLEMENTARY STRATEGY FOR ONCHOCERCIASIS ELIMINATION

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The control of onchocerciasis is considered a public health success story. The Onchocerciasis Control Programme in West Africa (OCP, 1974-2002), initially an anti-vectorial intervention in 11 countries, averted 600,000 cases of preventable blindness and made 25 million hectares of land habitable and productive. The African Programme for Onchocerciasis Control (APOC, 1995-2015), mainly an anti-parasitic intervention in the remaining endemic African countries, averted 17.4 million disability-adjusted life years (DALYs). None of these programmes, however, led to regional elimination of *Onchocerca volvulus* by the time of their closure. In 2012 the World Health Organization set goals for the elimination of onchocerciasis transmission by 2020 in selected African countries. Mathematical modelling has indicated that elimination will not be achieved with annual ivermectin distribution in all endemic foci; alternative treatment strategies (ATS) will be required. One such ATS is localized vector control in foci with intense transmission. Models need to consider the population dynamics and ecology of the main vector, *Simulium damnosum* sensu lato (s.l.), to evaluate the potential epidemiological impact of vector control in combination with current and complementary treatment strategies. We refined our simuliid population dynamics model to explore the impact of larviciding on *S. damnosum* s.l. biting rates. We calibrated the model with datasets from: a) OCP-era savanna sites in Ghana where larviciding was interrupted and then resumed; b) sites in Nigeria that underwent early-stage larviciding, and c) forested APOC sites in Equatorial Guinea and Cameroon. We explored the impact of varying the frequency, duration and efficacy of larvicidal treatments on the effectiveness of vector control, quantified as the number of blackfly bites prevented. We discuss our results in the context of implementing ATS for the control and elimination of onchocerciasis in Africa, and integrate our vector model into the EPIONCHO transmission model to explore how localized, low-cost, vector control might complement ivermectin-based interventions.

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## SYSTEMIC INSECTICIDE TREATMENT OF THE CANINE RESERVOIR OF *TRYPANOSOMA CRUZI* INDUCES HIGH LEVELS OF LETHALITY IN *TRITOMA INFESTANS*, A PRINCIPAL VECTOR OF CHAGAS DISEASE IN BOLIVIA

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Despite large-scale reductions in Chagas disease prevalence across Central and South America, *Trypanosoma cruzi* infection remains a considerable public health problem in the Bolivian Gran Chaco region where vector-

borne transmission persists. In these communities, peridomestic animals are major blood-meal sources for triatomines, and dogs play an important role in maintaining bug populations, potentially compromising the long-term sustainability of current control efforts. This study evaluated the systemic activity of three commercial oral insecticides (Bravecto®, fluralaner; NexGard®, afoxolaner; and Comfortis®, spinosad) in canine feed-through assays against *Triatoma infestans*. Twelve healthy, outbred dogs were recruited from the national rabies control program in Santa Cruz, Bolivia, and randomized to three groups, containing one control and three treated dogs. Following treatment, starved 2nd and 3rd stage *T. infestans* instars were exposed to dogs for 30 minutes at 2, 7, 21, 34 and 51 days post-treatment. Eighty-five per cent (768/907) of *T. infestans* successfully blood-fed during bioassays, with significantly more bugs becoming fully-engorged when exposed to Bravecto® dogs ( $p < 0.001$ ). Bravecto® or NexGard® treatment induced 100% triatomine mortality in fully- or semi-engorged bugs within 5 days of feeding at every time point. The lethality effect for Comfortis® was much lower (50-70%) and declined almost entirely after 51 days. Instead Comfortis® resulted in substantial morbidity; a third of bugs fully recovered after 120 hours but were unable to feed even 30 days later. A single oral dose of Bravecto® or NexGard® was safe and well tolerated, producing complete triatomine mortality on treated dogs over 7.3 weeks. While both drugs were highly efficacious, more bugs exposed to Bravecto® were immediately knocked-down and took complete blood-meals. Coupled with its longer residual activity, Bravecto® represents an ideal insecticide to develop into an operationally-feasible, community-level method of reducing triatomine infestation and controlling *T. cruzi* transmission in the Bolivian Gran Chaco region.

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## RENEWED MASS DRUG ADMINISTRATION'S IMPACT ON LYMPHATIC FILARIASIS IN A POPULATION WITH LONG LASTING INSECTICIDAL BEDNETS IN PAPUA NEW GUINEA

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A community of 5000 people in Dreikikir, Papua New Guinea (PNG) received 5 annual Mass Drug Administrations (MDA) 20 years ago, dramatically decreasing lymphatic filariasis (LF) prevalence but failing to end transmission. Subsequent vector control against the anopheline vector with long lasting Insecticidal bednets (LLIN) in 2009 reduced mosquito density and LF vectorial transmission potential to the point where transmission cessation was considered possible. To assess the impact of renewed MDA in 2015, night-time finger prick blood samples were collected from a sentinel group of 530 children  $\leq 10$  years-of-age (y) and 732 individuals 11-80y in 14 villages characterized as "moderate" or "high" transmission (32-63% and 63-85% MF+ prior to any interventions, respectively) before MDA (January-May 2015) and one year after MDA. Samples were examined for Mf by microscopy and filarial antigen by rapid tests (ICT in 2015 and the more sensitive FTS in 2016; because the tests are not directly comparable only FTS are reported). Additional monitoring assays (BM14, WB123 and Og4C3) are ongoing. In the "moderate transmission" sites prior to renewing MDA, children  $\leq 10$ y had 0% MF+ and individuals  $> 10$ y 2.8% MF+. In 2016 ALL individuals were MF negative, however infections persisted: children  $\leq 10$ y 1.7% were FTS+ and individuals  $> 10$ y 21.0% were FTS+. In "high transmission" sites MF prevalence decreased from 2.8% to 0.6% in  $\leq 10$ y after MDA and from 22.0% MF+ to 15.1% MF+ in  $> 10$ y. FTS positivity remained high in both age groups, 12.2% FTS+ in  $\leq 10$ y and 59.9% in  $> 10$ y. Though MF+ fell below limits of detection in moderate transmission sites, sustained antigen positivity in the population, especially in sentinel age children, highlights ongoing transmission. The adjacent higher transmission villages (2 to

15 km away in the same treatment unit) remained above programmatic thresholds to continue MDA. Factors contributing to ongoing transmission are failure of current MDA combinations to completely eliminate Mf and the inability to achieve adequate coverage of individuals with high Mf counts. Continuing studies aim to address these limitations for current MDA programs.

## 23

### MAPPING OF LYMPHATIC FILARIASIS IN LOIASIS AREAS: A NEW STRATEGY SHOWS NO EVIDENCE FOR *WUCHERERIA BANCROFTI* ENDEMICITY IN CAMEROON

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Mapping of lymphatic filariasis (LF) caused by *Wuchereria bancrofti* largely relied on the detection of circulating antigen using the ICT card test. Several studies have recently shown that this test is cross-reactive with sera of subjects heavily infected with *Loa loa* and that mapping results in loiasis endemic areas may be inaccurate. In order to develop an LF mapping strategy for areas with high loiasis prevalence, we collected day blood samples from 5,000 subjects residing in 50 villages in 5 areas throughout Cameroon (south, central, south-east North-west and far-north). Antigen testing using FTS (a novel platform that uses the same reagents as ICT), revealed an average positivity rate of 1.1% and *L. loa* microfilaria (Mf) rates between 0.1 and 43.4%. Among the subjects with 0 to 8,000 Mf/ml only 0.5% were FTS positive, while 25% of 118 subjects with >8,000 Mf/ml were also FTS positive. None of the FTS positive samples was positive for *W. bancrofti* antibodies as measured by two different PoC Wb123 tests that use an antigen not found in *L. loa*. Night blood examination of the FTS positive subjects showed also a high prevalence of *L. loa* Mf with average densities of up to 9,180 Mf/ml, but no *W. bancrofti* Mf (confirmed by qPCR). Our results show that high *L. loa* Mf in day blood are a reliable predictor for FTS positivity and rapid Wb123 tests can be used to confirm the absence of *W. bancrofti*. Lymphedema cases that were encountered during our surveys were most likely not due to *W. bancrofti*, but due to podoconiosis or other factors. Our study provides a simple day blood-based algorithm for LF mapping in loiasis areas and indicates that Cameroon is mostly non-endemic for LF and does not require mass drug administration for the elimination of LF.

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### IVERMECTIN PROTECTS AGAINST EPILEPSY IN ONCHOCERCIASIS ENDEMIC REGIONS IN THE DEMOCRATIC REPUBLIC OF THE CONGO

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An increased prevalence of epilepsy has been reported in many onchocerciasis endemic areas. To determine the prevalence of epilepsy in onchocerciasis endemic areas in the Democratic Republic of the Congo (DRC) and investigate whether a higher annual intake of Ivermectin was associated with a lower prevalence of epilepsy. Between July 2014 and February 2016, house-to-house epilepsy prevalence surveys were carried out in areas with a high level of onchocerciasis endemicity: 3 localities in

the Bas-Uele, 24 in the Tshopo and 21 in the Ituri province. Ivermectin uptake was recorded for every household member. A case of epilepsy was defined as a patient who reported at least 2 unprovoked seizures without fever or any acute illness. This database allowed a village, age and gender matched case-control pair subset to be created that enabled putative risk factors for epilepsy to be tested using univariate logistic regression models. Risk factors relating to onchocerciasis were tested using a multivariate random effects model. Of 12, 408 people examined in the different health areas 407 (3.3%) were found to have a history of epilepsy. A high prevalence of epilepsy was observed in health areas in the 3 provinces: 6.8-8.5% in Bas-Uele, 0.8-7.4% in Tshopo and 3.6-6.2% in Ituri. Median age of epilepsy onset was 9 years, and the modal age 12 years. Using the whole survey database, male gender, and onchocerciasis skin lesions were associated with a significantly higher risk of being a person with epilepsy. A case control analysis of 96 cases and 96 controls demonstrated that before the appearance of epilepsy, compared to the same life period in controls, persons with epilepsy were around two times less likely (OR: 0.52; 95%CI: (0.28, 0.98) to have taken Ivermectin than controls. After the appearance of epilepsy, there was no difference of Ivermectin intake between cases and controls. In conclusion, our study showed that the epilepsy prevalence in onchocerciasis endemic regions in the DRC was 2-10 times higher than in most non-onchocerciasis endemic regions in Africa and that in these onchocerciasis endemic regions Ivermectin intake protects against epilepsy.

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### A NOVEL RAPID TEST FOR DETECTING ANTIBODY RESPONSES TO *LOA LOA* INFECTIONS

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*Loa loa* affects over 10 million people in sub-Saharan Africa and is of particular significance in the context mass drug administrations (MDAs) aimed at eliminating onchocerciasis. Ivermectin is the only approved drug for such purposes but can induce severe adverse events in a subset of individuals with high levels of *L. loa* microfilariae. Extending MDAs to areas with coincident *L. loa* infection is problematic, and inexpensive point-of-care tests for *L. loa* are acutely needed to support programmatic decisions. Herein, we present the first lateral flow assay (LFA) to detect subjects serological response to *L. loa*. The assay detects antibodies directed against *L1-SXP-1*, a specific and validated marker of *L. loa* infection. The test was evaluated on serum samples from patients infected with *L. loa* (n=109) and other helminths (n=187), as well as on uninfected controls (n=78). When read with the naked eye, the test was 94% sensitive for *L. loa* infection and was 100% specific when sera from healthy endemic and non-endemic controls or from those with *S. stercoralis* infections were used as the comparators. When sera of patients with *O. volvulus* or *W. bancrofti* were used as the comparators, the specificity of the LFA was 82% and 87% respectively. A companion smartphone reader allowed measurement of the test line intensities and establishment of cutoff values. With a cutoff of 800 reader units, the assay sensitivity decreased to 56%, but the specificity increased to 97% for *O. volvulus* and 100% for *W. bancrofti*-infected individuals. The LFA may find applications in refining the current maps of *L. loa* prevalence, which are needed to eliminate onchocerciasis from the African continent.

## DEVELOPING THE FIRST NATIONAL DATABASE AND MAP OF LYMPHATIC FILARIASIS CLINICAL CASES IN BANGLADESH

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The Bangladesh Filarial Elimination Programme (FEP) is on track to meet the Global Programme to Eliminate Lymphatic Filariasis (GPELF) targets of 2020 by interrupting transmission with mass drug administration (MDA) and alleviating suffering by managing morbidity and preventing disability (MMDP) for 70 million people at risk. This study aimed to highlight FEP's success in determining the number of people affected by lymphoedema and hydrocoele, and to develop risk maps for targeted interventions across the 34 endemic districts (19 high; 15 low prevalence). In the 19 high endemic districts, 8,145 community clinic (CC) staff were trained to search for LF cases household census in catchment areas between 2013-2016 and registering all cases. In the 15 low endemic districts a team of 10 trained field assistants conducted active case finding utilising health facility records, health worker and community informants to identify and medically verify cases, with cases reported via a SMS mHealth database. Data recorded included general demographic (location, patient name, address, age, gender), and clinical information (lymphoedema, hydrocoele, severity, acute dermato-lymphangioadenitis (ADLAs). In the 19 high endemic districts, a total of 43,678 clinical cases were identified; 30,616 lymphoedema [70.1%; female 55.3%], 12,824 hydrocoele [29.4%], and 238 breast/female genital swelling [0.5%]. Rangpur district reported the highest number (8,545 lymphoedema; 2,654 hydrocoele). Overall the prevalence rate was 126/100,000 [highest in Lalmonirhat district 583/100,000]. In the 15 low endemic districts, 733 cases were identified; 661 lymphoedema [90.2%; female 39.6%], 57 hydrocoele [7.8%], and 15 both conditions [2.0%]. Risk maps, spatial and statistical analysis found district clinical prevalence was geographically and statistically correlated to baseline microfilaria (Mf) prevalence at  $r=0.904$  [ $p<0.01$ ]. FEP's remarkable efforts to identify LF cases across all districts represents one of the most comprehensive national databases on clinical LF in the world and provides a template for other countries to learn from and develop.

## PROJECTED NUMBER OF PEOPLE WITH ONCHOCERCIASIS-LOIASIS CO-INFECTION IN AFRICA, 1995-2025

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In Central Africa, community-directed treatment with ivermectin (CDTI) against onchocerciasis is hampered by co-endemicity of *Loa loa*, as people with  $\geq 30,000$  *Loa* microfilariae/mL of blood can develop potentially fatal severe adverse events (SAEs). To be conservative, test and treat approaches

will likely use a threshold of  $\geq 20,000$  *Loa* microfilariae/mL for exclusion from ivermectin treatment. We estimate the number of individuals with *Onchocerca volvulus* and *Loa* co-infections who are at risk ( $\geq 20,000$  *Loa* microfilariae/mL) for post-ivermectin SAEs, for 1995, 2015 and 2025. We combined pre-control rapid assessment data (REMO and RAPLOA) on onchocerciasis and loiasis prevalence. The loiasis data were categorised by proportions of people with  $\geq 20,000$  *Loa* microfilariae/mL. We used the mathematical model ONCHOSIM to calculate the expected trends in *O. volvulus* prevalence for 1995 to 2025, accounting for local treatment history. The impact of ivermectin treatment on loiasis was considered based on published data, with a one-time reduction in *Loa* prevalence and intensity after one round of CDTI. Among areas where *Loa* is potentially endemic, the number of cases with *O. volvulus* declined from 19.5 million people in 1995, to 14.2 million in 2015 and 3.3 million in 2025. Of those, 114,771 people were co-infected with *Loa* microfilaraemia  $\geq 20,000$ /mL in 1995; 44,370 predicted in 2015 and 20,477 in 2025. In 2025, 89% of cases (N=18,320) will live in onchocerciasis hypoendemic areas which would not benefit from control/elimination programmes. Democratic Republic of Congo and Cameroon will contribute to 78% of all cases in 2025. Mass distribution of ivermectin as part of the onchocerciasis elimination efforts is problematic in many countries. We predict that in 2025 over 20,000 people will require treatment for onchocerciasis while being at high risk of SAEs, justifying increased effort in research and development for safer drugs and control strategies targeted especially towards onchocerciasis hypoendemic areas which are co-endemic for loiasis.

## PREVALENCE OF EXPOSITION TO RIVER BLINDNESS IN THE GAROUA-BOULAI HEALTH DISTRICT (EAST REGION, CAMEROON): POTENTIAL CROSS-BORDER ISSUE IN THE CONTEXT OF ELIMINATION

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The control of onchocerciasis has been a success in many *foci* where the disease is endemic. However, large-scale preventive chemotherapy with ivermectin in *Loa* endemic areas to target hypo-endemic onchocerciasis is not recommended due to the risk of severe adverse events (SAE), thus slowing the momentum towards elimination. Since a safe threshold below which ivermectin can be administered through mass campaigns in communities was identified, additional epidemiologic investigations appeared essential to re-assess the distribution of infection in hypo-endemic areas that were not included in the original mass treatments, and particularly in the cross-border context. This study was carried out in the Garoua Boulai health district, belonging to the group of ivermectin naïve areas, and bordered to the east by the Central African Republic (CAR) where civil war led to an important refugee flow. A cross sectional survey following a community-based random cluster design was carried out to seek for exposure to *O. volvulus* using the newly developed SD Bioline onchocerciasis IgG4 rapid diagnostic test. The prevalence of exposure to river blindness ranged between 0.0% (95% CI: 0.0 - 8.0) and 20.0% (95% CI: 13.0 - 29.4), males being most exposed than females ( $p = 0.048$ ). An important geographical heterogeneity in the distribution of exposure to river blindness was observed among the clusters surveyed ( $p < 0.0001$ ). Amongst individuals with proven exposure to onchocerciasis, 30.2% were not native of the survey area, of whom 64.3% (95% CI: 49.2 - 77.0) were from CAR. The complete elimination of onchocerciasis implies the expansion of mass preventive chemotherapy in hypo-onchocerciasis areas which are up to now not targeted by mass treatments. This study

reveals a relatively high contribution of people from CAR, raising interest on effective collaboration between Cameroon and the CAR while envisioning the elimination of onchocerciasis in cross-border situations

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### EVALUATION OF MORPHOLOGICAL CRITERIA DUE TO HEPATIC ALVEOLAR ECHINOCOCCOSIS BASED ON THE ECHINOCOCCUS MULTILOCULARIS ULM CLASSIFICATION

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Computed tomography, mostly combined with PET, provides one of the most important diagnostic tools in suspected alveolar echinococcosis (AE). The recently established "Echinococcus multilocularis Ulm classification for computed tomography" (EMUC-CT) depicts the various morphologies of liver lesions due to AE. The classification scheme assigns morphological criteria of hepatic AE to five groups of primary morphologies (type I-V), including subcriteria and to six calcification patterns. Aim of this pilot study was to analyse several CT-morphological criteria of AE lesions in 72 patients based on the EMUC-CT classification and to make a comparison of those criteria in the clinical course under antihelminthic medication. The primary morphology showed to be very constant, whereas the subcriteria exhibited varying behavior. Between the patterns of calcification only slight changings could be registered. However the degree of calcification increases considerably over time. A slight regression of size in lesions under therapy could be noted. The extend and the count of lesions differ significantly between the various types. Obvious differences among the types were revealed regarding extrahepatic as well as biliar involvement. In conclusion the study could illustrate clear connections between the different morphological criteria within EMUC-CT and depicted morphological changings of AE liver lesions in the clinical course. Especially the observations concerning the extend and count of lesions in the different types and their varying behavior regarding extrahepatic as well as biliar involvement appears to be of clinical relevance. Larger studies should be done to reveal whether in future conclusions could be drawn thereout concerning prognosis of the disease and therapy planning.

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### LUNG AND LIVER CYSTIC ECHINOCOCCOSIS - CUMULATIVE INCIDENCE OF NEW HYDATID CYSTIC LESION AFTER SURGICAL TREATMENT

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Cystic echinococcosis (CE) is endemic in livestock-raising regions where surgery has been the main treatment approach used. Clinical series reported a prevalence of new cystic lesion after treatment between 5 and 15%; however they did not account those who developed a new lesion and never return. A prospective evaluation of subjects was performed to evaluate the cumulative incidence of new cystic lesion and the factors associated. A survival analysis was performed using subjects from a historical cohort of patients with CE between 01/2000 and 12/2011 in two referral hospitals. Subjects were evaluated by imaging methods. The primary study endpoint was the imaging evidence of a new cystic lesion in the same organ or cavity where the previous cyst was treated (index surgery). The "time to event" was the period elapsed between the index surgery and the time of diagnosis. Survival function was estimated using the algorithm developed by Turnbull for interval censored data and Cox

proportional hazard models were performed. One hundred eighty five subjects from the historical cohort were enrolled, 171 and 14 subjects registered one and two index surgeries, respectively. Therefore, we had 199 surgeries. Seventeen (8.54%) subjects developed a new cystic lesion after between 3 to 15 years (median 9 years; IQR 7-11) of index surgery; cumulative incidence was higher among liver involvement (14/115, 12.4% vs 3/86, 3.5%, p=0.026). Cox analysis, showed that the risk to develop a new cystic lesion was roughly 4 times higher among those who were operated at a hospital located in endemic area than those operated at a hospital located in a non-endemic area (HR: 3.45; 95% CI: 1.25 - 9.5). According to the results, there is a high proportion of subjects surgically treated who develop a new cystic lesion and were not account in clinical series

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### GENETIC VARIABILITY OF TAENIA SOLIUM CYSTICERCI RECOVERED FROM EXPERIMENTALLY INFECTED PIGS AND NATURALLY INFECTED PIGS USING MICROSATELLITE MARKERS

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The adult *Taenia solium*, the pork tapeworm, usually lives as a single individual in the small intestine of humans, its only known definitive host. Mechanisms of genetic variation in *T. solium* are poorly understood, but could be useful for tracing transmission. This study aimed to explore the utility of three microsatellite markers as potential transmission tracking tool. We first explored the genetic variability of *T. solium* from cysticerci recovered from experimentally infected pigs. We then explored the genetic epidemiology and transmission in naturally infected pigs and adult tapeworms recovered from human carriers from an endemic rural community in Peru. In the initial study on experimental infection, two groups of three piglets were each infected with proglottids from one of two genetically different tapeworms for each of the microsatellites. After 7 weeks, pigs were slaughtered, and necropsy was performed. Thirty-six (92.3%) out of 39 cysts originated from one tapeworm, and 27 (100%) out of 27 cysts from the other had exactly the same genotype as the parental tapeworm. This suggests that the microsatellite markers may be a useful tool for studying the transmission of *T. solium*. In the second study, we analyzed the genetic variation of *T. solium* in cysts recovered from eight naturally infected pigs, and from adult tapeworms recovered from four human carriers in the community; they showed low genetic variability. Six pigs harbored cysts with a genotype corresponding to one of the identified tapeworms from the human carriers. In the dendrogram, cysticerci appeared to cluster within the corresponding pigs as well as with the geographical origin, but this association was not statistically significant. Four pigs had cysts with only one genotype, and four pigs had cysts with two different genotypes, suggesting that multiple infections of genetically distinct parental tapeworms are possible. We conclude that genotyping of microsatellite size polymorphisms is a potentially important tool to trace the spread of infection and pinpoint sources of infection as pigs spread cysts with a shared parental genotype.

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### INFLAMMATION IS A KEY RISK FACTOR FOR REFRACTORY SEIZURES IN PATIENTS WITH NEUROCYSTICERCOSIS

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Neurocysticercosis (NCC) is the number one cause of adult epilepsy worldwide; 70-80% of people with NCC experience seizures that can persist (SP) despite treatment. Early intervention before recurrent seizures establish abnormal electrical circuits could impact disease progression, but to do so predictors of SP in NCC patients need to be identified. To identify such predictors, we conducted a retrospective review of 39 randomly selected subjects from one arm of a large clinical trial of patients with NCC and seizures. All patients in the study were treated with steroids, anti-parasitic, and anticonvulsant medications. Eleven subjects went on to develop SP (defined as seizures > 2 months after treatment for NCC despite anticonvulsant therapy) and 28 did not (seizure negative, SN, group). We examined a number of clinical factors in these patients in order to identify variables associated with SP. The primary risk factor identified was a strong and sustained inflammatory response to the parasite. This was demonstrated by an increased volume of edema on follow-up MRI scans (quantified by measuring pixels in Photoshop) in the SP group (8.2 cm<sup>3</sup>) compared to the SN group (0.6 cm<sup>3</sup>, p=0.02). Additionally, those in the SP group had twice as many degenerating lesions at the time of presentation compared to the SN group (median 2 in SP group, 1 in the SN group, p=0.002). There was a significantly increased number of bands seen on the initial NCC western blot in subjects who developed SP (GM 5.8 bands) compared to those who did not (GM 3.8 bands, p<0.05). As has been seen in prior studies, calcified lesions were also a risk factor for seizures; the SP group had a significantly increased number of calcified lesions on baseline imaging (median 4.9) compared to the SN group (median 0.9, p=0.005). Interestingly, edema around calcified lesions was also more likely in the SP group (13/120 calcified lesions had perilesional edema on baseline imaging, 10.8%) compared to the SN group (4/120, 3%, OR= 3.5, p=0.04). These findings provide a compelling rationale for further exploring signs and symptoms of ongoing inflammation as predictors of epilepsy development in NCC patients.

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### BANDING PATTERNS OF THE ENZYME-LINKED IMMUNOELECTROTRANSFER BLOT (EITB) AND BRAIN IMAGING FINDINGS IN PATIENTS WITH NEUROCYSTICERCOSIS

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Neurocysticercosis (NCC) is the infection of the central nervous system (CNS) by the larval form of the pork tapeworm *Taenia solium* (cysticercus). The enzyme-linked immunoelectrotransfer blot (EITB) is the reference serological test for NCC, detecting antibody bands reacting to seven defined parasite antigens belonging to three antigen families (GP50, T24-42 and 8kDa). A positive EITB result does not always correlate with

the presence of viable infections in the CNS, and individuals with a single viable brain cyst may be EITB negative. However, EITB banding patterns appear to be related with *T. solium* antigen families, and in turn with the characteristics of NCC in the CNS. This study evaluated the relationship between the EITB banding patterns and the characteristics of the infection in the CNS (location, stage and number of viable cysticerci). EITB banding patterns and brain images of 548 NCC cases were analyzed. Patients with similar banding patterns were grouped into homogenous classes using latent class analysis (LCA). The association between classes and brain image findings was assessed. A four-class model was specified by LCA as the best. Extra parenchymal NCC was strongly associated with banding patterns of class 4 (strongly positive to the 8kDa antigen family). Intra parenchymal infections with viable cysticerci was also strongly associated with banding patterns of classes 4 and 3 (positive to the 8kDa antigen family), and less with class 2 (positive to the T24-42 antigen family). Non-viable infections and single cysticerci in the brain were associated with banding patterns of class 1 (EITB negative or positive to the GP50 antigen family only). EITB banding patterns correlate with brain imaging and complement imaging information for the diagnosis of NCC and for staging NCC patients.

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### STANDARDIZATION OF A DIRECT ELISA USING MONOCLONAL ANTIBODIES FOR THE DETECTION OF PARASITE ANTIGEN IN URINE SAMPLES OF PATIENTS WITH NEUROCYSTICERCOSIS

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Neurocysticercosis (NCC), a parasitic disease caused by the larval stage of *Taenia solium*, is the main cause of acquired epilepsy and thus a public health problem in developing countries such as Peru. Immunodiagnosis of NCC consists on the detection of either antibodies or antigens, usually in serum samples. Serological tests based on the detection of antibodies cannot differentiate between active and past infections and thus may not be used for the follow-up of treated subjects. We standardized a direct ELISA technique using monoclonal antibodies (mAbs) to detect antigens in urine samples as an alternative method that utilizes a sample which is easier to be obtained by noninvasive methods. Four mAbs: three IgM isotypes and one IgG isotype obtained against crude antigen of *T. solium* were purified and biotinylated; then, all potential combinations between these 4 mAbs were evaluated. Finally, using the best combination (two IgM isotypes: TsW8 [4ug/ml] for capture and TsW5 [4ug/ml] for detection), a preliminary exploratory evaluation was carried out with urine samples from 20 healthy volunteers and 20 patients with NCC (six with subarachnoid NCC and 14 with calcified NCC). All samples from subjects with subarachnoid cyst (6/6, 100%) tested positive for *T. solium* antigen, compared with only one subject with calcified NCC (1/14, 7%) and none of the controls (specificity 20/20 100%). These promising results suggest a good sensitivity and specificity of the ELISA test in the detection of antigens in urine samples from subjects with subarachnoid NCC. Further work will extend this evaluation to patients with other types of cysticercosis.

## RING STRATEGY AS AN EFFECTIVE ALTERNATIVE TO MASS DRUG ADMINISTRATION FOR CONTROL OF *TAENIA SOLIUM* TAENIASIS/CYSTICERCOSIS

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Despite recent calls by the World Health Organization for increased efforts to control transmission of *Taenia solium* taeniasis/cysticercosis, optimal strategies have not been determined. In a previous small non-randomized study in Peru, we piloted a risk-based approach (Ring Strategy) in which screening and treatment for taeniasis targeted to residents living within 100 meters of heavily infected pigs reduced parasite transmission. Here we report on a larger cluster randomized trial that compared ring strategy vs. mass drug administration (MDA) in 23 villages (pop 9,183) randomly assigned to receive one of 3 interventions. In the Ring-Screening arm (n=8 villages), people living within 100-meters of a heavily-infected pig were offered screening for taeniasis using stool coproantigen ELISA followed by treatment of positives with niclosamide. In the Ring-Treatment arm (n=8), residents living within 100 meters of a heavily infected pig were offered presumptive treatment with niclosamide. Study teams examined tongues of all pigs every 4 months to determine intervention areas. In the MDA arm (n=7), all village residents were offered treatment with niclosamide every 6 months. A subgroup of villages within each arm also received oxfendazole for porcine cysticercosis, either within 100-meter rings or as MDA. We followed a cohort of >6000 pigs born during the study period to monitor parasite transmission using LLGP EITB. Preliminary analysis shows seroincidence reduced in all groups from baseline after 2 ½ years. The greatest and most rapid effect was seen in Ring-Screening, with 87.8% reduction in seroincidence after 8 months, a level maintained for the remainder of the study. This reduction was 1.4 times greater vs. Ring-Treatment (IRR 1.4 95% CI 0.8 – 2.4) and 2.1 times greater vs. MDA (IRR 2.1 95% CI 1.2 – 3.6). These results show that ring strategies are an effective alternative to MDA. Ring strategies have potential to operate as community-based programs given sufficient incentives for residents to report heavily infected pigs. Final results including prevalence of taeniasis and porcine cysticercosis determined by necropsy will be presented.

## MODIFIED MDA DID NOT SIGNIFICANTLY IMPROVE COVERAGE IN LGAS TREATING TWICE-PER-YEAR IN SOUTHERN NIGERIA

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The area along the border between Edo and Ondo states in southern Nigeria is highly endemic for onchocerciasis and parts are also co-endemic for lymphatic filariasis. Despite years of relatively high reported MDA coverage, anecdotal evidence and parasitological monitoring suggested that coverage may be poor, with persistent transmission of onchocerciasis. In 2016, twice-per-year treatment with ivermectin (with albendazole for

lymphatic filariasis added to the first round) began in five LGAs in Edo state bordering Ondo. We undertook a multistage cluster survey within three months after each round of MDA to assess coverage. In the first round, we randomly selected 30 clusters per LGA, then 7 households per cluster using systematic sampling at both stages; all household members present were interviewed. We found first-round coverage was very poor; among 4942 people interviewed from 145 clusters, coverage was 31.1% (95% CI 24.1% - 38.0%). To improve coverage in the second round, three LGAs were randomized to receive MDA through a "modified campaign" approach. This entailed doubling the travel reimbursement for drug distributors (to 3000 Naira), using a rapid coverage assessment tool to guide post MDA "mop up" activities, shortening the time for MDA delivery, and increasing supervision. The other two LGAs continued with the same MDA activities used in the first round. The coverage survey methodology after the second round differed from the first in that we randomly selected 48 clusters per group of LGAs and 9 households per cluster using systematic sampling; again, all household members present were interviewed (n = 3362 people in 87 clusters). Second-round coverage improved, but was not statistically different than in the first round [40.0% (95% CI 31.0% - 49.0%)] and there was no significant difference between the standard MDA and modified campaign groups (p = 0.7). The additional cost per treatment in the campaign was 1.6 times that of standard MDA. Different strategies must be deployed to improve treatment coverage in Edo state if transmission elimination is to be achieved.

## IMPLEMENTING THE SUPERVISOR'S COVERAGE TOOL IN THE PHILIPPINES: PILOTING TWO NOVEL ADDITIONS

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The Supervisor's Coverage Tool (SCT) is a rapid survey tool designed to help neglected tropical disease programs monitor and supervise the mass drug administration (MDA). In 2016, the Philippines conducted a harmonized MDA between the Ministry of Education and Department of Health for lymphatic filariasis (LF) and soil-transmitted helminthiasis (STH). Six villages, representing six different municipalities in two LF-STH-co-endemic provinces, were selected for the SCT. To supervise both the school- and community-based MDAs, a novel integrated SCT was conducted, whereby the survey teams randomly picked one school-aged child (5-18 years old), and one community member per household. For another innovation, the SCT was conducted *during* the current MDA (as opposed to after). Of the 12 SCTs conducted - six among school-aged children and six among community members - four of six among children resulted in a "good" coverage classification, meaning that MDA coverage was likely above the target threshold. One child and five community SCTs classified the villages as having "indeterminate" coverage, meaning that one cannot conclude if the coverage is above the target threshold; and one child and one community SCT classified village coverage as "inadequate". The results of the SCT highlighted several important issues with the MDA distributions including the need for stronger insistence on directly observed treatment, teaching the contraindications of the drugs, addressing fears of side effects, and improving coverage among private-school children. These issues were discussed with the local health workers during the SCT and action plans to address the issues was created for each participating municipality. Overall, the results from the SCT in the Philippines suggest that an integrated SCT is not only feasible but can save the program time and resources. By conducting the SCT during the latter half of the MDA, the program was able to identify coverage challenges and assess whether the target threshold was met in real-time and to take corrective actions to improve coverage during the remainder of the MDA and avoid the need for separate mop-up campaigns.

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### INTEGRATED PREVALENCE SURVEY OF SKIN NTDs AND COMMON SKIN DISEASES AMONG SCHOOLCHILDREN IN GAGNOA, CÔTE D'IVOIRE: DIAGNOSIS AND RISK FACTOR ANALYSIS

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Many neglected tropical diseases - including Buruli ulcer (BU), leprosy, and yaws - present with skin symptom(s) (skin NTDs). They are co-endemic in Côte d'Ivoire which reports the highest number of BU cases globally. Children are especially vulnerable to these, as well as to a large number of common skin diseases (CSDs), but their epidemiology is incompletely understood. In the Gagnoa district of Cote d'Ivoire, we performed a school-based skin survey for early detection and treatment of skin NTDs and CSDs; and to describe the distribution and the risk factors of these diseases. The program consisted of two phases: 1) screening by a team of village nurses of all primary schoolchildren aged 5 to 15 in a total of 38 schools, and selection of those presenting with any skin lesion(s); 2) sensitization campaign followed by in-school examination of screened children by two medical teams including dermatologists, leprosy and BU experts, and laboratory technicians. A total of 9,930 children (9.2% of all schools in the district) were pre-screened by the village nurses, yielding 1,781 children with skin conditions. These, and an additional 883 children who self-reported skin disease(s) following sensitization campaign were consulted by the medical teams. Among these, we identified 8 cases of skin NTDs: 3 BU and 1 post-BU with contracture; 1 confirmed and 1 suspected leprosy; and 2 suspected yaws were found. For CSDs, the majority of diagnoses were fungal infections including tinea capitis (n=1220, 46%) and pityriasis versicolor (n=1052, 39%), but others included such diseases as scabies (n=68, 2.6%) and eczema (n=35, 1.3%). Survey on demographics and personal hygiene, e.g., washing hands, use of soap, nail cutting, was conducted. Treatment for skin NTDs and prescription for CSDs were provided. The program had a high rate of community acceptability. This was the first attempt at an integrated, multi-skin NTD and CSD screening and diagnosis in the country. This strategy has the potential of improving early detection of NTDs, especially in areas of co-endemicity. CSDs are highly prevalent and should be integrated in skin NTD control measures.

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### DRAMATIC INCREASE IN THE PARTICIPATION WITH MECTIZAN TREATMENT IN SECOND ROUND OF TEST AND TREAT IN AN AREA COENDEMIC FOR LOIASIS AND ONCHOCERCIASIS

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Ivermectin-based mass drug administration has revolutionized onchocerciasis control over the last 30 years, and the target shifted from control to elimination. Although transmission of infection has been interrupted in some foci, the fear of serious adverse events (SAEs) in Loa loa co-endemic areas in Central Africa has been an obstacle to elimination. Millions of people were excluded from mass drug administration, because the risk of SAE in people with high Loa loa mf counts (>20000 mf/ml) was considered too high. The Test and (not) Treat (TNT) strategy has been developed as a way forward. TNT relies on the LoaScope to rapidly identify Loa loa-infected people "at risk" for SAEs and exclude them from ivermectin treatment. To provide proof of principle, TNT strategy was implemented in Okola Health District, Cameroon in 2015, where 15469 people were treated with ivermectin without SAEs occurring. The therapeutic coverage rate in Okola was 67.7%, a level felt to reflect a reluctance of the population to participate due to the fact that 23 SAEs occurred in this district during the first (and only) Community-Directed Treatment with Ivermectin in 1999. As a prelude to the second TNT campaign in March 2017, a press briefing was held by the Cameroonian Minister of Public Health and Minister of Communication emphasizing the success of the TNT strategy in 2015 and the absence of SAEs. Traditional rulers of the villages with the best participation rates in 2015 were awarded during the launching of the 2017 campaign. With this additional sensitization, between 13 March and 9 April, 11472 individuals participated in the TNT screening. The mean number of patients treated daily in 2017 was significantly higher than in 2015 (459 vs. 352, P=0.002), and total population coverage in the district is expected to exceed that seen in 2015. Our data suggest that both the absence of SAEs in the 2015 TNT campaign and the communication efforts made prior to the launching of the 2017 campaign were important factors in increasing adherence to the TNT strategy. This is very encouraging for the implementation of the TNT strategy across the Loa endemic regions in Central Africa.

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### LYMPHATIC FILARIASIS TRANSMISSION ASSESSMENT SURVEYS (TAS) AS AN OPPORTUNITY TO EVALUATE THE IMPACT OF MASS DRUG ADMINISTRATION (MDA) ON TRANSMISSION OF ONCHOCERCIASIS AND SOIL TRANSMITTED HELMINTHIASIS

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The control of neglected tropical diseases (NTDs) reached new possibilities when MDA became the platform to simultaneously target multiple NTDs amenable to preventive chemotherapy (PC). The rationale for this integrated approach is the geographic overlap of highly-prevalent NTDs and the availability of donated PC drugs. It has been shown that Albendazole, Mebendazole, Ivermectin or Diethylcarbamazine can be safely co-administered in almost any combination, rendering feasible the control of Soil Transmitted Helminthiasis (STH), onchocerciasis (Oncho) and lymphatic filariasis (LF). A comprehensive algorithm was built by the GPELF

for coordinated implementation of PCs. Unlike STH and Oncho, six years MDA is enough to halt LF transmission, and programmatic steps (with regular assessments) are clearly defined to achieve LF elimination goals. This study aimed to utilize the implementation of LF TAS as a framework for integrating Oncho and STH evaluations. Eligible individuals were children aged 6-7 years, anticipated to be free from *Wuchereria bancrofti* circulating antigen if MDA had been successful. A community-based cluster survey was used because of the low school enrolment rate (<75%). Finger prick blood samples were collected for LF (filaria test strip, FTS) and Oncho/LF (Biplex IgG4 antibodies against *O. volvulus* Ov16 and *W. bancrofti* Wb123 antigens). Stool samples were collected and examined using the Kato-Katz technique. A total of 1,646 children originating from eight health districts provided both blood and stool samples. None was positive for FTS and Ov16 antigen, and only 1 (0.1%; 95% CI: 0.0-0.3) child harbored antibodies against Wb123 antigen. *Ascaris lumbricoides* was the only STH species found in stools, with a prevalence of 0.8% (95% CI: 0.5-1.3). These results indicate that it was very likely that the PC had significantly lowered LF, Oncho and STH prevalence toward elimination targets. They also show that LF TAS can provide a suitable and cost-effective platform to assess the situation of other PC-NTDs.

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### LYMPHATIC FILARIASIS AND PODOCONIOSIS: INTEGRATED MORBIDITY MANAGEMENT AND DISABILITY PREVENTION SERVICES FOR LYMPHOEDEMA AND HYDROCOELE PATIENTS IN THREE CO-ENDEMIC DISTRICTS OF ETHIOPIA

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Lymphatic filariasis (LF) and podoconiosis are endemic in Ethiopia and cause painful and disabling lymphoedema. A burden assessment conducted in 2015 found 26,123 cases of lymphoedema and hydrocoele across 20 districts of Ethiopia. To increase access to quality, integrated, morbidity management and disability prevention (MMDP) services for lymphoedema and hydrocoele cases, a pilot study was conducted in three co-endemic districts of the Southern Nations, Nationalities, and Peoples' Region (SNNPR) over a twelve-month period from September 2016. This included: i) training health professionals at each health facility; ii) cascade training of health extension workers (HEWs) and health development army (HDA); iii) patient mobilisation and recruitment; iv) provision of supplies and training on lymphoedema care; v) referral of hydrocoele patients to hospitals for surgery and; vi) patient prospective monitoring with key progress indicators such as severity of condition, wounds present and frequency of acute attacks. A mid-term review found a total of 483 health professionals (60.2% female), 163 HEWs and 2,330 HDA from 22 health facilities had been trained on MMDP and patient referrals; with 2,033 lymphoedema cases (68% female), and 82 men with hydrocoeles having accessed the MMDP service. Conditions ranged from mild (n=655, 32.2%), moderate (n=1,062, 52.2%), to severe (n=317, 15.6%). Two thirds of lymphoedema patients (n=1,344, 66.1%) had experienced an acute attack in the month prior to their enrolment, and 333 (16.4%) had visible wounds on the affected limb. The main challenge reported was a weakness in the referral of hydrocoele patients for surgery from health facilities to hospitals, however, this is being addressed through consultations with the hospitals. Overall, the mid-term results show promise for an MMDP service integrated into the existing health structure, which may be scaled-up across Ethiopia. This will be a significant contribution to patient care and will help to ensure all lymphoedema and hydrocoele patients have access to quality care. Full results will be presented at the meeting.

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### ADDING ONCHOCERCIASIS MONITORING TO LYMPHATIC FILARIASIS TRANSMISSION ASSESSMENT SURVEYS: THE TANZANIA EXPERIENCE

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The Tanzania NTD Control Program (TZNTDCP) has a goal of lymphatic filariasis (LF) elimination by 2020 and onchocerciasis (OV) by 2025. For LF, WHO recommends at least five rounds of MDA to reduce infections to levels below a threshold at which mosquitoes are unable to continue spreading the parasites from person-to-person. For OV elimination, annual treatment with ivermectin with coverage rates of at least 80% for a period of 12-15 years is recommended. In January 2017, the TZNTDCP added the OV16 rapid diagnostic test (RDT) to planned LF transmission assessment surveys (TAS) in Bumbuli and Lushoto districts where 15 treatment rounds were completed with sufficient coverage. The main objective of the surveys was to assess transmission of lymphatic filariasis and monitor onchocerca volvulus among school children in the two evaluation units (EUs). Using the TAS platform, thirty primary schools were selected in each EU. Filariasis test strips (FTS) and OV 16 RDTs were tested on site, while dried blood spots (DBS) for OV16 ELISA were collected for laboratory testing. Children in grades 1 and 2 (6 and 7 year olds) were tested for circulating filarial antigens and the presence of IgG4 antibodies to the OV16 antigen, and DBS was collected. To increase the sample size for OV monitoring, grades 3 and 4 (8 and 9 year olds) were tested by OV16 RDT and DBS only. Out of 3,537 children tested for CFA, only 2 (0.11%) were found positive for LF in Lushoto EU. For onchocerciasis, 2,123 children were tested for OV16 RDT and prevalence was found to be 0% and 0.06% by OV16 Bumbuli (n=1343) and Lushoto (n=780) EUs respectively. The fact that the positive cases for both LF and OV were found in children from one district may suggest ongoing transmission of the two infections. OV16 ELISA testing will provide more insight. Adding OV monitoring in TAS was not difficult, but did need careful planning and carried an extra cost (expanded sampling, extra field days, more diagnostics and larger team size). However, the added cost was less than a standalone OV survey, while yielding valuable information. Given the dearth of current OV data, this approach could be used in other LF surveys.

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### REALIZING THE POTENTIAL OF COMMUNITY HEALTH WORKERS TO PROVIDE MALARIA CASE MANAGEMENT: SENEGAL'S SCALE UP OF PROACTIVE COMMUNITY CASE MANAGEMENT

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In 2008, the Senegal National Malaria Control Program (NMCP) introduced home-based malaria management, with diagnosis by rapid diagnostic test (RDT) and treatment with artemisinin-based combination therapy (ACT), known as prise en charge a domicile (PECADOM), and expanded to over 2000 villages by 2015. Together with district health officials and the American Peace Corps, the NMCP piloted an active model (called PECADOM Plus in Senegal) in one village in 2012 and 15 villages in 2013, in a rural area of seasonal, moderately high transmission, during the

approximately 20 weeks of high transmission season. Every week, CHWs visited every household in their village to identify fever cases, and offered case management based on RDTs and ACTs. In comparison villages, CHWs did their regular work, but households received visits at the beginning and end of the season. The proportion of cases treated by CHWs increased over 300% in intervention villages, but during the last week of household visits, there were far fewer cases of malaria in intervention than in comparison villages. Based on this evidence, the NMCP scaled the PECADOM Plus program to three districts in 2014 (132 villages), six districts in 2015 (246 villages), and 16 districts in 2016 (708 villages). In PECADOM Plus villages during active sweeps, DSDOMs tested 10,141 patients in 2014, 22,838 patients in 2015, and 63,603 patients in 2016, and diagnosed 8,071, 15,068, and 30,554 patients with malaria in 2014, 2015, and 2016, respectively. Over 96% of those with a positive RDT were treated with ACT, 1.5% referred for severe disease, with most of the rest referred for stock out of ACT. Patients who sought care between sweeps increased, with a 104% increase in the number of RDTs performed during regular CHW work and a 77% increase in the number of positive RDTs and patients treated with ACT. Overall the number of patients receiving an RDT increased by 307% and the number cases of malaria detected and treated by DSDOMs increased 274%, from the year prior to PECADOM Plus introduction to its first year of implementation. Based on these results, approximately 700 additional DSDOMs (in 24 districts) will be added in 2017.

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### MALARIA COMMUNITY-BASED MANAGEMENT IN INFORMAL KORANIC RESIDENTIAL SCHOOLS: A PILOT IN SENEGAL

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Since 2008, the Senegal National Malaria Control Program (NMCP) has implemented malaria community case management to ensure rapid access to diagnosis and treatment. An analysis of reported malaria cases in Diourbel health district identified high morbidity and mortality within informal residential Koranic schools (*daaras*) where students often receive inadequate nutrition and health care, and poor living conditions are often favorable to malaria transmission. A new community case management initiative called *PECA Daara* was piloted to target the high malaria burden in *daaras*. School-based care providers (*DSDaaras*) are volunteers selected within the *daaras* and provided with theoretical and practical training. An installation ceremony with involvement of religious, administrative and community authorities was organized and *DSDaaras* received a kit containing malaria rapid diagnostic tests (RDTs) and first line medication. *DSDaaras* recorded all consultations in a registry. Monitoring included supervisory visits, coordination meetings and reviews with all partners and the NMCP. To date, 17 *DSDaaras* in eight health post catchment zones were trained and provide care to ~ 4,500 residential students (4% of the catchment population). In the first year, 1,586 patients sought care, of which 1,237 (78%) had a fever and were tested by RDT; all had sought care within 24 hours of symptom onset. There were 684 confirmed malaria cases (8% of catchment malaria cases for the year) and all were appropriately treated and recovered. Test positivity rate was > 50% in eight *daaras* and four *daaras* accounted for 72% of cases. After *PECA Daara* implementation, reported severe malaria cases within health facilities serving the *daaras* decreased by 35% within a year. On average, 18% of sick patients consulted by the *DSDaara* were referred to a health center for a negative RDT. The promising 1-year results of *PECA Daara* highlight the value of scaling-up this program to guarantee rapid access to quality malaria diagnosis and treatment to the vulnerable population of *daara* students and ensure equitable access to care throughout Senegal.

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### IMPROVING EARLY ANTENATAL CARE (ANC) ATTENDANCE AND IPT UPTAKE THROUGH COMMUNITY HEALTH VOLUNTEERS (CHVS)

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Kenya adopted the use of intermittent preventive treatment with sulfadoxine pyrimethamine in 1998 but the proportion of pregnant women receiving at least two doses (22% (2010) and 56% (2015) has remained below the national target of 80%. In 2015, the country adopted an IPTp3 indicator for monitoring IPTp uptake; that year, the proportion of women taking at least 3 doses was 38% (2015). Some of the factors leading to low IPTp coverage include poor knowledge on the need for early antenatal care (ANC), distances to health facilities, sociocultural practices and a lack of financial resources. In 2012, community health volunteers (CHVs) were enlisted through a pilot program in one county to deliver messages aimed at increasing the proportion of women starting ANC ≤ 20 weeks of gestation and thus expand the proportion of women receiving IPTp early in the second trimester. A community survey in 2013 showed an increase in IPTp2 from 22% in 2010 to 63%. The practice was considered a success story, and was subsequently replicated in 30 sub-counties, in 4 out of 14 malaria endemic counties. The rollout involved training of 9,042 CHVs, in 761 community units. Between 2015 and 2016, the CHVs reached 86,433 women with MiP messages. During this time, there was an average increase in IPTp1 from 51% to 68%, and IPTp2 increased from 42% to 55% (p < 0.001). This could be attributed to early ANC attendance, which increased from 32% to 48% in the same period. The use of CHVs to sensitize pregnant women to start IPTp early in the second trimester and continue with scheduled ANC visits increases the probability that women will receive the recommended IPTp-SP doses. The rollout of the practice to other malaria endemic counties is likely to have contributed to increase in IPTp uptake in the four target counties.

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### INCREASE IN DELIVERY OF INTERVENTIONS TO DECREASE MALARIA AMONG PREGNANT WOMEN IN SENEGAL

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Pregnant women are known to be at high risk for malaria, and intermittent preventive treatment during pregnancy and long-lasting insecticidal nets (LLINs) are key interventions to reduce that risk. Intermittent preventive treatment during pregnancy (IPTp) consists of a treatment course of sulfadoxine/pyrimethamine (SP) given at intervals of at least 30 days during the second and third trimesters. In 2013, the Senegal National Malaria Control Program (NMCP) adopted the revised WHO guidelines for IPTp to increase number of doses of SP provided to pregnant women, introduced treatment of pregnant women with uncomplicated malaria with artemisinin-based combination therapy, and put increased emphasis on provision of LLINs during antenatal care. We analyzed routine malaria information system data reported to the NMCP from all publicly supported health facilities to evaluate the change in interventions delivered, calculated using to the estimated number of expected pregnancies as denominator, and the impact on incidence of uncomplicated malaria, severe malaria, and deaths among pregnant women. The number of pregnant women that received an LLIN during an antenatal visit increased from 105,686 in 2014 to 219,212 in 2016, 19% and 38% of expected

pregnancies, respectively. The proportion of expected pregnancies who received at least two doses of SP increased from 54% in 2014 to 58% in 2016, and those that received at least three doses of SP increased from 20% in 2014 to 40% in 2016. While the number of pregnant women diagnosed with malaria increased 9%, from 6,465 to 7,044, the test positivity rate fell from 28% to 20%. Hospitalizations of pregnant women for malaria fell 36%, from 1,414 to 900, and deaths of pregnant women due to confirmed malaria fell from 17 in 2014 to one in 2016. There is need for improvement in provision of LLINs and IPTp to pregnant women. More pregnant women were diagnosed with uncomplicated malaria, however, test positivity rate, hospitalizations, and deaths of pregnant women with confirmed malaria decreased from 2014 to 2016.

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### ASSESSMENT OF FACILITATORS AND BARRIERS TO ACHIEVING THE TARGET IPTP MUTASA DISTRICT, MANICALAND PROVINCE, ZIMBABWE: A FORMATIVE ASSESSMENT

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Use of intermittent preventive treatment (IPTp) is an evidence-based intervention for preventing malaria during pregnancy. Zimbabwe began implementing IPTp in 2004; however it has not achieved its target of 85% of eligible women receiving 2 or more doses. In 2016, the Ministry of Health and Child Care, with support from the President's Malaria Initiative, explored barriers and drivers of IPTp program uptake in Mutasa District, Zimbabwe. This preliminary assessment aimed to inform the questions and methods of a larger research study; methods included document review, secondary data review, facility visits and key informant interviews. The assessment identified factors within the health system requiring further study, for example: 1) limited physical availability of updated national IPTp guideline documents, 2) barriers to access such as antenatal care (ANC) services only offered on selected days and long wait times, and 3) non-provision of IPTp at private facilities. There were missed opportunities for IPTp (about 5% of 3,904 ANC visits reviewed), most due to facility stock-outs of sulfadoxine-pyrimethamine (SP). Data reporting was inaccurate, with facilities both under-reporting and over-reporting IPTp 1, 2 and 3 as compared to ANC registers. Additionally, IPTp coverage calculations need to factor in women ineligible to receive SP due to cotrimoxazole prophylaxis, which was 5% overall, but varied greatly by facility. Community health workers, community leaders and men were identified as influencing IPTp uptake, although how they drive or impede IPTp and ANC uptake and the best approaches to engage these groups require further study. Early ANC attendance was significantly associated with receiving more IPTp doses ( $p < 0.01$ ), thus examining factors influencing timing of ANC initiation is critical. Preliminary findings on supply and demand side factors influencing IPTp uptake were used to develop a conceptual framework for a research study to explore these and other factors influencing IPTp coverage in greater depth, using mixed research methods.

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### TREATMENT OF YOUNG INFANT INFECTION IN NTCHU DISTRICT (TYIIN): IMPLEMENTATION RESEARCH ON SIMPLIFIED TREATMENT OF POSSIBLE SERIOUS BACTERIAL INFECTIONS AND FAST BREATHING AMONG YOUNG INFANTS IN NTCHU DISTRICT, MALAWI

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Serious bacterial infection is a leading cause of newborn mortality and recommended inpatient treatment options are inaccessible to most families in low-income settings. In July 2015, the World Health Organization released guidelines for outpatient treatment of young infants with possible serious bacterial infection (PSBI) with simplified antibiotic regimens in cases where the caregiver refuses or is unable to complete referral to hospital. If implemented at scale, the outpatient regimen has the potential to prevent many young infant deaths in Malawi. We present results of implementation research to demonstrate the feasibility and acceptability of delivering simplified treatment for young infants in Malawi. The prospective cohort study is being conducted from December 2016 to September 2017 in 12 first level health facilities in Ntcheu district and will enroll ~368 sick young infants 0-59 days for whom referral is not possible. Health workers at study facilities are trained to treat eligible PSBI cases with injection gentamicin for 2 days and oral amoxicillin for 7 days and eligible fast breathing only cases with oral amoxicillin for 7 days. Health Surveillance Assistants (HSAs), trained in community-based maternal and newborn care, conduct home visits on days 3 and 6 to assess the child, encourage treatment adherence and remind the caregiver to take the child for facility follow up. Enrolled infants are followed up at health facility on day 4 and 8. The primary outcome is proportion of enrolled cases completing treatment per protocol. Secondary outcomes include proportion of eligible cases accepting outpatient treatment, completion of day 4 follow up, and completion of HSA follow up visits. Pilot phase results show that of 53 eligible cases, caregivers of 45 cases (85%) refused referral to district hospital and accepted outpatient treatment. Among enrolled cases (30 PSBI and 15 fast breathing only), 90% PSBI cases (27/30) completed day one and two gentamicin injections, 78% (35/45) completed day 4 follow up, and 53% (24/45) received at least one HSA home visit. Study results will inform replication and scale-up in Malawi and other countries.

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### THE RESILIENCE OF INTEGRATED COMMUNITY CASE MANAGEMENT IN ACUTE EMERGENCY: A CASE STUDY FROM SOUTH SUDAN

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The International Rescue Committee (IRC) was the first NGO to introduce integrated Community Case Management (iCCM) of childhood illness to South Sudan, then Southern Sudan, in 2005. An active conflict that began in December 2013 displaced approximately 2 million people over the course of few months. The IRC conducted in May 2015 a mixed-methods case study of the impact of the acute emergency in early 2014 on iCCM programming in Payinjar County, Unity State. The objective was to document the experience of operating an iCCM program during acute

crisis and to assess the ability of the program to continue operations. Semi-structured interviews and focus group discussions were conducted with key stakeholders such as policymakers, community health workers (CHW), and caregivers. Routine program data were examined to assess the effect of the crisis on key indicators. Internally displaced persons nearly doubled the population in Payinjar from 44,224 to 83,433. Some CHWs continued working despite displacement if they were able to take supplies with them. Despite no formal community mobilization effort by the iCCM program, the information that CHWs had drugs spread throughout displaced communities, with caregivers subsequently seeking care. The total number of treatments provided per month dropped from the July-December 2013 average of 3226 to the lowest level of 1420 in February 2014, but recovered to 3270 by August 2014. CHW supervisors attempted to continue supervision by utilizing their networks to track down displaced CHWs and by assessing the security situation prior to visits. The monthly supervision rate dropped from the July-December 2013 average of 93% to the lowest level of 77% in February 2014, but recovered to 91% by August 2014. Several CHWs and community leaders qualitatively validated this claim of sustained supervision. International donors and humanitarian actors should recognize iCCM as a potentially high-impact humanitarian response. Flexible funding from donors would allow for development of more evidence on iCCM approaches and improvements that can both sustain and enhance programming in acute crisis.

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### PRIMED INNATE IMMUNE RESPONSES IN MONOCYTES FROM KENYAN CHILDREN WITH UNCOMPLICATED FALCIPARUM MALARIA

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Monocytes are innate immune cells that play a key role in host protection and pathogenesis during malaria. We sought to determine whether uncomplicated falciparum malaria in children modulates Toll-like receptor (TLR) responsiveness in monocytes. Freshly isolated monocytes were obtained from 8 children in western Kenya at presentation with acute uncomplicated malaria and 6 weeks following treatment and from 4 malaria-naïve North Americans (NAM). Monocytes were cultured for 18 hours with media alone, a TLR4 agonist (LPS), or a TLR2/TLR1 agonist (Pam3CSK4). Supernatant cytokine concentrations were measured using a magnetic bead-based immunoassay. Genomic DNA was isolated from monocytes from the same 8 acute-recovery pairs for DNA methylation analysis using the MethylationEPIC array. Monocyte gene expression profiles were analyzed in another 6 acute-recovery pairs and 5 NAM using a targeted digital RNA sequencing panel. Both acute and recovery monocytes showed robust and equivalent responses to LPS and Pam3CSK4, with markedly increased production over media alone of IL-1 $\beta$ , IL-6, IL-8, IL-10, IL-12p40, and TNF (Friedman test  $P$  values < 0.05). Compared to NAM, acute and recovery monocytes showed greater magnitude of responses to LPS and Pam3CSK4, especially for IL-6, IL-12p40, and TNF (Kruskal-Wallis test  $P$  values < 0.05). Monocyte gene expression for the cytokines IL-1 $\alpha$ , IL-1 $\beta$ , IL-6, IL-8, and TNF was not different between acute and recovery, though these genes were significantly overexpressed in acute and recovery monocytes compared to NAM (adjusted  $P$  values < 0.0001). Preliminary analysis of DNA methylation showed significant differential methylation in acute vs. recovery monocytes in the promoter regions for *IL1A*, *IL1R1*, *IL18R1*, *IL10*, *TNF*, *TLR1*, and *TLR6*. Complete methylation analysis is ongoing and will be correlated with functional and transcriptional data. These data suggest that uncomplicated malaria in children has a priming effect on innate immune responses that is maintained several weeks after clinical recovery, which may be mediated in part by epigenetic changes such as altered DNA methylation patterns.

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### INDIVIDUAL AND COMPOSITE AMA-1 CELLULAR RESPONSES AND THEIR ASSOCIATION WITH CLINICAL MALARIA IN A PEDIATRIC COHORT IN MOZAMBIQUE AND TANZANIA

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Naturally acquired cellular immunity (NAI) in malaria is based on the interplay of pro- and anti-inflammatory responses that control parasite load while moderating deleterious responses. Although responses involve many cellular mediators, studies of NAI have focused on a limited panel of markers. We aimed to identify individual and combination of responses elicited by exposure to *P.falciparum* and those protective from clinical malaria based on several markers. Peripheral blood mononuclear cells (PBMC) were isolated 3 months before and at start of follow-up in a cohort of 459 children aged 6 weeks to 17 months nested within the RTS,S/AS01 Phase III trial conducted in Mozambique and Tanzania. PBMC were stimulated with AMA-1 and DMSO (mock stimulation) and 28 cytokines, chemokines, and growth factors were measured. Children were prospectively followed and the association of individual and combinations of AMA-1 specific responses with 12-month risk of malaria was estimated using logistic regression fitted with elastic net and group lasso. Baseline AMA-1 specific responses were elicited and many were correlated with age including pro-inflammatory TNF and regulatory IL-10; chemokines (e.g., CXCL9); growth factors (e.g., G-CSF); and IFN- $\alpha$ . Of these, levels of markers did not change significantly over time, except TNF ( $P = 0.006$ ). At the start of follow-up, TNF and a cluster composed of IL-7, IL-17, MIG, IL-2, and HGF were consistently detected by principal component analysis and K-means clustering. However, these clusters were not correlated with risk of malaria. In single and multi-marker analyses adjusting by vaccination status, IFN- $\alpha$  (Odds ratio [OR]=0.1), G-CSF (OR=0.8), and the TH1-related cytokines IL-12 (OR=0.7), and IL-15 (OR=0.8) decreased the risk of malaria. Conversely, the chemokine eotaxin (OR=3.4) increased the risk of malaria, perhaps due to its association with apoptosis of B cells and/or its negative impact on production of anti-*Plasmodium* IgG. These results provide novel information about cellular responses, such as the importance of IFN- $\alpha$  and G-CSF, highlighting the role of innate responses in protection from malaria.

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### ATYPICAL ACTIVATION OF DENDRITIC CELLS BY PLASMODIUM FALCIPARUM

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Malaria is characterized by high levels of inflammation and while an early inflammatory response is important for parasite clearance, excessive and persistent inflammation can contribute to severe forms of the disease. At the same time, *Plasmodium falciparum* infections fail to induce sterile immunity and clinical immunity is only acquired after years of exposure. Reports about the role of dendritic cells (DCs) in the immune response to

*P. falciparum* and how they contribute to the activation of CD4+ T cells during blood stage have been contradictory. We found that the activation of DCs by *P. falciparum* induces a highly unusual phenotype by which DCs up-regulate co-stimulatory molecules and secretion of chemokines, but not of cytokines typical of inflammatory responses (IL-1 $\beta$ , IL-6, IL-10, TNF). Contact-dependent cross-talk between the main DC subsets, plasmacytoid DCs and myeloid DCs, was necessary for increased chemokine and IFN $\alpha$  secretion in response to the parasite. Despite their atypical activation phenotype, myeloid DCs incubated with *P. falciparum*-infected erythrocytes activated autologous antigen-specific naive CD4+ T cells to proliferate and secrete high levels of the Th1 cytokines IFN $\gamma$  and TNF, but also IL-10 and IL-5, characteristic for a T regulatory type 1 (Tr1) response. Initial experiments with DCs obtained from uninfected malaria-experienced donors from Mali suggest a comparable activation phenotype in malaria-naïve and malaria-experienced individuals. We will further analyze the DC response to the parasite in Malian individuals in a longitudinal study comparing responses during the dry season to malaria season. Our findings might contribute to a better understanding of the mechanisms involved in the initiation of the adaptive immune response against *P. falciparum*.

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### TO KILL PARASITE THE NATURAL KILLER WAY: ANTIBODY MEDIATED CELLULAR IMMUNE RESPONSE AGAINST BLOOD STAGE MALARIA

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Antibodies effectuate immunity to malaria, however, there is incomplete understanding of protective mechanisms. Susceptibility of *Plasmodium falciparum* living inside infected red blood cells (RBC) to antibody-dependent cellular cytotoxicity (ADCC) remains unexplored. ADCC can be triggered by interaction of antibody Fc region to activating receptor Fc $\gamma$ R11 (CD16) present on natural killer (NK) cells. During ADCC, NK cells degranulate and deliver serine proteases, such as granzyme, into target cells where they activate apoptotic pathways. Here, we have evaluated the response of primary human NK cells towards parasitized RBC, its effect on RBC integrity and parasite viability. In the presence of serum from individuals clinically immune to malaria, NK cells degranulated and produced pro-inflammatory cytokines in response to parasitized RBC. Addition of immune plasma led to NK cell-mediated selective lysis of infected RBC as confirmed by live cell imaging and several lysis assays. Importantly, NK cell-mediated ADCC also inhibited the growth of *P. falciparum* in RBC. Intracellular parasite susceptibility to NK-mediated ADCC prompted us to further explore the cellular and molecular basis of parasite death. The variant surface protein PfEMP1 is the immunodominant antigen expressed at the surface of infected RBC, and parasites switch PfEMP1 expression to evade variant-specific antibody responses. RBC infected with a transgenic *P. falciparum* lacking PfEMP1 were not sensitive to NK cells in presence of immune plasma. In addition, ADCC was effective when PfEMP1-specific antibodies were bound to wild-type parasitized RBC, reaffirming the role of PfEMP1 as antigen during NK-mediated ADCC. ADCC affected the integrity of parasitized RBC, as shown by hemoglobin release, RBC membrane damage, granzyme-B delivery and caspase-3 activation. NK cells treated with granzyme inhibitor lose their cytotoxic potential towards infected RBC and the parasite survives. These findings suggest additional immune mechanisms that could contribute to natural protection to malaria.

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### IMPACT OF PLACENTAL MALARIA ON CORD BLOOD V $\delta$ 2 T LYMPHOCYTES IN MALAWI

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*P. falciparum* (Pf) placental infection primes the fetal immune system and in some instances reduces infant immunity against malaria and other pathogens. The mechanisms leading to these outcomes are not completely understood. We focused on V $\delta$ 2 cells, which respond to a broad range of pathogens including mycobacteria and Pf. These unconventional lymphocytes are triggered by small, non-peptidic antigens (including metabolites of Pf) independently of MHC presentation. We hypothesized that placental malaria (PM), which may cause fetal exposure to high levels of Pf metabolites, induces measurable changes in neonatal V $\delta$ 2 lymphocytes. We analyzed cord blood mononuclear cells from Malawian neonates born to women with PM, peripheral malaria or no malaria during pregnancy, using North American specimens as an external reference group. The PM group was stratified into past, chronic and acute infections. V $\delta$ 2 cell phenotype, repertoire and function were compared for Pf-exposed and unexposed newborns. A preliminary analysis showed that neonates exposed to chronic PM had increased frequencies of V $\delta$ 2 cells in cord blood compared to the acute PM group. Neonates exposed to active PM (including both chronic and acute infections) had higher proportions of CD16+ V $\delta$ 2 T cells than the past PM group; moreover, they displayed higher proportions of CD25+ V $\delta$ 2 cells and lower proportions of PD1+ V $\delta$ 2 cells than unexposed controls, overall indicating altered activation/differentiation state. We hypothesize that *in utero* exposure to Pf, at least in some cases, attenuates V $\delta$ 2 cell function in early life, thus impairing responses to BCG vaccination and innate protection against some infectious agents; we are currently testing whether the observed phenotypic differences for PM-exposed neonates are associated with altered function and/or perturbed T cell receptor gamma repertoire at birth. By determining whether V $\delta$ 2 cells represent a useful biomarker or possibly a key mediator of the impact of PM on neonatal immune system, we can contribute to public health interventions directed at mitigating the negative effects of PM on infant immunity.

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### ANTIBODIES TO PLASMODIUM VIVAX PVDBP REVEAL A MECHANISM FOR CROSS-SPECIES IMMUNITY TO P. FALCIPARUM PLACENTAL MALARIA

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During infection with *Plasmodium falciparum* in pregnancy, parasites express the surface antigen VAR2CSA that mediates adherence of infected erythrocytes to chondroitin sulphate A (CSA) in the placenta.

Antibodies to VAR2CSA are acquired with successive exposures to *P. falciparum* in pregnancy and the presence of VAR2CSA antibodies has been associated with protection from placental malaria. In Colombia, we previously observed a high frequency of antibodies to VAR2CSA in non-pregnant populations. However, the origin of these VAR2CSA cross-reactive antibodies and their role in protecting against placental malaria is unknown. Here, we tested the hypothesis that acquisition of VAR2CSA antibodies outside of pregnancy can arise due to exposure to *P. vivax*. We tested over 200 sera from men and children living in malaria-endemic regions of Colombia and Brazil and over 50% of individuals exposed only to *P. vivax* had antibodies that recognized VAR2CSA. We further showed that antibodies against the PvDBP protein from *P. vivax* can mediate this cross-reactivity. PvDBP affinity-purified antibodies from human sera and a monoclonal antibody raised against PvDBP recognized VAR2CSA through shared epitopes in DBL domains, showing that protective antigenic determinants can be evolutionarily conserved between these proteins. The monoclonal antibody also recognized the native protein on the surface of infected erythrocytes by IFA and flow cytometry, and significantly inhibited the binding of infected erythrocytes to CSA. Inhibition was similar to the level observed with sera from multigravid African women, which is the primary *in vitro* correlate of protection from placental malaria. These data show that exposure to PvDBP can induce antibodies that functionally recognize VAR2CSA. We thus identify a novel mechanism for cross-species immunity to falciparum malaria that can be exploited as a new approach to develop a vaccine against malaria in pregnancy.

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### DECLINING MALARIA TRANSMISSION DIFFERENTIALLY IMPACTS ON THE MAINTENANCE OF HUMORAL IMMUNITY TO *PLASMODIUM FALCIPARUM* IN CHILDREN

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Antibodies play a major role in naturally-acquired immunity to *Plasmodium falciparum* malaria and predominantly target the blood-stages, including merozoites and infected erythrocytes (IEs). As a result of intensified control efforts, and other factors, *P. falciparum* transmission has declined in many regions in recent years, and these declines have been associated with higher rates and severity of clinical malaria, which may be attributed to declining naturally-acquired immunity in the population. While there is evidence that declines in malaria transmission are associated with reductions in antibodies to blood-stage antigens, what is less clear is whether significant humoral immune responses to key targets are maintained after reductions in transmission, or the impact on functional protective antibody responses. In a 3-year longitudinal cohort of 300 Kenyan children, levels of antibodies to different antigens were measured by ELISA, and levels of functional antibodies promoting opsonic phagocytosis of merozoites, fixation of complement to merozoites and antibodies to IEs were quantified. Over a period in which malaria transmission declined substantially, antibodies to merozoite antigens AMA1 and MSP2 decreased substantially (antibody half-lives 0.8 and 1-3 years); however, ~70% of children maintained their sero-positivity to AMA1 and ~45% to MSP2. Antibodies promoting opsonic phagocytosis of merozoites also declined rapidly (half-life 0.15 years). In contrast, complement-fixing antibodies to merozoites did not decline over the study period and antibodies to surface antigens of IEs expressing virulent phenotypes, targeting PfEMP1, were much better maintained (half-life 4-10 years). These data suggest that the loss of immunity as malaria transmission declines is not universal as some key functional responses and antibodies to IEs were well maintained and may continue to provide some protection. These findings have implications for malaria surveillance and

control measures. Understanding why some responses are well maintained may inform the development of vaccines that generate sustained protection.

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### SINGLE-CELL RNA-SEQ REVEALS ACTIVATION OF CHROMATIN REGULATORS BY AP2-G DURING SEXUAL COMMITMENT IN MALARIA PARASITES

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Sexual commitment requires activation of *ap2-g*, the transcriptional master regulator of sexual development, from its epigenetically silenced-state during asexual replication. Expression of the AP2-G transcription factor during this "commitment cycle" poises gene expression in nascent merozoites to initiate sexual development through a hitherto unknown mechanism. In order to maintain a persistent infection, *ap2-g* expression is limited to a sub-population of parasites ranging from 1-30%, depending on genetic background and growth conditions. As sexually-committed schizonts comprise only a sub-population and are morphologically indistinguishable from their asexually-committed counterparts, defining their characteristic gene expression has been difficult using traditional, bulk transcriptome profiling. To determine the transcriptional changes induced by AP2-G within this sub-population, we applied highly-parallel, single-cell RNA sequencing to malaria cultures undergoing sexual commitment. In this first application of single-cell RNA-seq to malaria parasites, we surveyed the transcriptomes of over 16,000 parasites from multiple stages of development and found that, during merozoite formation, AP2-G robustly up-regulates key transcription factors, including histone modifying enzymes and regulators of nucleosome positioning. These epigenetic regulators act to poise the expression of genes necessary for initiation of gametocyte development in the subsequent cell cycle.

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### A SATURATION-LEVEL PIGGYBAC MUTAGENESIS SCREEN OF THE *PLASMODIUM FALCIPARUM* GENOME DEFINES GENES IMPORTANT FOR *IN VITRO* ASEXUAL BLOOD-STAGE GROWTH

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*Plasmodium falciparum* is the most lethal eukaryotic microbial pathogen and yet the genetic basis of many of its unique biological properties and important metabolic pathways remains unknown. In this study, we fulfill the critical need for a robust analytical method to systematically define the genes and pathways indispensable for *in vitro* survival of asexual blood-stage *P. falciparum* NF54 at a whole-genome scale. This study exploits the efficiency of the *piggyBac* transposon method that primarily produces single-insertion mutant parasites and Quantitative Insertion-site Sequencing (Qlseq), our next-gen mutagenesis sequencing tool to identify *piggyBac* insertion sites, to achieve for the first time saturation-level mutagenesis of *P. falciparum*. At this level of mutagenesis each gene (intergenic + ORF) in the genome was targeted by multiple *piggyBac* insertions. Even at this high density of mutagenesis, no *piggyBac* insertions were recovered from half of the genes in *P. falciparum* genome and so disruption of the ORF of these genes was presumed deleterious to parasite survival. Therefore, the genes without insertions disrupting their ORFs are considered essential for asexual blood-stage growth. Examples of essential genes includes K13 implicated in ART-R, the essential egress kinase gene CDPK5, and the primary drug target gene DHFR. In contrast, about half of

the genes in genome had one of more ORF-disrupting *piggyBac* insertion and these genes are presumed to be dispensable for asexual blood-stage growth. Analysis of this dispensable gene set indicated about half of these genes (>1000) are likely to be important in sexual stage development, based upon peak gene expression patterns. Overall, by generation and analysis of saturation-level, whole-genome coverage of unique *piggyBac* insertions, we can discern genes and pathways most important for asexual intraerythrocytic growth of *P. falciparum* NF54 under ideal *in vitro* culture conditions. The *pB-Qseq* method now provides the robust genomic tool needed to accelerate progress to identify and validate essential candidate targets to develop the most effective new antimalarial therapies.

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### FUNCTIONAL ANALYSIS OF A SPOROZOITE RHOPTRY PROTEIN DURING HEPATOCYTE INFECTION

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Rhoptries, the apical end organelle in *Plasmodium* and *Toxoplasma* invasive forms, are known as containing secretory proteins required for parasite invasion of target cells. It has been well demonstrated in *Plasmodium* that rhoptry neck protein members (e.g. RON2, RON4, and RON5) are essential for merozoite invasion of erythrocyte by forming the tight junction between parasites and host cells, however their roles during sporozoite invasion of hepatocytes remain largely unknown. We demonstrated that most merozoite rhoptry proteins are also localized to sporozoite rhoptries. By applying sporozoite stage-specific gene silencing system in *Plasmodium berghei*, we revealed that several rhoptry proteins, including one containing EF hand domains (named RON), are involved in sporozoite invasion of mosquito salivary glands. Here, we examined whether RON in sporozoites is also required for the invasion of different target cells, mammalian hepatocytes. RON repressed sporozoites were collected from hemolymph of infected mosquitoes and injected into c57bl/6 mice intravenously. Livers were harvested at 24 h post inoculation and parasite burden was examined by real time RT-PCR. Compared with control sporozoites, RON repressed sporozoites have approximately 1000 times lower infectivity to mice. Furthermore, the sporozoite invasive ability to hepatoma culture cells was almost abolished by RON repression. Since RON repressed sporozoites demonstrated far less gliding motility, which is required for invasion of mosquito salivary glands and mammalian hepatocytes, sporozoite RON might contribute to the both invasion processes by either regulating sporozoite motility or attachment ability to the target cells.

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### PLASMODIUM FALCIPARUM GENETIC COMPLEXITY INFLUENCES TRANSCRIBED VAR REPERTOIRE AND IMMUNE RECOGNITION AMONG HIGHLY RELATED GENOTYPIC CLUSTERS

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As transmission intensity has declined in Senegal, so has the genotypic complexity of circulating *Plasmodium falciparum* parasites, resulting in specific genotypes emerging and persisting over many years. Such genetically related clusters could be transcriptionally and antigenically related as well, potentially representing related immunogenic types, having implications for population level immunity and vaccine development strategies. We directly test the hypothesis that changes in parasite genetic signatures can alter the host population's immune repertoire to variant surface antigens, and that the degree of genomic relatedness influences the expression of variant antigens and the degree of immune recognition. We characterize parasites within genotypic clusters, defined as identical by the 24-SNP molecular barcode and assigned a haplotype identifier, for other highly polymorphic *loci*; we measure expression of variant surface antigens (VSA) such as PfEMP-1 by upstream-region (Ups) *var* group expression typing and expressed *var* DBL1 $\alpha$  sequencing in *ex vivo* RNA samples and short-term adapted RNA samples; and we measure variant surface antigen IgG responses against short-term adapted parasite isolates. We quantify the extent to which isolates within clusters diversify their genomic *var* repertoires over time. Parasites within genotypic clusters are genetically identical at other highly polymorphic *loci* and are extremely similar in their genomic *var* repertoire, transcribed *var* repertoire, and host antibody recognition of their VSAs. The degree to which parasites share IgG recognition of VSAs is related to the level of genome-wide identity in addition to the specific transcribed *var* repertoires. Monitoring changes in population-level parasite genomics and transmission dynamics is critical, as fluctuations will influence the breadth of resulting host immune responses to circulating parasite genotypes. These findings suggest shared immune recognition of genetically similar parasites, which has implications for both our understanding of immunity and vaccine development strategies in malaria elimination settings.

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### ALBA4 COORDINATES STAGE-SPECIFIC INTERACTIONS AND MRNA FATES DURING PLASMODIUM GROWTH AND TRANSMISSION

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Transmission of the malaria parasite depends upon an unpredictable moment where a mosquito must take a blood meal from a host. *Plasmodium* parasites have therefore evolved strategies to be prepared for successful transmission, including translationally repressing and protecting the mRNAs they will need to establish the infection following transmission. This phenomenon has been well-described in sexual stage parasites for the DOZI/DDX6, CITH, and PUF RNA-binding proteins, and recently in sporozoites through investigations of PUF2. However, much of the translational repression mechanism is not worked out, including whether translational repressors change their protein binding partners and mRNA targets in transmission stages, or other portions of the life cycle. This is in large part due to severe technical limitations of biochemical, proteomic, and transcriptomic studies of sporozoites, where substantial mosquito contamination has stymied these efforts. We have recently described the means to produce minimally perturbed, purified sporozoites that are finally amenable to these types of investigations. Here, for the first time, we provide a proteomic comparison of a protein complex across asexual blood, sexual, and sporozoite stages, along with a transcriptomic comparison of the mRNAs that are affected in these stages. We find that the Apicomplexan-specific ALBA4 RNA-binding protein acts to regulate development of the parasite's transmission stages, and that ALBA4

associates with both stage-specific and stage-independent partners to produce opposing mRNA fates. These efforts expand our understanding and ability to interrogate both sexual and sporozoite transmission stages and the molecular preparations they evolved to perpetuate their infectious cycle.

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### INVESTIGATING THE VECTOR COMPETENCE OF *CULEX QUINQUEFASCIATUS* FOR ZIKA VIRUS

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Following the recent outbreak of Zika virus (ZIKV) in the Americas, there has been a rapid investigation into the factors determining ZIKV transmission during this epidemic. While *Aedes aegypti* was demonstrated by several groups to be the major vector of ZIKV in the Americas, our collaborators have shown that a strain of *Culex quinquefasciatus* from the south of China may also be competent to transmit this pathogen. We were interested in investigating the vector competence of different strains of *Cx. quinquefasciatus* for an Asian ZIKV isolate. In order to assess viral infection, midguts and carcasses were dissected after infection via feeding on a ZIKV infected blood meal and viral titers were determined via plaque assay. We did not observe viral plaques in any *Cx. quinquefasciatus* tissues at the time points assayed, whereas plaques were found in *Ae. aegypti* control midguts as early as 3 days post-infection (dpi). We then infected *Cx. quinquefasciatus* via injection of ZIKV into the thorax and dissected salivary glands at 3, 5, and 7 dpi and virus was detected in the salivary glands at all time points. After infection of 2 *Cx. quinquefasciatus* strains via blood feeding, ZIKV RNA was detected in the saliva via RT-PCR. Alignment of the different ZIKV strains genomes identified potential polyprotein variations in geographic isolates that may explain varying competence phenotypes that have been observed in *Cx. quinquefasciatus*. Current studies focus on clarifying whether the discrepancy between plaque assay and RT-PCR results is due to a mosquito species-specific modification of the virus or a severe infection bottleneck in the *Cx. quinquefasciatus* midgut. This work is critical for understanding the role of *Cx. quinquefasciatus* in the transmission of ZIKV.

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### LONG-TERM SURVEILLANCE DEFINES SPATIAL, TEMPORAL AND ENVIRONMENTAL PATTERNS THAT IMPLICATE *CULEX TARSALIS* AS THE PRIMARY VECTOR OF WEST NILE VIRUS TRANSMISSION

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West Nile virus (WNV) has become the most epidemiologically important mosquito-borne disease in the United States with ~46,000 cases since its introduction in 1999. Transmitted primarily by *Culex* species, WNV transmission requires the complex interplay between bird reservoirs and mosquito vectors that results in human cases. To better understand these interactions, we have compiled data over the last 15 years (2002-2016) in Iowa examining WNV seroprevalence in sentinel chickens, mosquito infection rates, and their impacts on human disease cases. By quantifying infection rates of these hosts from spatial and temporal perspectives, we have constructed an overall picture of when and where WNV is most actively being transmitted. Overlaying these findings with mosquito infection rates and abundance data, we provide strong support that *Culex tarsalis* is a more competent vector of WNV and that its abundance is highly correlated to human WNV cases. In addition, we have identified underlying climatic factors such as over-wintering temperatures and prolonged periods of drought as integral components that influence WNV transmission from year to year. From these insights, we have generated predictive models to determine the intensity of WNV infection

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### MOSQUITO IMMUNITY BEFORE AND AFTER METAMORPHOSIS: EVIDENCE OF ADAPTIVE DECOUPLING IN *ANOPHELES GAMBIAE*

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As holometabolous insects, mosquitoes undergo dramatic changes in morphology and ecological niche as they transition from aquatic larvae to terrestrial adults. Because larvae encounter a high density and diversity of pathogens in their microbe-rich aquatic environments and selection pressures are strongest on younger individuals that have yet to reproduce, we hypothesized that larval immunity is stronger than that of adults. To test this, we surveyed a broad array of cellular and humoral immune parameters in *Anopheles gambiae* larvae and compared the strength of these responses to that of newly-emerged (1-day-old) adults and older (5-day-old) adults. Overall, we found that larvae display more robust immune responses compared to adults with respect to bacteria killing efficiency, circulating and sessile hemocyte numbers, phagocytic properties of hemocytes, antimicrobial and melanization activity of hemolymph, and infection-induced transcription of immunity genes. Furthermore, we found that adult immune responses tend to decline with age. These findings are suggestive of both immune senescence and adaptive decoupling, or the independent evolution of larval and adult traits made possible by metamorphosis.

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### USING MOBILE PHONES AS ACOUSTIC SENSORS FOR HIGH-THROUGHPUT SURVEILLANCE OF MOSQUITO ECOLOGY

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Frequent, widespread, and high resolution surveillance of mosquitoes is essential to understanding their complex ecology and behaviour, in order to formulate strategies to mitigate or prevent mosquito-borne diseases. However, there is a scarcity of data on the abundance, temporal variation, and spatial distribution of mosquito vector species, due to the absence of high-throughput, low-cost surveillance techniques. Acoustic measurement of the species-specific wingbeat frequencies of mosquitoes has been proposed decades ago as a solution to automate surveillance. Yet, this approach has not taken flight on a large scale, due to technological limitations in signal acquisition and hardware scalability. In this work, we demonstrate that commercially available mobile phones are a powerful tool for acoustically mapping mosquito species distributions, thus establishing a new citizen-driven paradigm for crowdsourced mosquito surveillance that takes advantage of the existing and widespread mobile network infrastructure. We show that even low-cost mobile phones with basic functionality are capable of sensitively acquiring acoustic data on species-specific mosquito wingbeat sounds, while simultaneously recording the time and location of the human-mosquito encounter. We survey a wide range of medically important mosquito species, to quantitatively demonstrate how acoustic recordings supported by spatio-temporal metadata enable rapid, non-invasive species identification. As proof-of-concept, we carry out field demonstrations where minimally-trained users map local mosquito fauna using their personal phones. Thus, by leveraging the global mobile phone infrastructure with the potential for engaging citizen scientists, our approach enables continuous large-scale acquisition of mosquito surveillance data in resource-constrained areas.

## INTERROGATION OF THE SEASONAL MICROBIOME OF *ANOPHELES COLUZZII* IN MALI

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The poorly understood mechanisms of the seasonal maintenance of *Anopheles* spp. mosquitoes through the dry season in West Africa remain a critical gap in our knowledge of *Plasmodium* disease transmission. While it is thought some mosquitoes remain in a dormant state throughout this seven-month dry season, the nature of this state remains unknown and has largely not been recapitulated in laboratory settings. To elucidate possible life history traits allowing for this phenotype, this study used molecular tools to investigate the spatiotemporal change in the microbiome of mosquitoes in the dry and wet seasons in Mali. We analyzed the 16S ribosome bacterial and internal transcribed spacer (ITS) fungal sequences present in mosquitoes collected from two locations with varying water availability. These locations were a village near the Niger River with year-round water sources (N'Gabakoro), and a Sahelian area with highly seasonal breeding sites (Thierola). Using 16S next-generation sequencing data, we found 449 unique genera across 29 families with 2771 unique 16S sequence variants. With these, we could discriminate several compositional differences that were seasonally and spatially linked. Counter to our initial hypothesis, we found a more pronounced seasonal difference in the bacterial microbiome in the area with year-round water sources (N'Gabakoro). These major seasonal shifts came in *Ralstonia* and *Duganella* spp. bacteria that are classically soil and water-associated, indicating that these changes may be from bacteria acquired in the larval environment, rather than during adulthood. Additionally, with fungal ITS-specific reads we found 209 unique fungal genera that showed a high degree of variability between individuals. Lastly, through 16S and cytochrome B analysis we found a greater heterogeneity in host choice of *An. coluzzii* in the dry season, which may indicate a relaxation of anthropophily in some locations during this time. With these findings, we help to further refine our knowledge of *Anopheles* spp. maintenance strategies during this cryptic period critical to the maintenance of the *Plasmodium* spp. transmission cycle.

## LARVAL BREEDING WATER: MICROORGANISMAL HETEROGENEITIES EFFECTS ON ADULT VECTOR COMPETENCE OF HUMAN PATHOGENS AND IMMUNITY

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The biotic characteristics of larval habitats have been shown to contribute to the growth and development of mosquito larvae and to adult fitness. The accretion of the current understanding of the role of bacteria in modulating mosquito permissiveness to human pathogens, although significant, still lacks understanding on how the microorganismal heterogeneity in the larval habitat may influence vector competence of the adult. In the current study, we tested the hypothesis that larval exposure to different Gram-positive and Gram-negative bacteria will result in a bacteria species-specific modulation of permissiveness to human pathogens through a prolonged transstadial modulation of mosquito physiological systems that can influence infection, such as the immune system. Late developmental larval stages of both *Aedes aegypti* and *Anopheles gambiae* were exposed to Gram-positive *Bacillus* sp. and *Staphylococcus* sp., and Gram-negative *Pseudomonas* sp. and *Enterobacter* sp. Here we present vector competence of the resulting adult mosquitoes as a measure of permissiveness to dengue and Zika viruses and *Plasmodium falciparum*.

For each bacteria we included experimental groups that had either been challenged at the larval (primed larvae) or adult (primed adults) stage, or both (primed larvae and adults) stages. The pupae were sterilized to avoid transstadial carry-over of bacteria. We also compared immune gene expression of bacteria-challenged larvae to the resulting adults, and we compared the daily survival of each experimental group. Our results show that each experimental group had different permissiveness to the pathogens. For example, the adults derived from the primed larvae group showed greater permissiveness to both viruses, indicating the existence of bacteria challenge-mediated transstadial influence on vector competence. We also observed a transstadial activation of immune genes and a significant difference in the survival rate of adults emerging from bacteria-challenged larvae. Our study predicts that bacterial heterogeneity in the larval habitat can impact the vector competence, immunity and fitness of adult mosquitoes.

## NORTHERN RANGE EXPANSION OF THE ASIAN TIGER MOSQUITO (*Aedes albopictus*): ANALYSIS OF MOSQUITO DATA FROM CONNECTICUT, USA

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The Asian tiger mosquito (*Aedes albopictus*) is a highly invasive species that was introduced into the U.S. in the 1980's and continues to expand its range in the eastern half of the country. Winter temperature is an important constraint to this species northward expansion and Connecticut appears to be located at the thermal limit for overwintering survival of this species. In this study, we sampled mosquitoes from 91 statewide trapping sites from 1997-2016 to track the establishment and range expansion of *Ae. albopictus* in Connecticut. In addition, *Ae. albopictus* larvae were monitored in tire habitats and tires were retrieved from the field in the spring and flooded to evaluate overwintering success of hatching larvae. *Ae. albopictus* was first detected during statewide surveillance when a single specimen was collected in 2006. This species was not collected again until 2010 and was detected every year since then with increasing abundance and distribution except following exceptionally cold winters. *Ae. albopictus* mosquitoes were most abundant in urban and suburban locations along the Long Island Sound shoreline of southwestern Connecticut; however, single specimens were occasionally detected in central parts of the state. Field-collected females were also screened for arbovirus infection yielding two isolations of Cache Valley virus and one isolation of West Nile virus highlighting the threat posed by this mosquito. *Ae. albopictus* overwintered in Connecticut under mild winter conditions as shown by recovery of larvae hatching in spring and by early seasonal detection of larvae and adults. This study documents the establishment and expansion of *Ae. albopictus* at the northern boundary of its range in New England and provides a baseline for monitoring future range expansion and population increases anticipated under climate change.

## DENGUE VIRUS IN PATIENTS DIFFERS FROM CELL CULTURE DERIVED VIRUS

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Recent results from vaccine trials have demonstrated that the presence of antibodies that neutralize cell culture derived DENVs does not necessarily correlate with protective immunity. These and other observations highlight the importance of defining the properties of DENVs that circulate within infected individuals. The goal of this study was to characterize

and compare DENV serotype 1 (DENV1) viruses circulating in viremic patients and the same patient viruses after 1 passage in cell culture. We collected blood from 20 viremic patients exposed to primary DENV1 infections during an epidemic in Sri Lanka in 2014, isolated the virus on commonly used C6/36 insect cells and Vero mammalian cell lines. The specific infectivity (viral genome copy number/infectious virus) of the human plasma viruses and corresponding cell culture passaged virus was determined using a digital drop polymerase chain reaction (ddPCR) and a Vero cell focus assay. Our results demonstrated that the specific infectivity of plasma viruses is 47 fold and 615 fold greater compared to the same virus amplified on mammalian (Vero) and Insect (C6/36) cell lines respectively. We evaluated the maturation state of plasma and cell culture derived DENV1 using a preMembrane (prM) protein specific MAb, which will capture partially or fully immature virions but not completely mature virions. The majority of the cell culture derived virus (>75%) was captured by the prM MAb whereas none of the plasma virus bound to the MAb. Thus, DENV1 in plasma is fully mature unlike cell culture grown DENV1, which was mostly immature. As DENV maturation stage can strongly alter sensitivity to antibody neutralization, we compared the ability of seven well-characterized human and mouse MAbs to neutralize plasma and cell culture derived DENV1. One DENV1 specific human MAb equally neutralized plasma and cell culture viruses. The remaining 6 MAbs neutralized the cell culture viruses much better than the plasma viruses. Our results demonstrate that the specific infectivity, maturation state and sensitivity to antibody neutralization are profoundly altered even after a single passage of DENV1 in cell culture.

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### EVOLUTION OF B CELL RESPONSE IN PRIMARY DENGUE INFECTION

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Any of the four mosquito-borne dengue virus (DENV) serotypes can cause disease, which affects more than one hundred million people worldwide per year. Antibodies are a key mechanism by which DENV is controlled, and is a desired feature of vaccination. The antibody response in dengue infection is characterized by both serotype-specific (TS) and cross-reactive (CR) antibodies, but the relationship between these pools of antibodies at a cellular level over time is not known. While neutralizing monoclonal antibodies have been isolated from both of these categories, non-neutralizing CR antibodies have also been implicated in severe secondary dengue. Understanding the dynamics and contribution of TS and CR antibodies is critical to understand how protection versus disease enhancement is generated after a dengue infection. To address this question, we analyzed the acute plasmablast and convalescent memory B cell repertoires in longitudinal samples from three subjects enrolled in a controlled human infection model for DENV-2. The early plasmablast response peaked at approximately 2-3 weeks post-DENV-2 infection, and accounted for approximately 6% of total B cells. Paired immunoglobulin heavy and light chains from single plasmablasts were sequenced. From a representative cross-section of IGH/IGL repertoire, we expressed recombinant IgGs. Up to 60% of these plasmablast-derived antibodies were specific to dengue antigen. The majority (74%) of the antigen-specific clones were specific to the infecting DENV-2 serotype. We immortalized and cloned memory B cells collected six months post-

infection and found that the frequency of DENV-specific memory B cells had contracted to an estimate of 0.02% of total B cells and consisted of both TS and CR clones at comparable proportions, with many clones targeting quaternary epitopes on complex virions. The information gained from exploring the antibody evolution in this primary infection model provides a foundation to evaluate the antibody response induced by vaccination and to assess the contribution of CR and TS antibodies to durable protective immunity against DENV.

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### DENGUE VIRUS NS1-INDUCED ENDOTHELIAL CELL-INTRINSIC VASCULAR LEAK IS INDEPENDENT OF INFLAMMATORY CYTOKINES BUT DEPENDENT ON ENDOTHELIAL GLYCOCALYX INTEGRITY

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Dengue virus (DENV) is the most medically important arbovirus, affecting 40% of people worldwide and infecting as many as 390 million individuals annually. Up to 96 million cases occur each year, ranging from uncomplicated dengue fever (DF) to life-threatening dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS), which is characterized by endothelial dysfunction and vascular leakage. Previously, we demonstrated that DENV nonstructural protein 1 (NS1) can induce endothelial hyperpermeability in both human cell culture and a systemic mouse model. Additionally, DENV NS1 has been shown to disrupt the endothelial glycocalyx, as well as trigger release of inflammatory cytokines from peripheral blood mononuclear cells via TLR4. Here, we examined the relative contributions of inflammatory mediators and endothelial cell-intrinsic pathways to DENV NS1-induced endothelial hyperpermeability and vascular leak. *In vivo*, we demonstrated that DENV but not West Nile Virus NS1 triggers dose-dependent, localized vascular leak in the dorsal dermis of wild-type C57BL/6 (WTB6) mice as measured by Alexa Fluor 680-conjugated dextran. *In vitro*, we showed that the human dermal endothelial cell line HMEC-1 does not produce inflammatory cytokines (TNF- $\alpha$ , IL-6, IL-8) in response to DENV NS1 but does in response to lipopolysaccharide (LPS) and that specific monoclonal antibodies to these cytokines do not block DENV NS1-induced endothelial hyperpermeability as measured by trans-endothelial electrical resistance (TEER). Further, we demonstrated that DENV NS1 induces vascular leak in B6 mice genetically deficient for TLR4 or TNF- $\alpha$  receptor at similar levels to WTB6 animals. Finally, we blocked DENV NS1-induced vascular leak in WTB6 using an inhibitor cocktail targeting sialidase, cathepsin L, and heparanase, molecules involved in endothelial glycocalyx disruption. Taken together, our results indicate that DENV NS1-induced endothelial cell-intrinsic vascular leak is independent from inflammatory cytokines but dependent on endothelial glycocalyx integrity and suggest potential molecular targets for the treatment of severe dengue disease.

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### FLAVIVIRUS NONSTRUCTURAL PROTEIN 1 MODULATES ENDOTHELIAL PERMEABILITY AND VASCULAR LEAK IN A TISSUE- AND DISEASE-SPECIFIC MANNER

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The flavivirus genus includes the human pathogens dengue virus (DENV), yellow fever virus (YFV), Japanese encephalitis virus (JEV), West Nile virus (WNV), tick borne encephalitis virus (TBEV) and Zika virus (ZIKV). Flaviviral infections range from asymptomatic to self-limiting febrile illness to severe clinical manifestations, including encephalitis, hemorrhage, shock,

congenital birth defects and neurological complications. The flavivirus non-structural protein 1 (NS1) plays critical roles in virus replication and immune evasion. Recently, we showed that DENV NS1 alters the integrity of endothelial monolayers, leading to endothelial barrier dysfunction and vascular leak. However, the ability of other flavivirus NS1 proteins to alter endothelial permeability remains unknown. Here, we examined the ability of NS1 proteins from DENV, WNV, JEV, YFV and ZIKV to interact with and modulate the barrier function of different human endothelial cell (EC) lines, as well as their ability to cause tissue-specific vascular leakage *in vivo*. We demonstrated that flavivirus NS1 proteins selectively alter the permeability of ECs from lung, liver, skin, brain, and umbilical vein *in vitro* and cause vascular leakage in a tissue-dependent manner *in vivo*, which reflects the disease pathogenesis. Mechanistically, we showed that flavivirus NS1 proteins differentially induce disruption of the endothelial glycocalyx and mislocalization of intercellular junction proteins of ECs. In preliminary *in vitro* studies, we found that only DENV NS1 facilitates DENV dissemination through polarized lung ECs, consistent with leakage in the lung in severe dengue disease. Interestingly, this was not observed with other flaviviral NS1 proteins. Conversely, ZIKV but not WNV NS1 increased ZIKV translocation across human umbilical vein ECs. Ongoing *in vivo* experiments will clarify the role of NS1 in virus dissemination. Our findings reveal a previously unidentified ability of flavivirus NS1 proteins to modulate endothelial barrier dysfunction in a tissue-specific manner both *in vitro* and *in vivo*, potentially influencing flavivirus pathogenesis and disease.

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### MAPPING THE TARGET EPITOPES OF THE TYPE SPECIFIC ANTIBODY RESPONSES INDUCED BY A LIVE-ATTENUATED DENGUE VACCINE

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The Laboratory of Infectious Diseases at the National Institutes of Health has developed, live attenuated Dengue (DENV) vaccines to each of the four serotypes (DENV1- DENV4). The LATV TV003, comprised of these four components, has been shown in a human challenge model, to completely protect all vaccinated subjects from infection following challenge with DENV2. An ideal DENV vaccine should elicit a balanced immune response against all four serotypes of DENV, in addition to being safe and inducing durable long-term protection. The safety, infectivity, and immunogenicity profiles of each monovalent DENV vaccine were characterized previously in healthy, flavivirus-naïve adult volunteers, however epitope-specific responses were not evaluated. To determine vaccine induced correlates of protection, we performed antibody depletions to determine the roles of serotype-specific and cross-reactive antibodies in the neutralization of each serotype. Once type-specific responses had been confirmed, we used recombinant DENV (rDENV) viruses to map the targets of type-specific neutralization epitopes targeted by the vaccine-induced antibody response. A portion of each of the type-specific neutralizing antibody responses were directed to previously identified epitope regions that mapped to strongly neutralizing quaternary type-specific antibodies against DENV1, DENV2, DENV4, and in limited cases, to DENV3. Interestingly, the importance of each epitope targeted by the type-specific response, appeared to be dependent on the cell type used for neutralization. The type-specific epitopes mapped for DENV1, DENV3, and DENV4 had a more significant impact on the neutralization in a human monocytic cell line expressing dendritic cell-specific intercellular adhesion molecule 3-grabbing non-integrin (DC SIGN). While, in the Vero cell line, the monovalent vaccine samples had an increased neutralization capacity against heterotypic DENV serotypes.

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### THE EARLY PLASMABLAST DERIVED ANTIBODY RESPONSE TO PRIMARY DENGUE VIRUS INFECTION

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The four mosquito-borne dengue virus (DENV) serotypes are responsible for dengue hemorrhagic fever. People exposed to acute viral infections develop a rapid but transient increase in antibody secreting plasmablasts. After recovery, memory B-cells (MBC) and antibody secreting long lived plasma cells (LLPC) are key components of protective immunity. For DENVs the origin and relationships between antibodies produced by the early plasmablast response and the MBC, LLPC responses are not well defined. The goal of this study was to characterize the plasmablast responses in primary dengue infection using a DENV2 human challenge model. Flavivirus-naïve subjects were challenged with a partially attenuated strain of DENV2. The plasmablast responses in three subjects were analyzed in terms of magnitude, antibody repertoire, antigen-specificity and neutralization properties. Peak plasmablast responses correlated with onset of peak viremia. The plasmablast antibody repertoire was determined using single-cell DNA barcoding and high throughput deep sequencing to determine the cognate heavy and light chain immunoglobulin genes expressed by a total of 1690 plasmablasts. The antibody sequences were analyzed to determine phylogenetic relationships of clones and antibody families within individuals as well as families convergent between individuals. A panel of 92 monoclonal antibodies representing families of expanded, proliferative clones were selected for antibody expression. Purified antibodies were screened for binding to and neutralization of the 4 DENV serotypes. A large fraction (60%) of the antibodies recognized one or more DENV serotypes. The majority of the DENV reactive antibodies were type-specific to serotype 2 and recognized epitopes displayed on intact DENV2 virions. Although lineages bound to DENV at a high rate, a small fraction of the DENV reactive antibodies strongly neutralized serotype 2. We will discuss the implications of our results for understanding the complex interplay of the early plasmablast and late memory B cell responses to primary DENV infection.

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### USE OF STRUCTURAL EQUATION MODELS TO PREDICT DENGUE ILLNESS PHENOTYPE

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Early recognition of dengue illness, particularly patients at high risk for plasma leakage syndrome, is of critical importance to optimal clinical management. However, substantial overlap in the clinical profile of mild and severe dengue illness continues to present significant challenges to this goal. The objective of this study was to build predictive models for dengue, dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS) using structural equation modelling. Using data from 284 children from a hospital-based, prospective study in Thailand, we performed structural equation modelling to capture mechanistic pathways culminating in dengue, DHF, and DSS. Fever day 0 was defined as the point when temperature was <38°C for a consecutive 12 hours and

considered the day of defervescence; days before and after defervescence were numbered consecutively. Study day 1 was defined as the day when a child was enrolled in the study, and days were numbered consecutively afterward. Illness day 1 was defined as the day when fever was  $\geq 38^{\circ}\text{C}$ , and days were numbered consecutively afterward. Models for dengue, DHF, and DSS were separately developed based on data from fever days (days -3 and -1) and validated by data from 834 children not used for model development. At fever day -3, predictors for dengue and DSS included age, tourniquet test, aspartate aminotransferase, and white blood cell, lymphocyte, and platelet counts. Predictors for DHF included age, aspartate aminotransferase, hematocrit, tourniquet test, and white blood cell and platelet counts. The models showed good predictive performances, regardless of disease phenotype and data collection time points as indicated by comparable areas under the receiver operating curves (AUC). For example, the AUC for any acute dengue illnesses based on fever day 0, study day 3, and illness day 3 data were in the range of 0.93-0.97, 0.90-0.94, and 0.80-0.85, respectively. These predictive models have potential use in estimating the probability that a child has dengue and whether the child will ultimately manifest DHF or DSS based on clinical laboratory data available before the critical phase of illness.

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### EFFECTIVENESS OF A COMBINED HOUSEHOLD-LEVEL PIPED WATER AND SANITATION INTERVENTION IN RURAL ODISHA, INDIA ON HEALTH: A MATCHED COHORT STUDY

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Globally, almost one billion people practice open defecation—the majority reside in India. While government campaigns have increased toilet access in India, recent studies found limited impact on health, possibly due to sub-optimal sanitation coverage and use. Although coverage of community-level improved water sources is high even in rural India, household-level water access may be critical to the use of toilets in this context. Gram Vikas, an NGO in Odisha, India, has implemented a combined intervention in over 1000 villages since 2002. Their approach includes household-level piped water connections contingent on full community-level coverage of household toilets. We undertook a matched-cohort study in Ganjam and Gajapati districts in Odisha, India to assess intervention impact on water and sanitation coverage and use, and on health. Surveys and environmental samples from eligible households (N=2384) from 45 randomly selected intervention and 45 matched control villages were collected at four times from May 2015–Oct 2016. The intervention was associated with increased access to improved toilets (85% v. 18%), as well as increased toilet use by adults (74% v. 13%) and for child feces disposal (35% v. 6%) ( $p < 0.001$ ). The intervention was also associated with increased access to piped water (73% v. 8%,  $p < 0.001$ ) although almost all households in both study arms stored drinking water—a possible source of continued exposure. Adjusting for sociodemographic variables, the intervention was not associated with reduced diarrhea in children  $< 5$  (OR: 0.94, 95% CI: 0.74-1.20). Children  $< 5$  in intervention villages had improved nutritional status as measured by height-for-age z-score (HAZ) (+0.17 HAZ, 95% CI: 0.03-0.31), though over a third of children in both arms were moderately stunted (40% v. 33%). This study offers evidence that a combined water and sanitation intervention can substantially decrease open defecation and improve child nutrition in India. The mixed effects on health may be due to continued exposure to fecal pathogens and to subclinical infections.

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### PREVALENCE AND ETIOLOGY OF ENTERIC INFECTIONS AMONG CHILDREN SHARING SANITATION IN LOW-INCOME NEIGHBORHOODS OF MAPUTO, MOZAMBIQUE: BASELINE DATA FROM THE MAPSAN TRIAL

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Persistent enteric infection can have long-term effects on child health. Risk of exposure to enteropathogens can be greater in low-income, informal, urban settlements where high population density and low-level access to safe sanitation coexist. The MapSan Trial examines the impact of providing shared sanitation on the risks of enteric infections in children living in Maputo, Mozambique. We report baseline data from MapSan, with a focus on etiologies of symptomatic and asymptomatic enteric infection. Stools collected from children  $< 4$  years were assayed using a multiplex, PCR-based, diagnostic assay to identify current infections of *Campylobacter jejuni*, *Salmonella* spp., *Shigella* spp., enterotoxigenic *Escherichia coli* [ETEC], Shiga toxin-producing *E. coli*, *E. coli* O157:H7, *Vibrio cholerae*, *Yersinia enterocolitica*, toxigenic *Clostridium difficile*, *Giardia lamblia*, *Cryptosporidium* spp., *Entamoeba histolytica*, Rotavirus A, Norovirus GI/GII, and Adenovirus 40/41. Self-reported diarrhea data were available for each child providing a stool. 87% of stools (n=684) were positive for  $\geq 1$  viral, bacterial, or protozoan target; only 14% of caregivers reported diarrheal symptoms in the 7 days prior to enrollment. The most frequently detected pathogens among both symptomatic and asymptomatic children included *Giardia* (52%), *Shigella* (44%), ETEC (30%), and *Salmonella* (21%); prevalence of *Giardia* and *Shigella* increased with age, and *Salmonella* prevalence decreased with age. 84% of detected infections were asymptomatic. Prevalence of coinfection was high: most (59%) children were infected with  $> 1$  pathogen and the average number of infections per child ranged from 1.3 in the youngest age stratum (0-11 months) to 2.1 in the eldest (24-48 months). Our results complement recent findings from the Global Enteric Multicenter Study and highlight the importance of surveillance for both symptomatic and asymptomatic infections as markers of child health. Chronic exposure to enteropathogens, regardless of symptomatology, may result long-term health effects in children including impairment of growth and cognitive development.

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### EFFECT OF A SANITATION INTERVENTION ON SOIL-TRANSMITTED HELMINTH PREVALENCE AND CONCENTRATION IN HOUSEHOLD SOIL: A CLUSTER-RANDOMIZED CONTROLLED TRIAL

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Sanitation interventions have been associated with a reduced prevalence of soil-transmitted helminth (STH) infection, and it has been hypothesized that improved sanitation interventions prevent fecal contamination from spreading throughout the household environment. Our study investigates the hypothesis that an improved sanitation intervention, a pit latrine with slab and child feces management tools, reduced STH eggs in household soil. We collected soil samples from 2107 households that were enrolled in the control (N=898), sanitation (N=613), and combined water, sanitation and hygiene (WSH) (N=596) intervention arms of the WASH Benefits cluster randomized controlled trial in rural Kenya. We combined the sanitation and WSH households into one improved sanitation intervention

group and found the intervention did not significantly reduce the prevalence (17.0% versus 18.9% in the control group) or concentration (geometric mean of 0.24 egg/g dry soil in both arms) of total STH eggs in soil. There was also no difference in the impact of the sanitation and combined WSH intervention arms on STH eggs in household soil, and there were no effects of the intervention on single STH species or viable eggs. Notably, most households (98.1%, 881 out of 898) in the control group had access to a sanitation facility; upgrading from unimproved to improved sanitation facilities may not be enough to affect the presence of STH eggs in household soil. In the control group, we found use of a shared latrine, past deworming of young children in the household, child open defecation practices, and an indicator of household wealth were associated with the presence of STH eggs in soil. Our results indicate that in areas with high access to latrines, where open defecation is rare and latrine sharing is common, improving latrines with slabs and drop hole covers and facilitating safe disposal of children's feces may not be enough to interrupt environmental transmission of STH.

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### SCHISTOSOMIASIS COUNTRYWIDE ASSESSMENT IN UGANDA: A NEGLECTED TROPICAL DISEASE OF CONCERN FOR WATER, SANITATION AND HYGIENE PRACTITIONERS

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Schistosomiasis is a neglected waterborne disease transmitted indirectly through freshwater snails, estimated to affect over 200 million people in Sub-Saharan Africa. In Uganda, where the disease is considered endemic, control programs have focused mainly on chemotherapeutic intervention with Praziquantel (PZQ) with less emphasis on interrupting environmental transmission. To understand the prevalence of Schistosomiasis in Uganda and the associated risk factors, we undertook the first nationally representative survey of an African country, which included testing, diagnosis and treatment at the household level. We conducted rapid data collection using mobile technology under the Performance Monitoring & Accountability 2020 platform that utilizes a multi-stage cluster design with fixed, Census-derived enumeration areas (EAs) and random selection of households. Infection with Schistosomiasis was the main outcome of interest and was positive in 1,097 participants of the 4,515 tested. After adjustment with sampling probabilities for EA selection and selection of households within each EA, the overall prevalence of Schistosomiasis in Uganda was 22.1% (95% CI: 18.7%, 25.6%). There was no statistical difference in prevalence when comparing EAs that had received mass drug administration with PZQ versus those that had not (31% vs 21%, respectively; p-value=0.14). In the adjusted analysis, the factors significantly associated with prevalence of Schistosomiasis were age, religion, region, and whether an individual self-reported openly defecating in water bodies. Wealth and education were not statistically significant factors indicating that the prevalence of Schistosomiasis is geographically and demographically widespread beyond the known endemic areas. This suggests that using a strategy to target certain communities for mass drug administrations may be less effective in interrupting the transmission of Schistosomiasis without water, sanitation and hygiene interventions designed to reduce the contamination of water bodies with human urine and feces.

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### IMPACT OF IMPROVED SANITATION AND HYGIENE ON STUNTING IN RURAL ZAMBIA IMPROVED SANITATION AND HYGIENE ON STUNTING IN RURAL ZAMBIA

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In 2015, an estimated 8.4 million people in Zambia lacked access to improved sanitation and 2.1 million practiced open defecation. Limited access to improved sanitation and safe water, and inadequate hand hygiene contribute to diarrhea and stunting among children under five in Zambia. The Zambian government, with support from DFID, UNICEF and other partners has been implementing the Zambia Sanitation and Hygiene Program to address these public health challenges. A key component of this program is the community led total sanitation (CLTS) initiative, which aims to have an additional 3 million people consistently using improved sanitation facilities and adopting hand washing with soap or ash. We conducted baseline and end-line cross-sectional surveys in 2013 and 2016 respectively, to measure relevant impact and outcome indicators. The use of improved not shared toilet facilities (defined as a facility that hygienically separates human excreta from human contact) improved from 36% (432/1204 households) to 51% (595/1170) and households having a specific place for handwashing improved from 21% (254/1204) to 33% (391/1170) between baseline and endline. While there was no impact of the CLTS intervention on two-week prevalence of diarrhea, there was a significant reduction in stunting (height-for-age Z score <-2 standard deviations) from 46% (309/670) to 41% (617/1507) (adjusted prevalence ratio [aPR] 1.18, 95%CI 1.06-1.31) and severe stunting (height-for-age Z score <-3 standard deviations) from 32% (217/670) to 18% (275/1507) (aPR 1.86, 95%CI 1.59-2.18) among under-five children. Stunting and severe stunting were significantly lower in households with improved not shared facilities and those with water/soap for handwashing relative to those without these improvements (stunting: 34% [78/228] vs. 41%, [539/1279]; aPR 0.75, 95%CI 0.61-0.93 and severe stunting: 14% [31/228] vs. 19% [244/1279] aPR 0.65, 95%CI 0.65-0.97). This impact evaluation demonstrates the importance of improved hygiene through development of facilities for handwashing coupled with improved sanitation for improving linear growth of children in Zambia.

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### THE ASSOCIATION BETWEEN FECAL CONTAMINATION AND ENVIRONMENTAL ANTIBIOTIC RESISTANCE IN RURAL BRAZIL

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Human and animal fecal contamination of soil and water is likely an important factor for distribution of antibiotic resistance in rural areas with inadequate sanitation. We sought to determine the relative contribution of each of these sources to the prevalence of antimicrobial resistant bacteria in the environment. In 2017, we studied a small rural community in Bahia, Brazil (pop 617) where 40% of toilets flush to the local rivers. We measured the resistance to ciprofloxacin (CipR) and beta-lactamases in lactose fermenting (LF) *Enterobacteriaceae* (ESBL-E) from water collected at 5 different sites along these rivers, the piped water system, as well as 50 stools collected from a random sample of residents and 13 farm animals. Water and stool were screened for CipR (growth on 10 µg/ml of ciprofloxacin) and ESBL-E (growth on 2 µg/ml of cefotaxime). Sanitary conditions were poor given the high coliform counts in river samples and treated water. For humans and animals, the majority of CipR bacteria were LF (78%), while ESBL-E were more evenly divided (54% LF). By contrast,

in water samples, CipR was evenly distributed between LFs and non-LFs, but 88% of the ESBL-E were lactose negative. The highest percentages of individuals harboring resistant Enterobacteriaceae were found in pigs (100% CipR, 75% ESBL-E) and humans (42% CipR, 38% ESBL-E). For humans, carriage of resistance did not differ by age. Cows and horses had carriage rates of 0% for CipR and 0-17% for ESBL-E. Unexpectedly, this rural community has similar rates of antibiotic resistance found in urban communities in other parts of the world. This suggests a significant use of antibiotics in the rural population. The high carriage rate in pigs was unexplained, but may be due to feeding habits in animals that root in the soil as opposed to grazing, the clandestine use of antibiotics in feed. Although this use is banned in Brazil, we did find feed containing antibiotics available in the neighboring town. Given the poor level of sanitation, pig and human fecal contamination are the likely sources resistant bacteria in the surface waters that the local treatment plant ineffectively removes.

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### IDENTIFYING ROBUST PROXY VARIABLES OF LATRINE USE: EXAMINING ACCESS TO IMPROVED SANITATION AS A PROXY

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Access to an improved sanitation facility is the standard proxy indicator for latrine use in the WASH sector. While there is growing evidence that latrine access does not ensure its use, there is little work that examines this relationship, due in part, to the difficulty in measuring latrine use behavior. The objective of this work is to create a proxy indicator for latrine use and test the association between estimated latrine use and sanitation facility. We use latent class analysis (LCA) with data from 253 individuals living in rural coastal Ecuador to model latrine use as a latent variable. The underlying probability of latrine use was predicted using 10 measured variables of attitudes, norms, and perceptions surrounding latrines. The LCA model did not show large uncertainty when predicting latrine use (entropy = 5.29). Using WHO/UNICEF Joint-Monitoring Programme definitions of household-level sanitation access, household access to an improved sanitation facility, relative to a unimproved facility, was regressed upon the latent variable of latrine use. The regression model accounted for household-level clustering and adjusted for individual-level educational attainment. Initial results show no association between access to a non-shared improved sanitation facility and the probability of latrine use (OR = 1.1, 95% CI = 0.60-2.1,  $p = 0.73$ ) when accounting for one's educational level. Further disaggregating access of improved sanitation between those that share and those that do not, this pattern holds: there is no association between shared, but improved household access and latrine use behavior (OR = 0.73, 95% CI = 0.25-2.2,  $p = 0.57$ ); and among non-latrine sharers with improved sanitation facilities, the association between improved sanitation access in the household and latrine use is null (OR = 0.90, 95% CI = 0.33-2.4,  $p = 0.83$ ). We show that latrine use can be quantitatively estimated through a set of proxy variables. Importantly, this analysis suggests that household-level access to sanitation infrastructure may be a poor proxy for one's behavior.

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### LONGITUDINAL IMPACT OF INTESTINAL PARASITES ON MICROBIOME DIVERSITY AND METAGENOMIC CHANGES IN CHILDREN FROM ECUADOR AND ARGENTINA

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Approximately 30% of children worldwide are infected with gastrointestinal (GI) parasites. Depending on the species, parasites can disrupt intestinal bacterial flora affecting nutritional status. We implemented a multi-parallel quantitative real-time PCR (qPCR) and whole genome sequencing analysis for bacterial microbiota and *Ascaris lumbricoides*, *Ancylostoma duodenale*, *Necator americanus*, *Strongyloides stercoralis*, *Trichuris trichiura*, *Cryptosporidium*, *Entamoeba histolytica*, and *Giardia lamblia*. Stool samples were collected from 122 asymptomatic children from rural Argentina and 85 children sampled at 3 and again at 5 years old from Ecuador. Separate analyses were done by country for uninfected, *Giardia* only, *Giardia*/helminth co-infections, and helminth only groups. For *Giardia* only infected children, longitudinal sequencing data showed a decrease in bacterial biodiversity compared to those uninfected that correlated with increasing *Giardia* burden (Shannon alpha diversity (*Giardia* only 2.1; uninfected 2.7,  $p = 0.0317$ ; Spearman  $r = -0.5491$ ,  $p = 0.0244$ )) within each age group but showed a significant increase in diversity from paired 3 to 5-year-old children ( $p = 0.01838$ ). In *Giardia* only infections, microbiome taxonomy shifted to *Prevotella copri*, with degree of shift related to intensity of infection compared to uninfected (43.2 % versus 12%,  $p = 0.012$ ). Abundance of *Prevotella copri* bacteria was decreased in the helminths only group, but increased for *Giardia*/helminth co-infections (12.5% versus 33.6%,  $p = 0.026$ ). Metagenomic analysis of the bacterial microbiota showed the proportion of vitamin B12 bacterial biosynthesis genes were diminished in the *Giardia* infected group compared to the non-infected group (0.98% to 1.89%, of total genes,  $p = 0.038$ ) with a compensatory increase in helminth only infected children. Our data provides possible evidence for an effect of parasitic infections on the intestinal environment allowing permissive growth of anaerobic bacteria such as *Prevotella*, decreasing in microbiota diversity and altering capacity of vitamin B12 biosynthesis.

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### A NATURAL MOUSE MODEL FOR CRYPTOSPORIDIOSIS

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Cryptosporidiosis is a leading cause of diarrhea and an important contributor to global infant mortality. There are no efficacious drugs or vaccines available and our knowledge of *Cryptosporidium* biology to drive their development is scant. *Cryptosporidium* research is greatly hindered by the lack of a continuous tissue culture system and poor animal models. To develop a more facile mouse model of *Cryptosporidium* infection, we have isolated a strain of *Cryptosporidium tyzzeri* (*C. parvum* mouse genotype I) from naturally infected *Mus domesticus*; strains which we now maintain continuously in our animal facility. De novo assembly of the *C. tyzzeri* genome shows 96% overall nucleotide identity to *C. parvum* and *hominis* and a high degree of synteny. The highest burden of *C. tyzzeri* infection is found in the distal ileum of the mouse small intestine, yet there is also significant infection of the jejunum and duodenum, similar to what is seen in human cryptosporidiosis. Perhaps of greatest importance, *C. tyzzeri* produces significant infections in healthy C57/BL6 mice. These infections produce high parasite burden but are self-limiting, and mice that have cleared *C. tyzzeri* appear resistant to future infection. Using CRISPR directed homology repair we have genetically engineered *C. tyzzeri* strains to express reporter genes for *in vivo* imaging and localization. In summary, we now have access to a natural mouse model that closely resembles the human infection in which both host and parasite are genetically tractable. We envision this model will lead to better understanding of cryptosporidiosis susceptibility, resolution, and subsequent protection in the context of a functioning immune system.

## CAREGIVERS AS A POTENTIAL SOURCE OF CRYPTOSPORIDIUM INFECTION IN KENYAN CHILDREN

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*Cryptosporidium* is a leading cause of diarrhea and linear growth faltering among children in sub-Saharan Africa. We aimed to determine whether caregivers are a potential source of *Cryptosporidium* infection. Between January-December 2015, Kenyan children aged 6-71 months of age presenting to two Nyanza province public hospitals with acute diarrhea, and their primary caregivers, provided stool samples for real-time PCR analysis. Univariate prevalence ratios (PR) estimating the association between child and caregiver *Cryptosporidium* infections were estimated using Poisson regression. Multivariable models adjusted for other potential transmission sources (unprotected water source, water improvement method, livestock ownership, and inadequate sanitation). To evaluate whether caregiver-child transmission was unique to *Cryptosporidium*, we also evaluated the association between child and caregiver *Giardia* infection accounting for other transmission sources. Among 212 child-caregiver pairs, 36% of children (median age: 25 months; interquartile range [IQR]: 12-38) and 23% of adults (median age: 24 years; [IQR]:22-28) had *Cryptosporidium* identified by PCR. Children with a *Cryptosporidium*-infected caregiver had a 1.7-fold higher prevalence of *Cryptosporidium* infection (95% confidence interval [CI]: 1.2, 2.4;  $p=0.006$ ). This relationship persisted after adjustment for other transmission sources (adjusted [a]PR: 1.7 [95%CI: 1.2, 2.4;  $p=0.004$ ]). Caregiver HIV-infection (12% of caregivers) was associated with child *Cryptosporidium* infection (PR: 1.7 [95%CI: 1.1, 2.6];  $p=0.009$ ) but not adult *Cryptosporidium* infection (PR: 1.0 [95%CI: 0.5, 2.2];  $p=0.9$ ). *Giardia* was commonly identified by PCR in children (54%) but less frequently in caregivers (14%); children of caregivers with *Giardia* were not more likely to have *Giardia* (aPR: 1.2 95%CI: 0.9-1.6),  $p=0.3$ ). There appears to be a unique relationship between caregiver and child *Cryptosporidium* infection that is unexplained by shared transmission sources. *Cryptosporidium* interventions may need to target both children and caregivers to achieve maximal benefit.

## NEW COMPOUND SERIES WITH POTENT AND SELECTIVE ACTIVITY AGAINST GIARDIA DUODENALIS

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*Giardia duodenalis* infects a wide array of hosts and is the most frequently reported human intestinal parasite. On an annual basis this parasite is responsible for ~1 billion human infections of which >200 million result in symptomatic disease. While infections are more prevalent in the developing world, they are ubiquitous. In developed countries the prevalence of giardiasis is ~7%. However rates are higher in sub-populations where the burden is primarily borne by children (>60% in Australian Aboriginal children). *Giardia* infection can result in severe and chronic disease. There is also evidence that it is linked to post-infection disorders including irritable bowel syndrome. However, treatment options are inadequate. The frontline drug, metronidazole (MTZ), is associated with side-effects and drug resistance. It is also distasteful and must be taken in multiple doses over 5-7 days, factors which result in poor compliance, treatment failure, rapid re-infection and parasite resistance. There is also increasing evidence that MTZ has a collateral effect on the host microbiome. New drugs that are effective against *Giardia* parasites

at compliant doses while having limited activity against host cells are needed. To improve giardiasis treatment options in the long-term we have developed a high-content imaging assay to assess the activity of compounds against trophozoites *in vitro*. This assay permits the direct assessment of parasite growth and morphology at multiple time-points and has been validated using control compounds. We have recently used this assay to screen compounds from the Compounds Australia Open Access Scaffold Library for anti-*Giardia* activity (2451 compound subset; 2/scaffold; Z-factor 0.75) and after rational selection based on activity, novelty, and chemical liabilities have identified three compound series with potent (IC<sub>50</sub>≤1μM) and selective activity against *G. duodenalis*. These compound series have been chosen as starting points of new anti-*Giardia* drug development. Significantly, the most promising compound identified is 290-fold more potent than MTZ and has *Giardia* vs human selectivity index of >8000.

## HOUSEHOLD TRANSMISSION OF CRYPTOSPORIDIOSIS IN BANGLADESH

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Diarrheal illness a leading killer of children under five years old worldwide, and *Cryptosporidium spp.* is a top contributor. *Cryptosporidium spp.* are apicomplexan protozoa, spread by fecal-oral contamination, with a very low infectious dose. As there is no vaccine and no approved drug for *Cryptosporidium spp.* in young children, a focus on prevention of infection is critical. We undertook a pilot case-control study to define the extent of person-to-person transmission of cryptosporidiosis within families in an urban (Mirpur) and rural (Mirzapur) community in Bangladesh. We enrolled 43 case families with a *Cryptosporidium*-infected child aged 6-18 months. Controls were age-sex matched *Cryptosporidium*-negative children (n=12). Once children were identified, we enrolled all household members (defined as persons sleeping under the same roof or eating from the same cooking pot). We then followed these individuals for 8 weeks, with weekly surveillance stools and testing with qPCR for *Cryptosporidium spp.* In the 43 case families, the rate of secondary infections with *Cryptosporidium* was 18.6% (22/118) compared to 0 new infections (0/35) in the 12 control families. In the 22 urban Mirpur households, the secondary attack rate was 30% (18/60) in cases compared to 0% (0/14) in controls (chi-square  $p = 0.018$ ). In contrast, in the 21 rural Mirzapur households, the secondary attack rate was 6.9% (4/58) in case households compared to 0% (0/21) in controls (chi-square  $p = 0.22$ ). In Mirpur, in 8 of 22 case families, multiple family members were infected at baseline, suggesting concurrent infections in household members may be commonplace. Person-to-person transmission is likely a major source of *Cryptosporidium* infection, and more important than environmental reservoirs, for children living in urban slums in Mirpur, Bangladesh. In rural households, there was more attenuated person-to-person transmission. Interventions aimed at interrupting anthroponotic spread of *Cryptosporidium* in children are needed to control transmission of this deleterious parasite.

## SEASONAL VARIATION OF CRYPTOSPORIDIUM GENOTYPES IN BANGLADESH

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Cryptosporidiosis is one of the top 5 causes of diarrhea in the first 2 years in the life in low-income countries. *Cryptosporidium hominis* positive stool samples have been collected from our on-going longitudinal study of infants from an urban slum of Dhaka, Bangladesh. This study surveys children in a well-defined population at monthly intervals with clinical and laboratory characterization of all diarrheal episodes. Combining molecular typing and clinical data will allow us to determine the frequency of specific subtypes in times of high and low transmission. DNA was extracted from all collected fecal samples and *Cryptosporidium* positive samples identified using a qPCR assay that can detect a broad range of *Cryptosporidium* species. Species specific assays were then used to identify infections with the three species common at our study site. Subgenotyping was performed using a polymorphic region within the GP60 gene. The subtypes were determined and associated with clinical symptoms and date of collection. In 86.6% of the cases, species subtyping was successful. Sixteen different subtypes were identified in the Bangladesh cohort. The most abundant subtypes in our population were *C. hominis* IaA18 (16.3%), IbA9G3R2 (16.3%), IaA11G3T3\_V (15%), IaA19 (13.2%), IdA15G1 (12.8%), and IaA25 (6.6%). The remaining 19.8% of the population were subtypes IbA9G3R2\_V, Bang\_IaA13G1, IaA27, IaA14, IIdA13G1, IlcA5G3R2, IaA26, IfA16G1, IaA22, IaA11G3T3. *Cryptosporidium* infections markedly increase during the rainy season (early June-late September of 2015 and 2016). While types Ia and Ib occur year round, they are more prevalent during the rainy seasons. We observed that type Id was only present during the first rainy season while type Ie was exclusively present beginning in mid-April of 2016, becoming abundant during the following rainy season. *Cryptosporidium* infection is much more prevalent during the rainy season than the winter or summer within our cohort. Longitudinal study of population genetics can help us better understand the genetic diversity, transmission, and rate of infection in the next rainy season.

## DEVELOPMENT OF DRUG CANDIDATES FOR CRYPTOSPORIDIOSIS TARGETING THE CRYPTOSPORIDIUM METHIONYL-TRNA SYNTHETASE

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Enteric infection by the protozoan parasite *Cryptosporidium* is the cause of an estimated 7.6 million cases and 200,000 deaths annually among children under two years old in low-resource settings. Chronic infections contribute to malnutrition, growth stunting, cognitive impairment, and oral vaccine failure. Current treatment options are extremely limited, with only one US FDA-approved drug that has poor efficacy in malnourished children. To address this issue we are developing inhibitors against a novel drug target, the *Cryptosporidium parvum* methionyl-tRNA synthetase (CpMetRS). This enzyme is essential for protein synthesis and differs substantially from the human MetRS orthologues. From an in-house library of MetRS inhibitors over 200 compounds were tested for activity

on the CpMetRS, and 55 compounds demonstrated IC50 values of <40 nM (the lower limit of detection in the enzyme assay). Nineteen of these compounds had EC50 values against *C. parvum* cells in culture of <1 µM including three below 0.1 µM. Selected compounds were tested in a murine model of *Cryptosporidium parvum* infection using NOD-SCID-gamma mice with the best compound (#2093 dosed at 50 mg/kg orally twice per day) demonstrating 98.6% reduction in stool oocysts measured by PCR. The results were confirmed in the interferon gamma knockout mouse model employing luciferase expressing parasites showing a 4-log decrease in stool parasite load following treatment, and comparable to the most potent available positive controls. The compounds do not show toxicity in mammalian cells nor in mice at the doses tested. The compounds represent promising drug candidates to target diarrheal disease caused by *Cryptosporidium* parasites.

## SCOPING REVIEW ON IDENTIFYING GLOBAL KNOWLEDGE GAPS IN ACUTE FEBRILE ILLNESS SURVEILLANCE

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Acute febrile illness (AFI), a common clinical syndrome among people seeking medical care globally, represents a spectrum of infectious disease etiologies with important variations geographically and by population. Yet, there is no methodological standardization on AFI surveillance, limiting interpretation of data in a global context. We conducted a scoping review to characterize current AFI research methodologies, identify global research gaps, and inform the future direction of AFI surveillance standardization. We searched Medline, Embase, and Global Health, for publications from 1 January 2005 - 30 April 2016; search terms included "undifferentiated fever," "acute febrile illness," and "non-specific fever" with proximity and Boolean operators. After identifying and reviewing 948 abstracts, 779 (82.2%) studies were excluded as they did not identify AFI etiology, or were modeling studies, clinical trials, lab procedures or economic impact evaluation, single case reports, and outbreak investigations. A full review conducted on the remaining 169 studies used a standardized tool to abstract AFI surveillance-related data. There was variability in study objectives, terms describing febrile illness, and case definitions. Studies originating in three countries made up 54 (31.9%) of the 169 articles. Of the 52 pathogens reported on in the 169 articles, 38 (73.1%) were investigated in fewer than 10 studies. Pathogens represented most commonly across the 169 studies were: Dengue (66 studies, 39.1%), *Leptospira* spp. (46, 27.2%), and *Plasmodium* spp. (43, 25.4%). AFI studies focused on single pathogens made up 93 of 169 (55.0%). Knowledge gaps in causes of AFI and their regional distribution limit quality of care and public health intervention effectiveness, especially in resource-limited settings where clinical management and disease control rely on knowing the proportional contribution of AFI etiologies. Wider implementation of standardized AFI surveillance with multi-pathogen disease detection is needed to improve knowledge of the range of underlying etiologies and their relative contributions to the global AFI burden.

## MAPPING CHILDHOOD DIARRHEA IN AFRICA

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Across Africa, diarrheal diseases are the 3rd leading cause of mortality and morbidity in children under the age of five. In this decade, there has

been, in aggregate, a reduction in death and disability-adjusted life-years (DALYs) associated with diarrheal diseases; however, these gains vary substantially from country to country as well as within countries. In 2013, UNICEF and the WHO set forth goals to reduce childhood mortality from diarrhea to 1 per 1000 live births and reduce severe diarrhea in children by 75% compared to 2010 levels. Here, leveraging over 50,000 geo-located household survey clusters, Bayesian geostatistical techniques, and the existing machinery of the Global Burden of Disease, we produce high-resolution spatio-temporal estimates of diarrheal prevalence, incidence, and death. Half-way to their 2025 deadline, the goals identified by UNICEF and the WHO in reducing childhood death and severe disease attributable to diarrhea appear achievable (or even achieved) in many regions of Africa, but difficult to achieve in other regions given progress so far. Targeted interventions are necessary to focus resources on locations where gains will be highest and as interventions reap diminishing returns, health professionals must be flexible enough to adapt to face the remaining challenges. Understanding at a scale where policy can act where these challenges remain is but the first step in reducing preventable child deaths from diarrhea.

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### ASSESSING THE NON-BIOLOGIC CONTRIBUTORS TO MORTALITY AMONG INPATIENTS WITH FEBRILE ILLNESS IN TANZANIA: A PROSPECTIVE COHORT SOCIAL BIOPSY STUDY

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Infectious diseases account for over half of annual deaths in Tanzania despite the existence of effective biomedical treatments. To quantify the impact of sociocultural and health systems factors on mortality from infection, we adapted the social autopsy framework for a prospective 'social biopsy' cohort study. From June 2015 to June 2016, we enrolled consecutive pediatric and adult febrile inpatients at two referral hospitals in Moshi, Tanzania. We interviewed participants to determine the burden of the 'Three Delays' in receiving health care: delays at home, in transport, and at health facilities. We assessed six-week mortality and matched fatal cases with non-fatal controls. We created two matched sets, one matched on age and gender for analyzing home and transport delays and one matched on age, gender, and severity of illness for health facility delays. We used chi-squared and Student's T tests to compare delays between cases and controls. We enrolled and obtained follow-up for 475 children, of whom 18 (3.8%) died, and 260 adults, of whom 34 (13%) died. The median age of participants was 2.9 (IQR 0.9-26.7) years and 326 (44%) participants were female. For home delays, there were no significant differences in the pediatric cohort, but adult cases took longer than controls to seek care outside the home (4.0 vs 2.9 days;  $p=0.038$ ). Twelve (35%) adult cases failed to recognize a severe symptom at home compared to 20 (17%) of 121 controls ( $p=0.017$ ). For health facility delays, cases were more likely to visit >2 facilities during their illness, with 7 (39%) pediatric cases versus 30 (18%) of 139 controls ( $p=0.038$ ) and 16 (47%) adult cases versus 7 (15%) of 48 controls ( $p=0.001$ ). Pediatric and adult cases reported more time between first facility presentation and study enrollment than controls (ped: 8.8 vs 3.5 days;  $p<0.001$ ; adult: 10.0 vs 4.4 days;  $p=0.027$ ). Sankey diagrams of care pathways show that cases were less likely to reach tertiary care within their first two facility visits

(ped:  $p=0.038$ ; adult:  $p=0.001$ ). Our findings suggest that delays at home and at health facilities are risk factors for mortality from febrile illness in northern Tanzania.

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### POPULATION ACCESS TO ITN IS A BETTER INDICATOR OF 'UNIVERSAL COVERAGE' THAN HOUSEHOLD OWNERSHIP OF AT LEAST ONE ITN FOR TWO PEOPLE

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The World Health Organization currently defines universal coverage as the proportion of households that own at least 1 ITN for every 2 household members. WHO also uses an indicator of population access to ITN to describe coverage; both targets are set at 80%. Many countries have implemented mass ITN campaigns expecting to reach 80% household universal coverage, but surveys conducted shortly after high quality campaigns show while population access to ITN can be relatively close to target levels, household universal coverage is consistently well below target. Eighty datasets from 37 countries in sub-Saharan Africa were used to explore the causes of this low performance and discrepancy between indicators. The proportion of households with at least 1 ITN for 2 people (enough ITNs) and population access to ITN were calculated. The proportion of households with enough ITNs did not exceed 60% at the national level, except in Uganda's 2014 MIS. Population access to ITN was systematically higher than household ownership of enough ITNs (coef 1.45;  $p<0.001$ ;  $R^2=0.84$ ). Regional estimates for these two indicators were calculated in three datasets in which fieldwork occurred shortly after mass campaigns. In these 46 regions, the proportion of households with enough ITNs ranged from 31%-48% in Mali, 26-81% in Tanzania, and 46-76% in Uganda, while population ITN access ranged from 63-78%, 52-91%, and 67-90%, respectively. Large households were found to drive low rates of enough ITNs and controlling for the level of population access, increasing mean household size was associated with a 3.0 percentage-point reduction in proportion of households with enough ITNs for each additional person ( $p<0.001$ ;  $R^2=0.94$ ). At 80% population ITN access, the proportion of households with 1 ITN for 2 people will be only 65%, because individuals in households with some but not enough ITNs are captured as having access, but the household doesn't qualify as having 1 ITN for 2 people. Population access to ITNs, because it is based on people as the unit of analysis, provides a better indication of ITN protection, and should be considered as the better indicator of "universal coverage".

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### SOCIAL BEHAVIOR CHANGE COMMUNICATION AND BEDNET RETENTION, CARE, REPAIR, USE AND IMPACT IN BENIN

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The use of durable, long-lasting insecticide treated nets (LLINs) is key to tackle malaria in Benin. In 2014, the President's Malaria Initiative (PMI) supported PADNET with an experimental random cluster study to increase net durability in a 900 household (HH) sample in Seme Podji commune, distributing one LLIN (PermaNet 3) per HH. The sample was split into 3 groups (arms) of 300 HH: arm1 received only Social Behavior Change Communication (SBCC); arm2 received SBCC and a net repair kit; and

arm3 was the control. SBCC consisted of monthly HH visits by Care Group Leaders, and community dialogues through interpersonal communication to promote continuous net use, reducing damage and repairing nets. For monitoring purposes, all HHs were visited every four months over a 33 month period. Data was collected using android tablets through Open Data Kit software. Databases were developed and analyzed using Excel pivot tables and SPSS 17. Results show that for net loss due to physical damage beyond repair as stated by net owners, the retention rate for arm1 was 90%, 94% for arm2 and 75% for arm3 ( $p < 0.0001$ ). Net washing more than once per trimester was more frequent in arm3 (60%) than arm1 (37%) and arm2 (30%) ( $p < 0.0001$ ). The % of nets with a proportional Hole Index (pHI) of 0 (no/few tiny holes) in arm3 was 20% while arm1 had 64% and arm2 had 75% ( $p < 0.0001$ ). The % of nets with a pHI>64 (not deemed a good barrier), was 38% in arm3, while arm1 had 4% and arm2 had 2%. The % of ever-damaged LLINs with signs of repair in arm3 was 34%, while arm1 had 95%, and arm2 had 98%  $p < 0.0001$ . The proportion of children <5 that slept under any LLIN with pHI=0 was 21% in arm3, 62% in arm1 and 55% in arm2. The proportion of <5 sleeping under a net deemed as a good barrier (pHI<65) was 51% in arm3, 88% in arm1 and 73% in arm2 ( $p < 0.0001$ ). The relative risk of developing fever in the fortnight prior to the survey associated with the lack of SBCC in arm3 was 1.9 ( $p < 0.001$ ), suggestive that SBCC cut fever risk by half via improved net integrity and continuous net use. SBCC for net use and preservation should be promoted in order to improve net durability in the protection against malaria

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### EFFECTIVENESS AND SUSTAINABILITY OF A COLLABORATIVE IMPROVEMENT METHOD TO INCREASE THE QUALITY OF ROUTINE MALARIA SURVEILLANCE DATA IN KAYUNGA DISTRICT, UGANDA

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Improving malaria surveillance data quality in Africa is a priority. We evaluated the effect of collaborative improvement method (CIM), an innovative quality improvement method, to increase malaria data quality in 5 health facilities (HFs) in Kayunga District, Uganda. Intervention began in November 2015 with in-service training, plan-do-study-act cycles to test changes by HF CIM teams, learning sessions, and CIM coaching. To assess data completeness and accuracy, we abstracted data from 156,707 entries in outpatient department (OPD) and lab registers and aggregate monthly reports (MR) for 12 months pre-intervention, 10 months of intervention, and 4 months of sustainability assessment. Monthly OPD register completeness was measured as the proportion of malaria patient entries with: 1) all data fields completed, and 2) clinically-relevant fields completed. Accuracy was calculated as the relative difference between: 1) monthly malaria patients in OPD registers versus MR, and 2) proportion of positive malaria tests in lab registers versus MR. Data were analyzed as interrupted time series with segmented linear regression modeling. Ethnographic observations and interviews with health workers were used to describe the context and inner processes of CIM. Baseline completeness for all data fields ranged from 0–19%, with an immediate increase by 71 %-points (95% CI: 62–81%) post-intervention to 85%. Completeness of clinically-relevant fields (17–34% at baseline) improved by 69 %-points (95% CI: 65–72%) immediately post-intervention to 98% and remained high for the duration of follow-up. Relative differences ranged from 3–21% for reporting malaria patients and 9–57% for the proportion of positive malaria tests at baseline, and only the latter improved with CIM (relative change of 77 %-points; 95% CI: 35–120%). All improvements were sustained 4 months post-CIM. Qualitative findings indicated that

healthcare hierarchy, financial incentives, and the relationship with the CIM mentor were key determinants of success. If these positive results are confirmed in larger studies, CIM may be a useful approach to improve malaria surveillance.

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### ASSOCIATION BETWEEN INCREASING MALARIA CONTROL INTERVENTIONS AND REDUCTIONS IN STUNTING CHILDREN 6-59 MONTHS OF AGE: A MULTI-COUNTRY DECOMPOSITION ANALYSIS

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Malaria prevention is one essential element of successful child health programs in malaria endemic countries. Malaria impacts child health through direct and indirect pathways. Directly, malaria infection in pregnant women and in children can lead to severe anemia, premature birth, low birth weight and ultimately to death. Indirectly, repeated or continuous malaria infection can contribute to chronic inflammation limiting childhood growth and causing impaired physical and cognitive performance and a reduction in years of healthy life. To investigate the contribution of increasing coverage of malaria control interventions on stunting in children less than five years of age we analyzed data from 15 countries that had two or more demographic health surveys (DHS) conducted between 2005 and 2016 with data on insecticide-treated net (ITN) use and anthropometric measurements of children under five. Multivariate Oaxaca-Blinder decomposition for nonlinear response models with deviation contrast normalization for categorical variables were used to estimate the proportion of the decline in stunting prevalence over time due to increases in malaria control interventions. ITN use increased from 36% in baseline surveys to 46% in endline surveys and stunting decreased from 38% to 35%. Although significant in bivariate models, in pooled, multi-country logistic regression models controlling for socioeconomic and child and maternal health variables and malaria transmission risk, odds of stunting were not significantly lower for children who used an ITN the night before interview compared to those who did not. Decomposition models reveal that the increase in ITN use between baseline and endline surveys accounted for an insignificant proportion of the decrease in stunting. Changes in vitamin A coverage explained the greatest proportion of the total change in stunting between baseline and endline as compared to other covariates. Results suggest that the scale-up of ITN use are unlikely to have had a measurable impact on stunting.

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### A MAJOR LOCUS ON CHR. 1 DETERMINES CERCARIAL SHEDDING TIME IN OMANI SCHISTOSOMES

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The timing of cercarial shedding from schistosome infected snails is critical for successful transmission. Most *Schistosoma mansoni* populations that infect humans shed cercariae larvae in late morning, while parasite populations that primarily infect rats shed cercariae in late afternoon or night. As reported previously, dramatic variation in shedding time in Oman, with parasites collected from rats shedding cercariae nocturnally (~8pm) while parasites from humans shed cercariae during the day (11am). This project was designed to understand the genetic basis for nocturnal shedding. We conducted reciprocal genetic crosses between nocturnal and diurnally shedding Omani schistosomes, determined cercarial shedding profiles in parents, individual F1 and F2 (n= 65 in each cross) progeny. We then sequenced exomes of parasites from each cross, revealing 5295

and 6657 SNPs that were fixed for alternative alleles in the two crosses and therefore fully informative. We used linkage mapping approaches to determine the genome regions underlying this trait. We found a strong quantitative trait locus on chr. 1 (LOD = 6.1) and a secondary peak on chr. 6. The chr. 1 peak contains a compelling candidate locus (*Hes-1*) that encodes a transcription repressor known to influence cell proliferation, embryogenesis and developmental timing in *Drosophila*. In future work, we will exploit the growing functional genetics toolbox for schistosomes to determine the *loci* underlying this trait and the mechanisms underlying timing of cercarial release. Our central goal is to understand, at the molecular level, a key parasite trait critical for transmission and host specificity.

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### COMPARISON OF THREE METHODS TO EVALUATE THE BURDEN OF INFECTION BY *FASCIOLA HEPATICA* IN SHEEP FROM AN ENDEMIC AREA

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Fascioliasis caused by *Fasciola hepatica* mainly affects liver of ruminants from endemic areas. Diagnosis of this disease is based on the eggs detection by coproparasitological evaluation; however, its performance is influenced by the burden of infection and the stage of the parasite. On the other hand, imagenological techniques have been used to evaluate the damage produced by the parasite and could be used as a proxy to determine the burden of disease. We compared results obtained by coproparasitological and imagenological evaluation using ultrasound and computed tomography (CT) scan of 50 sheep from an endemic area. The preliminary results from 10 sheep were presented in the current abstract, and the complete results including all sheep will be presented at the meeting. Coproparasitological evaluation was done by direct observation of fecal sample after sedimentation; ultrasound evaluation was performed using convex probe; and computed tomography (CT) evaluation was performed using a spiral CT scan; animals were anesthetized for CT procedure. A positive result for imagenological evaluation was the presence of a parenchymal calcification; and agreement between evaluations was determined by Kappa test. Coproparasitological evaluation was positive in 90% (9/10) sheep; 5 of them had a low burden infection (100 - 167 eggs per gram). Ultrasound evaluation found calcification in 60% (6/10) of sheep; whereas CT scan revealed calcification in 90% (9/10) sheep. In terms of burden of infection, sheep with low burden infection showed a higher number of calcification (more than 20 calcifications). Fair agreement (K=0,286) was found between coproparasitological and ultrasound evaluation, as well as between ultrasound and CT evaluation (K=0,286); and poor agreement was observed between coproparasitological and CT evaluation (K=0,111). Despite that we expected a good correlation between burden and number of calcifications, this was not observed in this preliminary evaluation. Results obtained support the high correlation between presence of at least one calcification and a positive result in coproparasitological evaluation.

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### MONITORING AND MEASURING SCHISTOSOMIASIS AT TRANSMISSION SITES IN KENYA: SENTINEL MICE COUPLED WITH GENOTYPING OF RECOVERED ADULT WORMS

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We lack quantifiable and spatially and temporally explicit estimates of the force of schistosome transmission from snails to their definitive hosts in natural transmission *foci* in Kenya. To address this problem, we have devised floating cages to expose sentinel mice and collect individual adult worms via perfusion for genotyping. With this approach, we can determine if some localities are more potent transmission *foci* than others, infer rates of acquisition of adult worms including at different times of the day or year, and learn if mice become infected with worms representing relatively few genotypes or if extensive mixing of genotypes occur. We can also learn how long some snails may persist in shedding cercariae in natural habitats. A preliminary study involving habitats along the shore of Lake Victoria and along Asao stream in west Kenya revealed that mice placed in the water between 10am-2pm were considerably more likely to become infected, coinciding with snail peak shedding times. Genotype clones were found across time points, suggesting that cercariae shed from one snail were in the water for up to 8 hours. Subsequent experiments were done along the course of Asao stream in a 7-week-long fine time scale and a year-long course time scale. In all experiments, a male-biased sex ratio was observed. The total number of worms acquired per mouse varied considerably among sites with transmission proving to be focal, but was as high as 1 worm per minute at Asao stream. Approximately 60% of mice had bisexual infections (at least one male and one female). A similar number of unique genotypes were seen at Asao stream and Lake Victoria but the average number of genotypes per mouse was almost 2-fold higher at the lake. At Asao stream, microhomogeneities were observed with identical genotypes found between sites. In total, we genotyped nearly 1,500 adult *S. mansoni* worms from sentinel mice experiments at Lake Victoria and Asao stream. This approach provides new insight into transmission dynamics that can be used to provide rate parameters in developing transmission models and guiding treatment programs.

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### WHY DOES OXAMNIQUINE KILL *SCHISTOSOMA MANSONI* BUT NOT *S. HAEMATOBIIUM* OR *S. JAPONICUM*?

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The major species of *Schistosoma* affecting humans are *S. mansoni*, *S. haematobium*, and *S. japonicum*. There is currently only one method of treatment (monotherapy), the drug Praziquantel. Constant selection pressure through mass chemotherapy - this year will see the administration of over 250 million doses - has yielded evidence of resistance to PZQ. Previous treatment of *S. mansoni* included the use of oxamniquine (OXA), a prodrug that is enzymatically activated in *S. mansoni* but is ineffective against *S. haematobium* and *S. japonicum*. The OXA activating enzyme was identified by our laboratories as being a sulfotransferase (SmSULT). One focus of this research is to understand why OXA does not kill *S. haematobium* or *S. japonicum* and with this information reengineer OXA to be effective against *S. haematobium* and *S. japonicum*. An alignment of the sulfotransferases (SULT) shows that SmSULT, ShSULT and SJSULT share considerable sequence identity (71% Sm/Sh; 58% Sm/Sj, and 58% Sh/Sj) and predicted structural similarity. We sought to understand how differences in the amino-acid composition of Sm-, Sh-, and SJSULTs gave

rise to species-specific drug action. Using site-directed mutagenesis, we demonstrated that SmSULT modified to look like ShSULT and vice versa each could activate OXA in an *in vitro* assay ie the SULTs were functional. We next evaluated the transcriptional differences between the SULTs by qPCR and Digital PCR. SmSULT transcription was 100X ShSULT and 1000X SjsULT. The differences in transcription account in part for the inability of OXA to be cidal in Sh and Sj. Next we employed an iterative process which lead to the identification an OXA derivative (CIDD790) that is effective against *S. mansoni* (100% killing), *S. haematobium* (80% killing) and *S. japonicum* (80% killing). These results demonstrate that understanding the mechanism of action of a drug and its structure function relationship can lead to novel drugs.

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### IMPACT OF DIFFERENT TREATMENT STRATEGIES OVER FIVE YEARS FOR SCHISTOSOMIASIS IN MOZAMBIQUE

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Schistosomiasis is highly endemic across Mozambique (overall prevalence of 47%), in particular the northern provinces. The recommended treatment strategy for schistosomiasis is preventive chemotherapy (PCT) with praziquantel, where frequency depends on the prevalence of infection among school-aged children. As recommended by the WHO, the treatment strategy usually implemented is school-based (SBT), however, in highly endemic areas community-wide treatment (CWT) may be necessary. Studies have found CWT in addition to SBT very effective, as often not all the children in a village attend school and CWT can help to reach them. The aim of SCORE is to provide an evidence base across several countries for programmatic decisions on how best to gain and sustain control of schistosomiasis. In Mozambique specifically, the goal was to understand the impact of alternative treatment strategies in a study area with prevalence of >21% urogenital schistosomiasis (*Schistosoma haematobium*) infection by hemastix. This was a cluster-randomised trial that compared a total of 150 schools in Cabo Delgado province. Each village was randomised to one of six possible combinations of CWT, SBT or drug holidays over four years. In each village two urine filtrations were carried out on 100 children aged 9-12 years, 100 first year students (5-8 years) and 50 adults (20-55 years) at baseline (2011) and the final year (2016). In total, data was collected from 81,167 individuals. Results showed *S. haematobium* was highly endemic throughout all schools, where many schools had 90% baseline prevalence. Praziquantel treatment resulted in a significant reduction in the prevalence of *S. haematobium* infection as well as the proportion of heavy infections over five years. Both gender and age had a significant effect, with higher prevalence and proportion of those heavily infected seen in males and among adolescents (9-12 years). There was no significant variation *S. haematobium* prevalence between the most intense treatment (CWT each year) and the least intense (SBT every two years). These results have a significant logistical and cost implications for a national control program.

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### CONTROLLED HUMAN INFECTION WITH SINGLE-SEX *SCHISTOSOMA MANSONI* CERCARIAE

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Controlled human infections (CHI) provide a core platform to accelerate the development of novel drugs and vaccines for infectious diseases and reduces the risk of failure in downstream clinical development. For schistosomiasis, no such model exists. To develop a CHI model for schistosomiasis we have taken the important conceptual step to produce single-sex *Schistosoma mansoni* cercariae according to regulatory requirements for human use. Single-sex worms cannot deposit eggs and consequently pathology is avoided when infecting healthy volunteers. We developed a multiplex real-time PCR to determine cercaria gender. In addition, we characterized the bioburden associated with a dose of male cercariae and produced the cercariae under highly controlled conditions. We now report the first data of a dose-escalating clinical trial to assess the safety, tolerability and infectivity male *Schistosoma mansoni* cercariae in a controlled human infection model. We exposed groups of three volunteers each to 10 and 30 male *Schistosoma mansoni* cercariae and present the safety data of these groups. Infection with 10 cercariae was well tolerated with mild adverse events mainly related to the skin penetration of the parasites. No serious adverse events occurred. Serum circulating anodic antigen (CAA) levels were measured in weekly samples and peaked at 6-8 weeks following infection. Peak CAA levels were close to the detection limit of the CAA lateral flow assay. All volunteers were treated with 40mg/kg praziquantel and CAA levels were followed over time. A second group was infected with 30 cercariae. Results from this group are still pending. In conclusion, we have established a male *Schistosoma mansoni* controlled human infection model. Initial results show that this model is safe and leads to detectable CAA levels in serum which can be followed over time. Future studies will focus on establishing a female controlled infection model as well as performing reinfection studies.

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### GENOME SCALE APPROACHES TO UNDERSTANDING THE PERSISTENCE OF SCHISTOSOMIASIS IN RESIDUAL TRANSMISSION HOTSPOTS

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In China, public health officials are attempting to interrupt transmission of schistosomiasis. However, schistosomiasis has reemerged and persisted in some areas despite aggressive control efforts, for reasons that are not well understood. We are using a two pronged approach that incorporates field-based epidemiological surveys and next-generation sequencing to understand why schistosomiasis persists in residual transmission hotspots, and how control programs might be modified to attain permanent disease reductions. We followed two counties from 2007 through 2016 in Sichuan, China where schistosomiasis previously reemerged in order to evaluate infection patterns following reemergence. Residents in study villages were tested for *Schistosoma japonicum* infection, *S. japonicum* miracidia were archived, and demographic and geospatial data were collected. Approximately 100 miracidia were whole-genome amplified and sequenced using double digest restriction site associated DNA sequencing (ddRADseq), an affordable next generation sequencing method that

reliably samples 10,000-30,000 *loci*. Using these data, we estimated schistosome relatedness within and between hosts, and evaluated parasite population structure over space and time. We found schistosomiasis infection prevalence exceeded 10% in several villages despite ongoing disease control programs (12/36 villages in 2007, 10/36 in 2010 and 3/10 in 2016). There was strong evidence for population structure by village. We identified probable schistosome siblings within the same host at different timepoints, suggesting some individuals may harbor the same adult worms over time despite referral for treatment. It is unclear whether these retained infections are due to non-compliance with treatment, sub-optimal dosing, or drug resistance. Collectively, our findings suggest a role for local sources in sustaining local transmission hotspots and raise questions about the efficacy of treatment. More generally, our findings demonstrate the potential to integrate field-based studies of schistosomiasis and high-resolution genomic methods to advance disease control efforts.

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### POST-DENGUE ACUTE DISSEMINATED ENCEPHALOMYELITIS: A NEW CASE REPORT, SYSTEMATIC REVIEW, AND META-ANALYSIS

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Dengue fever (DF) is a debilitating flavivirus disease which can spread by *Aedes aegypti* mosquitoes. Acute disseminated encephalomyelitis (ADEM) in dengue is very rare and it may occur during the dengue infection acute phase or post-infectious phase. The aim of this study was to systematically review and meta-analyze ADEM and to represent a new case. We retrieved the articles from nine databases. We used Fisher's exact and Mann-Whitney U tests. Classification and regression tree (CART) was used to find the predictors of the DF outcomes. A 13 years old girl was admitted to the hospital due to fever. The examinations found no focal neurologic deficits. She lost the urination sensation and have urinary retention. The neurological examinations revealed that she became lethargic and quadriplegic. She had upper limbs weakness and lower limbs complete paraplegia. Her alertness gradually promoted. Although urinary retention remained, her muscle strength of upper and lower extremities increased. Her limbs weakness was significantly improved after two weeks of oral prednisolone then sphincter function was back to normal. She was nearly intact with the proximal part of her legs had a mild weakness in discharge. Our meta-analysis revealed that the prevalence of ADEM among DF patients, of all neurological disorders among dengue and of ADEM among neurological disorders were 0.3% [0.1 - 0.9%], 2.6% [1.8 - 3.8%], and 6.8% [3.4 - 13%], respectively. The most frequent manifestation of ADEM was altered sensorium/consciousness (58%). There was a significant difference between cases having bad outcomes, including partial recovery or death, or complete recovery in the neurological manifestations onset, being earlier, and in temperature, being higher, in cases having bad

outcomes (P-value < 0.05). This was confirmed by CART which included these two variables. Our findings suggest that the prevalence of ADEM among DF and other DF-related neurological disorders are not too rare. The high fever of ADEM cases at admission and the earlier neurological manifestations onset are associated with the DF bad outcomes.

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### SECONDARY HETEROTYPIC DENV INFECTION OF DIFFERENT DENV GENOTYPES IN MARMOSETS

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Marmoset, a new world monkey, has been proved useful as an animal model for primary and secondary dengue virus infection. However, there have been limited data on heterologous infection of different DENV genotypes. In this study, 9 marmosets that had been previously inoculated with DENV-2 were used. During the secondary DENV-1 infection experiment, 4 marmosets were inoculated with clinical isolates of DENV-1 genotype I and 5 marmosets were inoculated with DENV-1 genotype IV. We found that all the marmosets developed viremia by day 2 post-inoculation and peak viremia was observed at day 4 post-inoculation. The mean viremia duration is 5.3 days (GI= 5 days and GIV= 5.6 days). There were no differences in the viremia levels between the 2 genotypes groups. The marmosets inoculated with G1 and GIV also exhibited high levels of neutralizing antibody in secondary infection. Three marmosets exhibited mild hemorrhagic manifestation in the form of petechiae on day 4, day 10, and day 14 post-inoculation. The antibody response in marmosets also similar as the antibody response during DENV infection in humans. We confirmed that the marmoset model is a suitable animal model to study dengue virus infection.

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### TRANSIENT MONOCYTOSIS SUBJUGATES LOW PLATELET COUNT IN ADULT DENGUE PATIENTS

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Dengue is one of the most important vector-borne human viral diseases globally. The kinetic changes of hematological parameters of dengue in adult Taiwanese patients have seldomly been systematically investigated and characterized. Serial laboratory data of 1,015 adult patients who were diagnosed with dengue virus serotype 2 (DENV2) and 3 (DENV3) infections in southern Taiwan were retrospectively examined. Prominent parameters were verified with specimens from a 2015 dengue outbreak. Higher absolute monocyte counts on day 5 in severe patients than mild fever subjects after the onset of fever was seen. The absolute number of monocytes was significantly greater in those with DENV2 than DENV3

infections in spite of subtle differences in laboratory tests. Platelet counts were lowest and activated partial thromboplastin time was highest on day 5 in patients with severe conditions. In addition, sudden downward platelet counts corresponding to a transient surge of monocytes on day 4 onward was observed. Fluorescence-activated cell sorting analysis of peripheral blood mononuclear cells obtained from acute dengue patients and experimental investigations revealed that phagocytic effects of innate immune cells contribute to thrombocytopenia in dengue patients. Innate phagocytic cells play an essential role in low platelet counts in adult patients with dengue virus infections.

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### HOUSEHOLD COSTS OF HOSPITALIZED DENGUE ILLNESS IN SEMI-RURAL THAILAND

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Dengue-related illness is a leading cause of hospitalization and death in Thailand and other Southeast Asian countries, imposing a major economic burden on households, health systems and governments. This study aims to assess the economic impact of hospitalized dengue cases on households in Chachoengsao province in eastern Thailand. We conducted a prospective cost-of-illness study of hospitalized pediatric and adult dengue patients at four public hospitals. We examined all hospitalized dengue cases regardless of disease severity. Patients or their legal guardians were interviewed using a standard questionnaire to determine household-level expenditures for medical and non-medical costs and income losses during the illness episode. Between March and September 2015, we recruited a total of 224 hospitalized patients (<5 yrs, 4%; 5-14 yrs, 20%, 15-24 yrs, 36%, 25-34 yrs, 15%; 35-44 yrs, 10%; 45+ yrs, 12%), who were clinically diagnosed with dengue. The total cost of a hospitalized case was higher for adult patients than pediatric patients and was US\$153.6 and US\$166.3 for pediatric DF and DHF patients and US\$171.2 and US\$226.1 for adult DF and DHF patients, respectively. The financial burden on households increased with the severity of dengue illness. Although 74% of the households reported that the patient received free medical care, hospitalized dengue illness cost approximately 20-25% of the monthly household income. These results indicated that dengue imposed a substantial financial burden on households in Thailand where a great majority of the population was covered by the Universal Coverage Scheme for health care.

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### PHENOTYPES OF STEM AND PROGENITOR CELLS ACCOUNTING FOR THE ACUTE AND PERSISTENT INFECTION OF DENGUE VIRUS

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Dengue is an arthropod-borne viral disease of global importance, other routes of transmission are recipients of donor organs or tissues, blood and vertical transmission. Mostly dengue infected patients are asymptomatic with overt clinical presentation. The disease manifest markedly hypocellular marrow and abnormal early megakaryopoiesis. New findings demonstrates that stem and progenitor cells are highly permissive to dengue infection, but their actual identity unknown. Therefore we address to investigate the phenotypes of dengue infected stem and progenitor cells and their sustainability of infection. We have proposed an *ex vivo* method that human cord blood supported dengue infection, though a

variation in individual donor was observed. After infecting  $1 \times 10^6$  cells with 1MOI (Multiplicity of infection) three distinct stages, productive, silent and reactivation, in dengue infected Stem Progenitor Cells was observed. In addition, hematopoietic stem cells and early megakaryocyte progenitor cells were the most permissive to support DENV infection. Multi-color flow cytometry analysis revealed that the phenotypes of cells infected by dengue for acute stages were CD133+CD34-CD45-CD41-CD61+CD14- population, while CD133+CD34+CD45- CD41+CD61+CD14-CD11c- were for latent. Infectious virus was recovered from the sorted latent cells by co-culturing with indicator vero cells, suggesting dengue established as latent in these cells. We also observed a transition shift of cells from acute to latent rising to early Megakaryocyte Progenitor populations. Characterization through fluorescent microscopy of infected stem cells like CD61 and CD34 expression with NS-1 showed increasing trend gradually by Day 30, indicating that dengue might equip a machinery to manipulate the differentiation of Hematopoietic stem cell for the transition. Therefore it is imperative to determine how CD34+ cells is lost during early differentiation in dengue infected cord blood cells. The significance of our study may lead to further understand the complexity of dengue and its interaction with Hematopoietic stem cells, providing an avenue for developing new therapy.

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### SEASONALITY OF ARBOVIRAL ILLNESS IN RURAL ECUADOR: 2009-2016

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Season is a major determinant of infectious disease rates, including mosquito-borne arboviruses, such as dengue, chikungunya, and Zika. Seasonal patterns of disease are driven by a combination of climate factors, such as temperature or rainfall, and human behavioral time trends, such as school year schedules, holidays, and weekday-weekend patterns. These factors affect both disease rates and healthcare-seeking behavior. Seasonality of mosquito-borne illnesses has been studied in the context of climate, but temporal trends are less well-understood. With 2009–2016 medical record data from patients diagnosed with arboviral illness at two hospitals in rural Ecuador, we used Poisson generalized linear modeling to determine short- and long-term seasonal patterns of mosquito-borne disease, and the effect of climate factors. The most important predictors of illness were annual fluctuations in disease, long-term trends, day of the week, and climate variables. Compared to Tuesday, weekends were the least likely days for arboviral illness to be diagnosed, with 34% ( $p=0.007$ ) and 40% ( $p=0.001$ ) less cases reported on Saturday and Sunday, respectively. Seasonal trends showed a single peak during April, with long-term trends showing an overall decrease in diagnoses and suggested inter-epidemic periods every two or three years. Important climate variables included total monthly precipitation ( $p=0.002$ ), Oceanic Niño Index ( $p=0.039$ ), interactions between total precipitation and monthly absolute minimum temperature ( $p=0.002$ ), total precipitation and monthly number of days with precipitation ( $p=0.019$ ), and three-way interaction between minimum temperature, total precipitation, and precipitation days ( $p=0.014$ ). Seasonality assessments revealed 10- to 30-day lags between peaks in climate variables and disease. This is the first report of arboviral disease seasonality in Ecuador, one of few reports from rural patients, and one of very few studies utilizing daily disease reports. These results can inform local disease prevention efforts, public health planning, and global or regional models of arboviral disease trends.

### FIRST REPORT OF COMPLETE GENOME ANALYSIS OF NEUROTROPIC DENGUE VIRUS SEROTYPE 3 ISOLATED FROM THE CEREBROSPINAL FLUID OF AN ENCEPHALITIS PATIENT

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Neurological manifestations associated with dengue viruses (DENV) infection have been reported. However, there is a very limited information on the genetic characteristics of neurotropic DENV. Here we described the isolation and complete genome analysis of DENV serotype 3 (DENV-3) from cerebrospinal fluid (CSF) of an encephalitis pediatric patient in Jakarta, Indonesia. The 10-year old boy was diagnosed with dengue encephalitis based on the clinical symptoms and detection of DENV in CSF, anti-DENV IgM in serum, hemoconcentration, and thrombocytopenia. Vero cells inoculated with the CSF sample showed cytopathic effects at day 5 after inoculation and next-generation sequencing was employed to deduce the complete sequence of this neurotropic DENV-3 isolate (201610225). Compared with DENV-3 prototype strain and all other reference strains from GenBank (n=787), 46 amino acid changes were found within the genome of 201610225, in which two novel amino acid changes, R3259K and F3369Y, located in the NS5 protein coding sequence were not observed in any other complete DENV-3 genome. Additionally, while the remaining 44 amino acid changes were also observed in other DENV-3 strains in our complete genome data set, nine amino acid changes were only found in relatively few other DENV-3 strains (less than 1% occurrence). A phylogenetic tree and molecular clock analysis revealed that the neurotropic virus was a member of the endemic Sumatran-Javan clade of DENV-3 genotype I which includes strains from Sumatra, Jawa, and Sulawesi islands in Indonesia as well as strains from Timor Leste (formerly East Timor). To our knowledge, this is the first report of neurotropic DENV-3 complete genome analysis. Genetic characterization of additional neurotropic DENVs will allow further insights into the role of unique amino acid changes found in isolate 201610225. In addition, further studies are warranted to study the virulence of this isolate in animal models and its tropism for neural cells *in vitro*.

### HUMAN MONOCLONAL ANTIBODIES AGAINST DENGUE VIRUSES: REVEALS FROM A NOVEL ASSAY

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Dengue is the most important arboviral illness in humans. It is estimated that 390 million infections occur on a yearly basis around the globe. Among them, about 96 million people develop symptoms, and there are approximately 20,000 deaths. Treatment for patients with dengue is mainly supportive, with anti-pyretic medication and fluid management to treat the symptoms as needed. Antivirals against dengue viruses (DENV), the causative agent of dengue, are not available. Passive therapy with antibodies is considered a potential antiviral approach. To have the greatest effect, antibodies would need to be administered early in the acute phase of DENV infection, when viremia levels are high. Effective antibodies, if available, would be desired to reduce disease severity, the symptomatology, and the duration of illness. Moreover, the potential for virus transmission from infected humans to mosquitoes could also be mitigated due to a more rapid decline in viremia, to a level below the

mosquito infectious dose. However, there is a lack of clinical evidence for the efficacy of antibody therapy in dengue. We have access to 14 well-described antibodies, shown to neutralize DENV in the Plaque Reduction Neutralization Test (PRNT). However, there are limitations to this conventional assay i.e. PRNT employs laboratory-cultured cells which do not mimic the natural infection of DENV. We develop a novel, more biologically-relevant approach, the Viremic Blood Neutralization Assay (ViBNA). This assay was used to assess the neutralization profiles of those 14 antibodies against DENV. Results showed that only five antibodies effectively neutralized DENV in our assay. The neutralization caused by the therapeutic antibody candidates followed a dose-response relationship. These results demonstrate the increased sensitivity of ViBNA and have implications for the pharmacokinetics and the dosage of the promising antibody candidates.

### DENGUE AS A RURAL DISEASE? FINDINGS FROM A HOUSEHOLD STUDY IN KAMPHAENG PHET, THAILAND

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With an estimated 3.9 billion people at risk of dengue virus infection, a better understanding of dengue epidemiology is needed to reduce the global burden of this life-threatening disease. Herein, we present an analysis of community- and household-level factors associated with risk of dengue infection. Data for this study is derived from the first year of a five-year prospective cohort study of dengue infection conducted in 500 family units in Kamphaeng Phet, Thailand. Acute dengue illnesses were identified by ELISA, RT-PCR, and HAI, and subclinical cases were identified by HAI during routine annual follow up. A total of 34 dengue infections were detected among 78 family units in the first year of the study; 22 infections were subclinical. Risk of dengue infection was compared in rural and urban areas with the finding that 31.7% of homes in agricultural areas experienced dengue infection, 33.3% within villages, and 0% within urban areas (p=0.15). The mean number of homes within a 100m radius was 9.6 for dengue-infected homes and 14.5 for non-dengue-infected homes (p=0.12). This suggestion of increased risk in rural areas was hypothesized to be primarily due to differences in housing construction and sanitation. Rural homes were more likely to be constructed primarily from corrugated metal sheeting, which was associated with an increased risk of dengue infection (62.5% of homes with metal sheeting experienced dengue versus 25.7%, p=0.04). Agricultural homes had more larval breeding sites, including water containers, organics such as coconut shells and bamboo, and tires (p<0.05 for all). These preliminary analyses were limited to enrolled houses that had completed annual follow-up at the time of writing; these findings will be further explored with accumulating data from this active prospective cohort study. Taken together, this data from Thailand suggests that dengue infection rates may be higher in rural areas and would challenge the current paradigm of dengue as an urban disease. This has implications for the appropriate targeting of vector control or community education campaigns, particularly in the absence of a readily-available vaccine.

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### IPS CELL DERIVED DENDRITIC CELL LIKE CELL IS INFECTED WITH DENGUE VIRUS, AND ACTS AS ANTIGEN PRESENTING CELL

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Dengue virus (DENV) infection poses a great threat to about 2 billion people globally. Although several vaccine trials have been launched, there is no protective vaccine against infection. The serotype specific humoral immunity has been reported to show a limited protection against infection, and this fact suggested the importance of the cellular immunity. Dendritic cell (DC) is defined as a professional cell to present antigenic peptide coupled with MHC to T cells, and also known as the first target cell of DENV infection. In this study, we tried to set up an *in vitro* antigen priming system for T cells using induced pluripotent stem (iPS) cell derived DC like cells. We found that DENV efficiently infected iPS DC like cells. Moreover, the *in vitro* experiments showed clearly that the infected iPS DC like cells gave a significant antigenic stimulation to T cell even from naïve donor. It is concluded that it is possible to use the iPS DC like cells to identify T cell antigenic epitopes and to enhance development of both T and B stimulating dengue vaccine.

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### PREVALENCE AND BURDEN OF DENGUE IN EUROPE: A SYSTEMATIC REVIEW AND META-ANALYSIS

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The increase of air travel was concomitant with the importation of dengue to non-endemic areas as Europe. The imported dengue cases are thought to be an important source for transmission of autochthonous dengue in Europe. This systematic review and meta-analysis aims to investigate the prevalence of dengue in Europe, evaluate the severity, predict the future risk, and propose potential solutions to overcome this problem. Out of 5287 reports, resulted from the search in nine electronic search engines, we included 198 reports which reported confirmed dengue cases. The screening processes and the data extraction were performed by three independent reviewers. The meta-analysis was performed by pooling the event rate and 95% confidence interval (CI). Meta-regression and subgroup meta-analysis were performed to test the effect of the covariates. The protocol was registered into PROSPERO (CRD42015015037). Autochthonous dengue cases were detected in nine countries with the highest number of cases reported in Croatia (n=44). However, the highest level of imported dengue cases was in Germany

(n=6736) and subsequently France (n=4223). Most cases were imported from South-East Asia (n= 8986) especially Thailand. Total dengue cases increased during the last six years (n=6413), especially in in Germany and France, compared to the detected cases during the previous decades. The pooled rate of dengue infection among other imported infections to Europe was 0.275 (95% CI [0.177-0.401]). The estimated rate of severe dengue among all European dengue infection was 0.110 (95% CI [0.061-0.193]), while the rate of dengue with warning signs was 0.217(95% CI [0.156-0.293]). Meta-regression using age and sex of patients didn't reveal any significant effect on the estimated proportions. Publication bias was detected only in dengue cases with warning signs. In conclusion, imported dengue is increasing in Europe which in turn participates in the appearance of autochthonous cases. Severe dengue is not common among European dengue cases. European travel authorities should pay more attention for diagnosis and control dengue cases among returning travelers.

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### A COMPARISON OF RAPID AND STANDARD DIAGNOSTIC ASSAY EFFICACY FOR THE DETECTION OF DENGUE VIRUS

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The burden of arthropod-borne viruses (arboviruses) is notoriously difficult to determine. A primary roadblock to determining the impact of these viruses in endemic regions and communities is the availability of reliable, accurate, and affordable diagnostic tests. Proteins targeted by diagnostic assays generally have a short window of expression, making it difficult to quickly and accurately diagnose acute infections. Viral proteins or viral RNA are identifiable within the first 3 days of infection, followed by IgM antibodies from days 3-10, and IgG antibodies thereafter to identify previous exposure to specific viral genera. We aimed to identify the sensitivity of a dengue virus (DENV) rapid diagnostic test (RDT), which functions as a triplicate assay providing results for DENV nonstructural glycoprotein 1 (NS1), IgM antibodies, and IgG antibodies in a single test, against traditional methods of detection. Febrile children participating in a larger study to estimate the burden of arboviruses in coastal Kenya provided blood samples during the initial and follow up visits. The follow-up visit samples were collected after one month. Initial blood samples were tested using the DENV IgG/IgM/NS1 RDT from Deangel Biological. Serum obtained from both initial and follow-up blood samples were tested for anti-DENV IgG antibodies by indirect ELISA. In total, samples from 180 children tested using both RDT and ELISA. All samples were negative for NS1, whereas 37% were IgM positive. RDT results for IgM should have correlated with IgG results obtained from follow-up indirect ELISA testing, yet only 5.3% of samples tested were positive for IgG antibodies during the follow-up testing. There were several discordant results when comparing IgG prevalence by RDT and indirect ELISA, with 7 false positives and 4 false negatives using indirect ELISA as the gold standard. We have concluded that the positive predictive and the negative predictive values for the RDT kit are 6.7% (CI95 1.9%-16.2%) and 95.4% (CI95 89.5%-98.5%), respectively. Improved rapid diagnostic testing is needed to facilitate diagnosis in remote settings, as current methods are substandard.

### IL-10 PROTECTS THE BLOOD-BRAIN BARRIER AGAINST LEAKAGE INDUCED BY SERUM FROM DENGUE PATIENTS

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Dengue is a viral infection disease characterized by systemic symptoms ranging from self-limited undifferentiated fever to life threatening symptoms, including plasma leakage. Yet, less than 5 % of dengue patients present dengue virus (DV) infection in cerebrospinal fluid, neurons, microglia, astrocytes or brain endothelial cells. The inflammatory cytokines from the immune response against DV are thought to be responsible for endothelial tight junction (TJ) opening and plasma leakage. Here, we used a blood-brain barrier (BBB) *in vitro* model to compare the effect on TJs of sera from patients with dengue fever (DF, without plasma leakage) and sera from patients with dengue hemorrhagic fever (DHF). A functional assay, based on the continuous measurement of transendothelial electrical resistance (TER) of the BBB, was used to evaluate sera effect. Sera cytokine levels were determined using a commercial bio-plex assay. TJ proteins expression and localization were evaluated by Western blot and immunofluorescence assays. Our results indicate that both, DF and DHF sera had a biphasic effect: at earlier times (4 h) they induced a rapid and significant decrease of TER, while at later times (after 24 h) an increase of TER was detected. However, with FHD sera, the TER recovered to control levels, while with DF serum, the TER increased above control values. Exposure of BBB for 48 h to FD or FHD sera, decreased CI-5 and increased occludin expression. The levels of cytokines IL-1Ra, IL-6, IL-8, TNF- $\alpha$  and INF- $\gamma$  were not significantly different between DF and DHF sera; however, significantly higher levels of IL-10 were observed in DF sera which correlated with higher TER values. Finally, we exposed BBB to purified IL-10 or FHD sera supplemented with IL-10 (0.5 ng/ml) and observed an increase in TJs seal, together with CI-5 and occludin preservation at cell borders. These results suggest that IL-10 in DF sera acts as a protective factor for TJs and prevents the opening induced by other serum factors.

### THERAPEUTIC POTENTIAL OF INTERFERON-A AND RIBAVIRIN AS COMBINATION THERAPY AGAINST DENGUE VIRUS IN DIFFERENT CELL LINES

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Dengue virus (DENV) is the most prevalent mosquito-borne viral illness in humans and is endemic in over 100 countries world-wide. Currently, there are no therapeutic agents available to prevent or treat DENV infections. Our objective was to fill this unmet medical need by evaluating the antiviral activity of interferon- $\alpha$  (IFN) and ribavirin (RBV) as combination therapy against DENV. IFN plus RBV is a currently approved therapy for the treatment of hepatitis C virus infections, a virus related to DENV. For these studies, Vero and Huh-7 cells were infected with DENV in the presence of increasing concentrations of IFN and/or RBV. Viral supernatants were harvested on the day of peak viral infection in the control arm and viral burden was quantified by plaque assay on Vero cells. A mathematical model was fit to the viral burden data to define drug-drug interactions for antiviral effect. In Vero cells, the effect of IFN and RBV was additive with EC50 values of 1,222 IU/ml and 100.7 mg/L for IFN and RBV, respectively. However, drug interactions were highly synergistic in Huh-7 cells, yielding EC50 values of 72.24 IU/ml for IFN and 46.58 mg/L for RBV. Despite additive and synergistic interactions, the EC50 values obtained for RBV in both cell lines are supraphysiological. We then evaluated the

antiviral activity of the IFN plus RBV at clinical exposures in an *in vitro* plate assay to further investigate the therapeutic promise of this regimen. Clinically-relevant exposures inhibited DENV by 2-log<sub>10</sub> at 24h and 48h post-treatment in Vero cells. Inhibition was much higher in Huh-7 cells, as viral burden was suppressed by 3.3-log<sub>10</sub> at 24h and 4.6-log<sub>10</sub> at 48h. Our findings highlight the importance of host cell selection for antiviral screening assays, as different cell lines may result in different conclusions. Since Huh-7 is a human hepatocyte cell line it may serve as a more relevant tissue culture model for DENV infection compared to Vero cells. Moreover, our results suggest that IFN plus RBV is a potential therapeutic strategy for the treatment of DENV, as it may be possible to achieve sufficient levels of viral inhibition at physiologic drug exposures.

### ASSESSING DENGUE VIRUS-INDUCED CHANGES IN GENE EXPRESSION PROFILES VIA RIBOSOME PROFILING

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A majority of the research on dengue (DENV) virus-induced changes in gene expression has focused on the role of the adaptive immune response, which is undeniably important. However, epidemiological data suggest that the host's genetic background may also contribute important susceptibility factors that could exert their effect in a manner independent from the adaptive immune response. Thus, an understanding of differential global gene expression at the proteome level is essential to understand how DENV infection can result in dramatically different disease outcomes. Ribosome profiling enables direct measurements of protein expression at the whole cell level. In so doing, it generates all the information needed for a comprehensive understanding of how global gene expression may influence particular disease phenotypes. We have recently completed a ribosome profiling-based study of DENV-2 in infected K562 and A549 cells. The two models of DENV infection allow us to test both low and high infection rates. In the K562 low infection model we found 471 differentially expressed (DE) genes in response to DENV infection, whereas in the A549 high infection model we detect over 3000. Interestingly we detected a large number of regulatory RNA species that may be novel regulators of dengue pathogenesis. Our data indicates that ribosome profiling is a gene discovery tool with great potential to inform understanding of disease pathogenesis.

### THE PHYLOGEOGRAPHY AND PHYLODYNAMICS OF THE DENV-2 AMERICAN-ASIAN GENOTYPE IN PERU

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Dengue virus and particularly the American-Asian genotype of Dengue virus type 2 (DENV-2) causes a large burden of morbidity in Peru and Latin America. Existing studies into the spatial epidemiology of DENV-2 in Peru have been limited by a relative paucity of Peruvian and Latin American sequence data. In particular, it is unclear what predicts diffusion of this virus within Peru and whether certain locales can serve as sources of persistent DENV-2 variants over time. We therefore sequenced the whole genome of 216 DENV-2 positive human sera collected from Peru and neighboring countries between 2007 and 2014, with additional background global sequence data obtained from GenBank. A maximum likelihood E-gene tree inferred under a GTR+I+G nucleotide substitution model confirmed the circulation of two large DENV-2 American-Asian (DENV-2 AA) lineages in Peru with a replacement of Lineage I by Lineage II in 2009. Overall, Peru experienced seven distinct DENV-2 AA introductions. Lineage I was most likely introduced into Peru via three different routes from either Brazil or Ecuador. Similarly, three geographic routes of lineage

Il into Peru were identified, a northern route from Ecuador/Colombia, and two eastern routes into Loreto and Madre de Dios from a Brazil/Paraguay/Bolivia mixed lineage. Regarding within-Peru viral traffic, the results show clear evidence of spatial division between coastal and Eastern regions of Peru and regional viral dispersal typically between departments sharing borders. A Loreto-specific analysis revealed Iquitos data in basal positions of multi-province Loreto sub-lineages, suggesting that Iquitos may have experienced multi-year persistence of DENV-2 strains and may have seeded other locations within Loreto. Taken together, our results indicate that Peruvian DENV-2 epidemics are spatially structured, that diffusion of DENV-2 into and within Peru is typically predicted by geographic distance and that the tropical city of Iquitos experiences multi-year persistence of DENV-2 variants and may act as a regional hub of viral traffic.

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### DENGUE AND OTHER ARBOVIRUSES IDENTIFIED IN RESPIRATORY SPECIMENS OF UNKNOWN ETIOLOGY

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Dengue viruses (Family *Flaviviridae*; genus *Flavivirus*) are transmitted to humans through the bites of infected female mosquitoes. Dengue viruses cause dengue fever, a severe, flu-like illness that can lead to complications and cause death. During periods of peak viremia dengue virus can be easily detected in serum samples by PCR. Following peak viremia, dengue can also be diagnosed using ELISA-based assays. Here, we report identification of dengue viruses, as well as other arboviruses, in oropharyngeal swabs collected from patients with respiratory symptoms. Briefly, while conducting routine respiratory surveillance in Peru, we identified a large number of samples that had shown cytopathic effect (CPE) while in culture, but tested negative for all known respiratory pathogens using either PCR- or ELISA-based approaches. These samples (196 total), entered a pathogen discovery pipeline that uses next-generation sequencing (NGS) to generate unbiased amplifications of viral nucleic acids. Sequencing libraries were prepared with Nextera kits and sequenced on the Illumina MiSeq platform. We detected previously undiagnosed viruses in 35 samples (18% total positives). As expected, the majority of viruses detected were respiratory viruses (20, or 57% of those detected). However, we also detected a number of arboviruses, including dengue (2, or 6%), Western Equine Encephalitis (WEE, 2 or 6%) and Venezuelan Equine Encephalitis (VEE, 1 or 3%). Arboviruses like dengue, WEE and VEE are not usually detected from oropharyngeal swabs, but given that these pathogens can cause flu-like symptoms, it is not surprising these patients would be sampled. The duration and role of viral shedding in the upper oropharynx of these arboviral diseases should be explored further. These results indicate that unbiased NGS approaches are an effective way to detect viral pathogens from patient samples when traditional methods fail to provide etiologic confirmation.

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### MODIFIED ELISPOT FOR THE ANALYSIS OF SPECIFIC AND CROSS-REACTIVE DENGUE VIRUS AND ZIKA VIRUS MEMORY B CELLS

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Dengue virus (DENV) and zika virus (ZIKV) are antigenically related flaviviruses that have a significant global health impact. Recently, immune sera and monoclonal antibodies from DENV patients were found to be cross-reactive with ZIKV suggesting that memory B-cells from a DENV infection or vaccination can be reactivated by a ZIKV infection. Conventional ELISpots have been used to effectively to measure antibody secreting B-cells (ASCs) but since it is limited to a single antigen at a

time, it is challenging to detect DENV serotype-specific and cross-reactive memory B cells in the same well. Furthermore, high concentrations of antigen are required to detect ASCs. Novel assays that measure flavivirus cross-reactive memory B cells with reduced assay cost are needed as they can be used to better understand the breadth and magnitude of cross-reactive flavivirus responses. Using a panel of DENV immune and naïve PBMC, we evaluated biotinylated recombinant Virus-like Particle (Bio-VLP) and fluorescently labeled viruses (FLVs) in modified ELISpot assays. Conjugation efficiency and recognition of Bio-VLPs and FLVs by virus specific antibodies were validated by flow cytometry. We were able to reduce the amount of antigen required 10 fold, without any loss of detection sensitivity, by utilizing biotinylated antigens as an alternative detection system. FLVs were able to simultaneously detect DENV serotype-specific and cross-reactive memory B cells. Our findings indicate that the biotinylated-DENV VLPs and fluorescently labeled viruses might provide sensitive and specific tools to detect DENV-specific and DENV-ZIKV cross-reactive memory B-cells.

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### THE EFFECT OF GARLIC IN REDUCING INFLAMMATION IN DENGUE INFECTION

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Dengue virus (DENV) is a mosquito-borne flavivirus that causes significant human disease and mortality in the tropics and subtropics. There has been a recent global trend of increased epidemic activity and DENV infection is considered a serious reemerging health problem worldwide. A relatively unexplored approach is to develop new treatments for dengue infection and prevent severe disease development through the investigation of alternative and natural medicines. Inflammation plays both beneficial and harmful roles during the host response to dengue virus infection. Several studies have proposed that the oxidative stress response induced by dengue virus infection is responsible for triggering the pro-inflammatory cytokine response. Thus, blocking the oxidative stress response and reducing inflammation in dengue patients could together reduce the likelihood of severe disease development. There are several plant products and herbal supplements that have been suggested or proven to reduce inflammation, including flavonoids, curcumin and garlic. Garlic also has effects on the oxidative stress response and prevents intracellular glutathione depletion. To investigate the effects of garlic on inflammation during dengue virus infection, we used three active organosulfur garlic compounds: DAS, DADS and Aliin. We examined the levels of inflammatory cytokines TNF-alpha, IL-8 and IL-10 in the supernatants of dengue virus-infected cells with and without the garlic compounds. We found that the addition of garlic compounds reduced the levels of all 3 inflammatory cytokines at all doses tested. Oxidative stress is thought to be the mechanism through which dengue virus acts to trigger the pro-inflammatory immune response during infection. As we examined the anti-oxidant activity of garlic during dengue infection we found that garlic can reduce inflammation through the reduction of the oxidative stress response.

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### CHARACTERIZATION OF IN VIVO T CELL ACTIVATION DURING ACUTE DENGUE ILLNESS

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Several studies suggest that T cells play an important role in both protection against dengue virus (DENV) infection and pathogenesis of

dengue disease. What remains unclear is the relationship between the responses of T cell subsets to primary or secondary DENV infection and disease severity. In addition, the role of peripheral T follicular helper (pTfh) cells during acute DENV infection has not been well studied. To further characterize T cell activation during acute DENV infection, we studied the expression of activation markers on CD4 and CD8 T cell and pTfh cell subsets using flow cytometry in blood samples collected from Thai children during acute DENV infection. We demonstrated a massive activation of T cells during acute DENV infection. The most pronounced T cell activation occurred during the critical stage (fever day +1, one day after defervescence), as defined by a large increase in CD38 and PD-1 expression. On average, ~50% of CD8 T cells and ~20% of CD4 T cells were CD38+ PD-1+ on fever day +1. pTfh cells were also highly activated with increased expression of PD-1, CD38, and OX40. Interestingly, we also observed a 61% increase in the frequency of pTfh cells on fever day +1 when compared to 1 yr post-infection ( $p \leq 0.05$ ). Our study demonstrates a robust activation of CD4 and CD8 T cells during acute DENV infection. The expansion and activation of pTfh cells may be related to plasmablast activation and DENV-specific antibody production. Overall, our study supports the model that T cells contribute to disease evolution during the critical stage of infection. Future analysis will be performed to determine if there is a correlation between T cell activation and virus serotype, primary versus secondary infection and disease severity.

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### PRE-EXISTING ANTI-DENGUE VIRUS ANTIBODY TITER PREDICTS SEVERITY OF DENGUE DISEASE IN A PEDIATRIC COHORT IN NICARAGUA: A CASE-CONTROL AND LONGITUDINAL STUDY

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The four dengue virus serotypes (DENV1-4) are mosquito-borne flaviviruses that cause up to ~100 million dengue cases and ~500,000 hospitalizations annually. Severe dengue disease, most common during secondary heterotypic DENV infection, is often attributed to antibody-dependent enhancement, but a direct relationship between pre-existing anti-DENV antibody (DENV-Ab) titer and disease severity has not been shown in human populations. Pre-existing DENV-Ab titers were measured in healthy annual blood samples from 6,684 children in the Nicaraguan Pediatric Dengue Cohort Study using Inhibition ELISA. Severe dengue disease was categorized by 1997 and 2009 WHO dengue severity criteria as well as a clinical management score. We studied the relationship between pre-existing DENV-Ab titer and 1) severe vs. non-severe secondary dengue using logistic regression; 2) severe and non-severe secondary dengue cases and matched controls using conditional logistic regression, Wilcoxon rank sum and signed rank tests; and 3) risk of severe and symptomatic dengue using Cox proportional hazards models. Statistical analyses adjusted for year of exposure, sex, previous infections, age, and serotype. Across all severity classification schema, dengue cases with pre-existing DENV-Ab titers between 21 and 80 had significantly greater odds of being severe (compared with non-severe) than primary dengue cases. Severe secondary dengue cases had lower DENV-Ab titers (peak 21-80) than non-severe dengue cases or matched controls (peak 81-320). Further, children with DENV-Ab titers of 21-80 had greater hazard of severe dengue as compared to DENV-naïve children (Hazard ratio=7.47 [95%CI 3.05-18.27]), whereas children with lower or higher DENV-Ab titers did not. In

contrast, children with DENV-Ab titers >320 had significantly lower hazard of symptomatic dengue. In sum, we observed the highest risk of severe dengue at pre-existing DENV-Ab titers of 21-80 and protection against symptomatic dengue at high DENV-Ab titers, thus providing a basis for identifying individuals at highest risk for severe disease in populations and vaccine trials.

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### USING CARTOGRAPHY TO DEFINE ANTIGENIC RELATIONSHIP AMONG DENGUE VIRUSES (DENV) IMPORTED BY TRAVELLERS

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The dengue viruses (DENV) are genetically divergent and grouped as four distinct serotypes (DENV-1-DENV-4), based on recognition of the viral envelope by anti-DENV antibodies. The four serotypes are more precisely classified, using phylogenetic approaches, into distinct genotypes which have been defined as clusters with nucleotide sequence divergence of not more than 6%, and lineages within the genotypes may represent strains with similar geographic origins. Such antigenic variation among the four dengue viruses influences epidemic virulence, immunopathogenesis, and vaccine design. We sought to understand the immunological significance of virus diversity by assessing DENV-specific cross-recognition and cross-neutralization using a panel of isolates and acute and convalescent sera collected over a period of up to 4 years, from travellers with well-defined monotypic DENV infection. DENV was isolated from acute-phase serum from Western Australian (WA) travellers returning from Bali, Indonesia, where they were infected with DENV; phylogenetic analysis identified serotype genotypes and lineages circulating at the time and imported by the travellers. Antigenic maps were constructed from hemagglutination inhibition (HI) and focus reduction neutralization tests (FRNT) titers. We show that all DENV, representing diverse genotypes and lineages, are recognized and neutralized by heterologous and homologous anti-sera, within 2 months of infection. By 2 years after infection, broad cross-recognition of all four serotypes is still apparent, however cross-neutralization is more restricted and homologous DENV and anti-sera are loosely clustered in the antigenic map. By 4 years after infection, there is clear clustering of DENV neutralized by homologous anti-sera, and also increased DENV recognition as measured by haemagglutination inhibition. In this study, we show that DENV genetic classification does not necessarily represent antigenicity and immune dynamics. It is essential to explore antigenic properties to understand the evolution and level of DENV-specific immune response.

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### USE OF NEEDLE-FREE JET INJECTION AND ELECTROPORATION TO ENHANCE THE IMMUNOGENICITY OF A TETRAVALENT DENGUE DNA VACCINE

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The Naval Medical Research Center has developed a tetravalent dengue DNA vaccine. Two phase 1 clinical trials, first with the monovalent dengue-1 product for proof-of-principle and a second with the tetravalent product, demonstrated that this vaccine is safe and well tolerated but produced less than optimal humoral immune responses. To determine if other methods of vaccine delivery could enhance the immunogenicity of this vaccine, we conducted a study in non-human primates. Animals were vaccinated at 0, 30 and 90 days with either 1 mg or 5 mg of a tetravalent dengue DNA vaccine via either the intradermal or intramuscular route using either a needle-free jet injection or electroporation device. Data

on neutralizing antibody titers assessed monthly up to five months after the last vaccination and cellular immune responses will be presented. Preliminary results suggest that intramuscular delivery of the tetravalent DNA vaccine by electroporation may generate the best anti-dengue neutralizing antibody response.

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### DEVELOPMENT AND VALIDATION OF CLINICAL ALGORITHMS FOR THE DIAGNOSIS OF DENGUE IN ENDEMIC AREAS OF COLOMBIA

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Due to the increase in the incidence and mortality of dengue, its prompt and correct diagnosis is a priority for endemic countries. Clinical classifications and existing laboratory tests for diagnosis have a variable performance with sensitivities between 45% - 98% and specificities between 4% - 71%. To develop and validate clinical algorithms for dengue diagnosis in endemic areas of Colombia. A Bayesian-adaptive quasi-experimental clinical trial is being conducted with a target of 2000 febrile subjects. In the first phase, diagnostic algorithms based on Bayesian methodologies are being developed and validated prospectively. Demographics, signs and symptoms of dengue, leukocytes and platelets are collected from all participants; clinical algorithms are compared to gold standard diagnostic dengue tests. In the second phase, the diagnostic accuracy of the best algorithm identified in the first phase will be validated in the routine clinical practice. Interim analyses will be performed every 5 false negatives for dengue. To date, 6 clinical diagnostic algorithms have been developed. Three based on signs and symptoms and three that include leukocytes and platelet counts. The latter have reached up to 75% sensitivity. In an external validation in databases of suspected dengue the sensitivity was 80%. In addition, a pilot test of prospective validation was performed, 65 febrile subjects were recruited and the clinical algorithm (general pain, skin rash, hemorrhagic manifestations, warning signs, neurological alterations, absence of respiratory symptoms, absence of jaundice, leukopenia <4.500/mm<sup>3</sup> and thrombocytopenia <160.000/mm<sup>3</sup>) had a specificity of 95%. There were no positive cases of dengue by gold standard tests but 2 were positive by the clinical algorithm. The inclusion of blood count parameters improves the sensitivity of dengue diagnostic algorithms based only on signs and symptoms. The development of highly sensitive and specific dengue diagnostic tests based on clinical criteria useful at point of care remains a challenge for dengue research.

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### TRIVALENT AND TETRAVALENT DENGUE VACCINES PROTECT AGAINST DENV-4 CHALLENGE IN NON-HUMAN PRIMATES

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A successful dengue vaccine should elicit protective immune responses against all 4 dengue serotypes. Developing a vaccine formulation which elicits balanced immune responses to all serotypes is challenging, and immune correlates of protection may differ by serotype. Takeda's candidate tetravalent dengue vaccine (TDV), is comprised of an attenuated DENV-2 virus strain (TDV-2) and three chimeric viruses containing the pre-membrane and envelope genes of DENV-1, DENV-3 and DENV-4 genetically engineered into the attenuated TDV-2 genome backbone (TDV-1, TDV-3 and TDV-4). TDV elicits neutralizing antibodies and cellular immunity to all 4 dengue serotypes, with magnitude of responses against DENV-2 higher than against DENV-4. In order to determine the relative contribution of the DENV-2 and DENV-4 component of TDV to immunogenicity and efficacy against DENV-4, we evaluated immunogenicity and efficacy of a trivalent

(TDV-1, -3 and -4) and a tetravalent (TDV-1, -2, -3 and -4) vaccine against DENV-4 challenge in cynomolgus (*Macaca fascicularis*) macaques. Infected cynomolgus macaques develop immune responses and a subclinical infection detected by viremia. Vaccine efficacy against subcutaneous DENV-4 1228 challenge was determined by assessing presence of DENV-4 viral RNA and isolation of replicating DENV-4 from serum. Both trivalent and tetravalent vaccines were efficacious against DENV-4 challenge. Neutralizing antibody titers measured using a reporter-virus particle assay demonstrated a correlation between DENV-4 antibody levels and protection against DENV-4 challenge. These results demonstrate that TDV immunogenicity and efficacy against DENV-4 does not require the TDV-2 vaccine component. Characterization of the quantitative and qualitative features of the immune response to DENV-4 may help identify surrogate markers related to correlates of protection.

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### COMPARISON OF ACTIVE AND PASSIVE SURVEILLANCE SYSTEMS FOR DENGUE FEVER IN MACHALA, ECUADOR IN 2014 AND 2015

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Dengue is a mosquito-borne pandemic with approximately 2.5 billion people at risk and approximately 400 million people infected annually. Although dengue reporting is mandatory in Ecuador, the current passive surveillance (PS) system is dependent upon people seeking care if symptomatic. Studies have shown that an active surveillance (AS) strategy allows for more cases to be detected and earlier within an outbreak. This could better inform prevention and control strategies. The purpose of this analysis was to compare an AS system to existing PS in Machala, Ecuador. Dengue cases reported to the Ecuadorian Ministry of Health's (MOH) PS data of from 2014 and 2015 were compared to data from an AS study of Dengue cases in Machala. All cases were laboratory confirmed. AS data were collected from individuals who were seen at one of four clinics or the sentinel hospital in Machala as well as family members and neighbors of the patient. Cases that were reported to both systems were identified based on matching on age, sex and date of visit. In both years, 218 cases were identified by PS, and 287 from AS. Of these 16 cases appeared in both systems. Younger patients were more frequently identified by PS while the AS system identified more dengue infections in the 20-64 age group, ( $p=0.014$  and  $0.358$  in 2014 and 2015). In 2014, the incidence rates were 8.35 per 10,000 for AS and 4.48 per 10,000 for the PS. In 2015, the incidence rates were 1.45 and 2.82 per 10,000 for active and passive surveillance, respectively. Combined active and passive incidence rates in 2014 and 2015 show the significantly higher incidence rates in the 5-19 age group than any other age group (22.97 per 10,000 in 2014 and 7.57 per 10,000 in 2015). Thus, the AS captured a different cohort possibly due to healthcare-seeking behaviors or disease dynamics such as differences in clinical presentation by age group. In addition, it helps improve understanding of the disease dynamics of Dengue infections for more effective, targeted interventions.

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### MOLECULAR EPIDEMIOLOGY OF DENGUE VIRUS SEROTYPES IN NEPAL

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Global expansion of dengue virus (DENV), especially towards previously non-endemic areas, is a serious public health concern. Although DENV was introduced in Nepal nearly a decade ago in 2006, its molecular epidemiology remains unclear. We adopted a phylogenetic approach to understand the genetic diversity and potential origin(s) of the Nepal DENV strains. For this, complete envelope (E) gene sequences of all four DENV serotypes found in Nepal were obtained and maximum-likelihood (M-L) trees were constructed along with relevant global sequences from GenBank. Blood samples (n = 1215) were collected from acute dengue patients during the major outbreaks in Nepal (2010-2012), affecting at least 18 districts, with an estimated case fatality rate of 1.5%. All four serotypes were detected in acute sera by reverse transcription-polymerase chain reaction (RT-PCR). However, DENV 1 and 2 (92%) were found responsible for the major outbreaks in the country. Nepal DENV 1 strains belonged to genotype-V and formed two distinct clades, which showed spacio-temporal variation during these outbreaks. DENV 2, 3, and 4 strains were clustered into cosmopolitan genotype, genotype-III, and genotype-IIIB, respectively. M-L trees revealed that the vast majority of Nepal DENV strains were closely related to India and contemporaneous Singapore strains. India is the most plausible origin of Nepal DENV due to its physical proximity, extensive cross-border activities (open border) and similar geo-climatic features supporting the phylogenetic relations. To this end, we have outlined genetic diversity of DENV serotypes in Nepal. This sequence-based information is not only useful for Nepal in epidemic preparedness but also aids in the understanding of evolutionary dynamics at regional and global level. Additionally, these findings underscore the need of cross-border collaboration in dengue control where high public mobility exists across the porous borders.

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### MUTAGENESIS OF DENGUE VIRUS ENVELOPE PROTEINS TO MAP ANTIBODY EPITOPES AND IDENTIFY RESIDUES ESSENTIAL FOR FUNCTION

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To characterize the immune response to dengue virus (DENV) infection, we have developed a high-throughput strategy that enables the rapid identification of both linear and conformational epitopes on DENV prM/E envelope proteins from all four DENV serotypes. For each DENV serotype (1-4), we used Shotgun Mutagenesis technology to create a comprehensive library of single mutations in DENV prM/E, 3,380 mutations in total. Each library of individual mutant expression plasmids was arrayed into 384 well plates and transfected into human cells to achieve native protein expression and folding. The immunoreactivity of MAbs to the prM/E variant in each individual well was quantified by high-throughput flow cytometry, resulting in approximately 200 MAb epitopes. The epitopes obtained have been correlated with their abilities to protect against DENV infection. A number of these anti-DENV MAbs were found to cross-react with ZIKV prM/E, predominantly within the fusion loop but also within Domain II of the E protein, identifying critical immunogenic residues shared by DENV and ZIKV. We have also produced DENV virions from all four mutation libraries using a previously developed DENV reporter virus particle (RVP) system, allowing us to screen each individual DENV Env variant protein for DENV particle budding and infectivity. For DENV3, analyses of budding and infectivity, along with the MAb binding studies, identified residues whose mutation eliminated virus infectivity, but did not impact E protein expression, antigenicity, virion assembly, or particle budding. We identified variants that showed increased DENV virion budding, up to 5-fold above wild-type, indicating the ability to engineer highly expressed, non-infectious DENV variants that can be used for

vaccine design. Our research has identified neutralizing epitopes in DENV prM/E and specific sites that are critical for DENV infectivity, providing new targets and opportunities for vaccine development.

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### ZIKA VIRUS QUANTITATIVE PCR RESULTS AMONG SYMPTOMATIC PEDIATRIC PATIENTS

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In 2015-2016, over 38,000 Zika virus (ZIKV) infections were reported in Puerto Rico (PR). We previously characterized the clinical manifestations of symptomatic children (<18 years (y) old) with confirmed ZIKV infection (positive qualitative ZIKV polymerase chain reaction (PCR) result) enrolled in the Sentinel Enhanced Dengue and Acute Febrile Illness Surveillance System (SEDSS) in Ponce, PR. Our objective was to further characterize these patients by evaluating quantitative ZIKV PCR results (viral loads, VLs). The study population comprised symptomatic children enrolled in SEDSS by December 31, 2016 with confirmed ZIKV infection. Available PCR+ specimens underwent VL testing (copies/mL) using an RNA standard curve generated from Trioplex Real Time RT-PCR Assay target amplicons. The Mann-Whitney-Wilcoxon test (comparison of medians), Bonferroni correction (pairwise comparisons among age groups and days post-onset of illness (DPO) groups), and Jonckheere-Terpstra test (assessment of trends) were used. Of 353 patients, 351 had ≥1 specimen available. Among 484 specimens, the median (interquartile range (IQR)) of VLs was 19,603 (5465-95,598). The median DPO for specimen collection was lower in serum (1 day) than urine (2 days) (p<0.001). Analyzing only serum and urine specimens collected on the same day (n=131 patients), the median (IQR) VL was higher in serum [21,790 (8784-88,242)] than urine [10,352 (2880-56,481)] (p=0.002). Analyzing one serum sample/subject, there were no statistically significant differences in median VLs according to sex [males (n=171) (25,436) vs. females (n=148) (26,558) (p=0.99)] or age [44,372 (infants, n=21), 26,781 (1-4y, n=60), 22,828 (5-9y, n=88), and 25,621 (10-17y, n=150) (p=0.46)]. The median VL varied significantly according to DPO: 106,778 (DPO=0, n=33); 46,299 (DPO=1, n=131); 20,780 (DPO=2, n=78); 15,876 (DPO≥3, n=77) (p=0.001). Among symptomatic pediatric patients, ZIKV VLs were higher in serum vs. urine. In serum, no statistically significant differences in median VLs were observed according to sex or age, but median VLs decreased significantly with increasing DPO.

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### NEUROLOGICAL OUTCOMES OF JAPANESE ENCEPHALITIS VIRUS INFECTION IN PEDIATRIC AND ADULT PATIENTS AT MAHOSOT HOSPITAL, VIENTIANE, LAO PDR

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Japanese encephalitis virus (JEV) infection is a serious disease with a fatality rate of ~20-30% and 30-50% of survivors have significant neurological sequelae. In Laos, the frequency of death and neurological sequelae have never been studied. Better understanding of the consequences of JEV

infection will help improve disease management and reduce mortality, complications and neurological sequelae. This study aimed to follow patients with JEV infection to determine the frequency of fatality and neurological sequelae. Patients' hospital charts, forms and reports from Mahosot Hospital between 2003 and 2013 were retrospectively reviewed and patients were followed prospectively. Positive anti-JEV IgM, PCR or culture in CSF or sera was considered diagnostic. 183 patients (91 = or <15 years old and 92 >15 years old) with confirmed JEV infections were admitted and included in the study. The median age was 18 (0.1-70) years. 57% of them were male. The mean days of fever before admission was 6.1 (5.3-6.9) days. The fatality rate was at least 22% (40/183) as 49 (27%) patients were lost to follow-up. The proportion of deaths was not significantly different between children (21/91) and adults (19/92),  $p=0.87$ . Among patients who were still alive at the last follow-up, the median duration of follow-up was 55 (18-156) months. 38% of patients completely recovered (without neurological sequelae), with significantly more adults (49%) than in children (28%) recovering,  $p=0.007$ . Of patients 61% (51% in adults and 72% in children,  $p=0.007$ ) had neurological sequelae at follow up. Severe and moderate functional impairment at follow-up was also significantly higher in children (24%) compared to adults (2.1%),  $p<0.001$ , while at discharge neurological sequelae was not significantly different based on Liverpool Outcome Scores (74 vs 72,  $p=0.018$ ). At least 1/5 of the patients with JEV infection in this study died. Among those who are still alive, nearly 2/3 of them had neurological sequelae at last follow-up. Although mortality was similar for children and adults, the severity and frequency of neurological sequelae were higher in children than in adults.

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### THE FIRST SEROLOGICAL EVIDENCE OF PREVIOUS ZIKA VIRUS TRANSMISSION IN ETHIOPIA

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The the genus *Flaviviridae* contain the most important mosquito transmitted viruses such as Dengue and West Nile viruses that affect humans and cause millions of infections every year around the world. However, many of the other viruses in this genus such as Zika virus are generally believed to have a more limited geographical range and to cause few infections annually but this believe has been refuted with the recent large epidemics of Zika virus infections especially in Latin American countries. We show here evidence of past Zika virus transmission in Ethiopia. The evidence of Zika virus transmission here was generated as part of the yellow fever risk assessment study done in Ethiopia in 2014 in which the laboratory investigation required differential diagnosis for flavi viruses including Zika virus. In the study, the country was divided in to five ecological zones based on factors that affect the distribution and abundance of flavi virus vectors. Serum samples were collected from 1643 study participants and tested for flavi viruses namely; yellow fever, dengue fever, Zika and West Nile viruses specific IgG by ELISA and confirmed by Plaque Reduction Neutralization Test (PRNT). Demographic and laboratory data was analyzed using excel spreadsheet. Of the total serum samples tested, 7 (0.43%) showed a confirmed PRNT IgG positivity for previous Zika virus infection. Three of the five Ecological Zones of the country showed IgG positivity for Zika virus. The three Ecologic Zones represent areas of the country that span from North-South and East-West. Five of the total 7 positive cases (71.4%) were Females and the median age for the positive cases was 28; with the youngest study participant infected with the virus being a nine year old girl and the oldest a 60 year old

woman. This is the first serological evidence showing previous Zika virus transmission in Ethiopia. Further studies are required to see if there is active infection in the areas with Zika positivity and understand the risk factors for Zika virus infection and transmission. This study also warrants existing surveillance system in Ethiopia to consider and follow Zika virus infection more closely.

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### YELLOW FEVER AND ARBOVIRUS SURVEILLANCE IN SYLVATIC AREAS FROM MISIONES PROVINCE, ARGENTINA

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Yellow fever (YF) is a viral hemorrhagic fever endemic to tropical regions. In South America, the sylvatic cycle of Yellow fever virus (YFV) is maintained by viral circulation between monkeys and diurnally active mosquitoes that breed in the forest canopy. In Argentina, circulation of YFV was detected during 2007-2009, where a sylvatic outbreak affected monkeys and humans. However, the transmission cycles are not completely understood, and identification of vectors and reservoirs remains poorly known. Since 2015, we have captured mosquitoes in four protected areas of the Atlantic Forest of Misiones province, Argentina. In order to evaluate the YFV enzootic circulation, mosquitoes were tested for YFV and other arbovirus. During the day, we captured adult mosquitoes from human bites, focusing on *Sabethes* and *Haemagogus* genera, because of their known implication in the YFV transmission. Specimens were sorted according to location, date and species. RT-PCR for YFV, generic Flavivirus, Alphavirus and Bunyavirus from supernatant of mosquitoes pool homogenates were performed. Inoculation into Vero and C6/36 cells for virus isolation attempts were done from those pools positives for genome detection. A total of 696 mosquitoes sorted in 134 pools were analyzed. *Sabethes* mosquitoes were the most abundant genus (40%, 10 species), followed by *Trichoprosopon* (18%, 4 species). Among the mosquitoes taxa implicated in YFV transmission, only 5 captured specimens belonged to *Hg. leucocelaenus*, 2 belonged to *Sa. chloopterus* and 36 specimens belonged to *Ae. serratus*. No specimen of *Sa. albiprivus*, *Ae. aegypti* or *Ae. albopictus* were captured. No YFV genome was detected in any of the analyzed mosquitoes pools. Nevertheless, several arbovirus genomes were detected, such as Mercadeo virus, Madariaga virus, and one new flavivirus strain. Currently, isolation attempts are still ongoing. Even though YFV was not detected in our survey, the current YF outbreak in Brazil could spread to bordering countries representing a threat for public health. Continuous maintenance of surveillance is crucial to detect the potential spread of YFV to Argentina.

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### INTERVALS OF POSITIVE AND NEGATIVE DETECTION OF THE ZIKV RNA IN THE URINE OF ZIKA-INFECTED PREGNANT WOMEN

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Zika virus (ZIKV) is a re-emerging flavivirus that was first isolated in Uganda in 1947, and before the Brazilian outbreak, ZIKV infection was considered a mild febrile illness that did not produce severe outcomes. However, the cases of congenital malformations or neurological alterations reported in

Brazil reshaped knowledge on the course of infection caused by a flavivirus and demanded a rapid approach to diagnosing the virus. The molecular diagnosis of flavivirus is usually performed using blood/serum samples; however, urine, saliva, and semen have been identified as additional sources since detection can be prolonged in these fluids even after viremia clearance. This study describes the long-term detection of ZIKV RNA in the urine from a group of ZIKV-positive pregnant women. From February to October of the 2016 ZIKV outbreak, pregnant patients with Zika-like symptoms, with 4 to 38 weeks into their pregnancies, were treated at the Public Health Authority in the Brazilian city of São José do Rio Preto. Serum and/or urine samples were collected from the mothers during their first visits to facility after the onset of symptoms, and the patients were tested for ZIKV. The viral RNA was extracted and TaqMan® RT-qPCR assay was performed using primers targeting the ZIKV envelope (E) gene. After their first ZIKV-positive RT-qPCR (ct ≤ 38.5), these pregnant women were monitored, and viruria was measured until delivery. The viral RNA was detected by RT-qPCR more than four weeks (and up to seven months) after the first detection, even with intervals of negative detection. The use of urine for diagnosis represents an additional tool for virus detection. It is easy to obtain (as it does not put physiological stress upon pregnant women), and it can be obtained more than five days after the onset of clinical symptomatology. Because microcephaly and fetal abnormalities have been attributed to ZIKV, the monitoring of ZIKV viruria in pregnant women through the regular collection of urine samples proved to be an important approach over the course of the pregnancy; however, the meaning and the consequences for newborns will need to be evaluated in the future.

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#### HOW IS ZIKA AFFECTING PREGNANT TRAVELERS? ZIKA VIRUS SURVEILLANCE IN A NON-ENDEMIC AREA

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Zika virus (ZIKV) represents a global threat with dramatic consequences on reproductive and infant's health. Epidemiological surveillance is key to ensure detection of imported cases in non-endemic areas. We aimed to assess the epidemiological characteristics and pregnancy outcomes in a cohort of pregnant travellers at risk of ZIKV and to evaluate its effects in their infants. An epidemiological surveillance system, which included screening for ZIKV (RT-PCR, serology and microneutralization assay) was established at the Hospital Clínic of Barcelona, Spain. Women were enrolled in the study and followed-up until delivery, as well as their infants during the first months of life. Infants' follow-up included weight, length, head circumference, hearing, eye fundus, physical, neurological and psychomotor evaluation, and ZIKV screening. We describe a cohort of 125 women screened for ZIKV; of them, nine were positive (3 by RT-PCR and 6 by microneutralization) and 22 were categorised as a suspected case (undetermined microneutralization or results pending). Infected pregnant women had travelled primarily to the Dominican Republic and Colombia, had been exposed to ZIKV predominantly during the peri-conceptual period or the first trimester, and the majority of them were symptomatic; being rash the most frequently reported symptom. Pregnancy outcomes included 15 live births at term, a miscarriage associated with ZIKV maternal viremia, 14 ongoing pregnancies and a termination of pregnancy. Subtle periventricular cysts were reported in one live birth. All placentas

tested negative, except the residues from the miscarried case that were positive by RT-PCR. All infants tested negative for ZIKV at birth. Psychomotor and anthropometric assessment of study infants during first months of life did not reveal any abnormal finding. Further data will be presented as recruitment is in process and both mothers and infants continue under follow up. Surveillance of ZIKV in pregnancy is important also in non-endemic areas. Prospective studies of infants born to ZIKV infected mothers are critical to understand long-term consequences of the infection.

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#### LOW DENSITY CIRCULATION OF ZIKA VIRUS IN THE PHILIPPINES, 2016

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The Zika virus (ZIKV) outbreak in Brazil in late 2015 and its association with microcephaly made the virus global public health concern. In the Philippines, a single ZIKV case was reported in 2012 despite the abundance of the mosquito vectors of the virus, *Ae. aegypti* and *Ae. albopictus* in most areas of the country. Awareness for ZIKV and its complications started in 2016. In this study, we aimed to characterize the lineage of ZIKV circulating in the Philippines and determine possible origin of the virus by comparing with available sequences in GenBank. Around 2000 urine and serum samples were collected from all areas of the country in 2016. Samples were tested using real-time PCR, in which 59 samples were ZIKV positive. Phylogenetic analysis was done based on the partial coding region of the envelope protein (E gene) using maximum likelihood method. A two-step RT-PCR was used to amplify the 1890 bp of the ZIKV E gene and sequenced using Sanger method. Our findings reveal that majority of the ZIKV detected in the Philippines were of Asian-lineage and that resulting sequences clustered together with ZIKV isolated from Micronesia in 2007. This suggests low-level local transmission but remained at low levels, as symptoms are similar to other mosquito-borne viruses such as Dengue and Chikungunya. Continued surveillance of ZIKV is necessary to track its spread in the Philippines; to assist public health authorities to instigate prevention and control strategies for vectors; and contribute information for its related complications.

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#### EVALUATION OF ACUTE ENCEPHALITIS SYNDROME/ JAPANESE ENCEPHALITIS SURVEILLANCE SYSTEM IN DEORIA AND GORAKHPUR DISTRICT, UTTAR PRADESH, 2016

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**Introduction:** Acute Encephalitis Syndrome (AES) including Japanese Encephalitis (JE) is public health problem due to high epidemicity, mortality and neuropsychiatric sequelae. In 2014, Uttar Pradesh accounted for 33% of 9693 AES cases and 1490 deaths in India. **Objectives:** We evaluated AES/JE surveillance system in Deoria and Gorakhpur Districts to improve surveillance as per CDC recommended surveillance evaluation attributes. **Methods:** We selected four blocks of Gorakhpur and Deoria districts to evaluate the AES surveillance based on selected CDC recommended attributes: data-quality, usefulness, representativeness, timeliness, acceptability, simplicity, and stability. We collected data using data abstraction forms by review of relevant reports and registers and interview of health workers. We studied 12 and 10 health facilities in Gorakhpur and Deoria districts respectively. **Results:** Of government health facilities in Gorakhpur and Deoria, 91% and 93% respectively were reporting, but no private facility was reporting. Of AES patients from Gorakhpur, 88% had JE tests compared to 77% from Deoria. Amongst AES deaths from Gorakhpur and Deoria, 83% and 66% respectively had been investigated. Among reported cases, 82% and 73% of cases were reported within

48hrs of onset in Gorakhpur and Deoria, and 91% and 82% of forms were complete. Most health workers (95% in Gorakhpur, 93% in Deoria) were able to recall correct case definition. Among health facilities, 88% and 80% didn't default in reporting >7 days consecutively anytime in one year. **Conclusion:** The AES / JE surveillance system in Deoria and Gorakhpur districts was useful for investigating deaths, representative of government health facilities, stable and timely for reporting, acceptable based on high reporting rates, and simple in terms of case definition recall. We recommend improving private provider representation and strengthening lab surveillance.

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### PHASE 1 STUDY OF MV-ZIKA, A LIVE RECOMBINANT MEASLES VIRUS VACCINE TO PREVENT ZIKA VIRUS INFECTION

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Zika virus is an emerging mosquito-borne flavivirus. The virus emerged in the past 70 years only sporadically with self-limiting small outbreaks. In 2013, a large outbreak in French Polynesia resulted in over 30,000 cases. Since early 2015 Zika virus spread in the Americas and to date caused autochthonous, vector-borne transmission in 48 countries and territories. This rapid emergence of the previously unknown pathogen raised the urgent need for a vaccine that can be rapidly produced in response to a newly emerging pathogen. Themis took the challenge and developed a vaccine candidate from design to Phase 1 clinical trial within 14 months. The MV-ZIKA vaccine candidate is a live attenuated recombinant viral vectored vaccine for the prophylaxis of Zika virus infection. The measles virus (MV) Schwarz vaccine strain was used as the backbone into which nucleotide sequences encoding Zika virus structural proteins glycoprotein precursor (prM) and the Envelope (E) were inserted to produce the MV-ZIKA. In measles virus susceptible mice, single or multiple vaccinations with MV-ZIKA induced a robust protective immunity, as shown by the induction of ZIKV E protein specific antibodies. The immunization of *Cynomolgus* macaques resulted in the induction of Zika virus neutralizing antibodies in all vaccinated animals. To evaluate the optimal dose of MV-ZIKA in regard to immunogenicity, safety, and tolerability we initiated a double blinded, randomized, placebo-controlled, multi-center, phase 1 trial in 48 healthy volunteer subjects. The subjects will receive one or two vaccinations. The immunogenicity as confirmed by the presence of functional antibodies will be determined on day 28 after the second immunization. The clinical trial is currently ongoing and preliminary data will be presented here for the first time.

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### IDENTIFICATION OF A NOVEL FLAVIVIRUS, NAKIWOGO VIRUS, IN KENYAN MOSQUITOES

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Nakiwogo flavivirus (NAKV) was first isolated in field collected *Mansonia* mosquitoes in Uganda and has never been described elsewhere. This is the second description of this virus. In this study we used NGS/BIO as a surveillance method for novel viruses in Kenyan female mosquitoes. Mosquitoes were collected from Budalangi in Western Kenya and pooled according to the species in 1-25 mosquitoes per pool. RNA was isolated, libraries prepared and sequenced on Illumina platform. Low quality read filtering and adapter trimming was done by dual index filter and Trimmomatic analysis softwares. CLC Genomics workbench was then used to align the reads to NAKV reference genome. After filtering the unaligned reads, variant calling, phylogenetic analysis and viral RNA structure

predictions were performed. NAKV was detected in 17 pools; two pools comprised of *Culex spp* while the rest of the pools had *Mansonia spp*. A full genome (10,122 bp) NAKV was isolated in one pool while the rest of the pools had sequence lengths ranging from 744 bp to 9,888 bp. The near full genome (9,888 bp) had 28 SNVs and the full genome had 89 SNVs occurring in both the structural and non-structural regions of the comparator NAKV. BLAST analysis of the highly conserved NS5 region showed highest homology (99%) to NAKV of Uganda, followed by CuaCua virus (92%), Palm creek virus (82%), and *Culex flavivirus* (74%). Phylogenetic analysis of the full genome indicates that the virus is genetically distant from mosquito and tick borne group of human pathogenic flaviviruses. The observed structural variations can be an adaptation to the Kenyan environment. To our knowledge this is the first report of NAKV in Kenyan *Mansonia* and *Culex* mosquitoes.

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### PORTABLE GENOMIC SURVEILLANCE OF ZIKA VIRUS IN BRAZIL

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Zika virus (ZIKV) transmission in the Americas was first confirmed in May 2015 in Northeast Brazil. Brazil has the highest number of reported ZIKV cases worldwide as well as the greatest number of cases associated with microcephaly and other birth defects. Following the initial detection of ZIKV in Brazil, 47 countries and territories in the Americas have reported local ZIKV transmission, with 24 of these reporting ZIKV-associated severe disease. Yet the origin and epidemic history of ZIKV in Brazil and the Americas remain poorly understood, despite the value of such information for interpreting past and future trends in reported microcephaly. To address this we generated 54 complete or partial ZIKV genomes, mostly from Brazil, and report data generated by the ZIBRA project - a mobile genomics lab that travelled across Northeast (NE) Brazil in 2016. One sequence represents the earliest confirmed ZIKV infection in Brazil. Joint analyses of viral genomes with ecological and epidemiological data estimate that ZIKV epidemic was present in NE Brazil by March 2014 and likely disseminated from there, both nationally and internationally, before the first detection of ZIKV in the Americas. Estimated dates of the international spread of ZIKV from Brazil indicate the duration of pre-detection cryptic transmission in recipient regions. NE Brazil's role in the establishment of ZIKV in the Americas is further supported by geographic analysis of ZIKV transmission potential and by estimates of the virus' basic reproduction number.

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### DYNAMICS OF ANTI-ZIKA VIRUS IGM ANTIBODY IN A PROSPECTIVE COHORT STUDY

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The duration of detection of anti-Zika virus (ZIKV) IgM antibody is necessary to inform diagnostic testing algorithms. CDC recommends testing serum specimens collected 4 days to 12 weeks after symptom onset or ZIKV exposure for anti-ZIKV IgM. With interim data from an ongoing cohort in Puerto Rico of patients with ZIKV infection confirmed by RT-PCR, we estimated the time to loss of detection of anti-ZIKV IgM antibody. Index patients were recruited following detection of ZIKV RNA in serum or urine specimens. Index-patients' household contacts were also

tested, and if positive by RT-PCR, were invited to join the cohort. Follow-up visits occurred every week for four weeks and at 2, 4, and 6 months post-enrollment. Serum collected at each visit was tested by anti-ZIKV IgM antibody capture enzyme-linked immunosorbent assay (MAC ELISA). Data from participants that reported a recent illness consistent with ZIKV disease were used to determine time in days post symptom onset to loss of anti-ZIKV IgM detection. We used Weibull regression models to address interval censoring and estimate medians and percentiles. Of 270 symptomatic cohort participants, 139 (51.5%) were female, including 9 pregnant women, and median age was 36 years (range: 9 months-83 years). Anti-ZIKV IgM antibody was detected in 263 (97.4%) participants, and through January 31, 2017, 114 (43.4%) had become IgM-negative. Median time-to-loss of anti-ZIKV IgM was estimated as 117.5 days post symptom onset (95%CI: 107.4, 127.7), and the 75th percentile was 169.9 days (95%CI: 153.7, 186.1). Twelve weeks after illness onset, 69% of participants still had detectable anti-ZIKV IgM. No significant differences in duration of detection of anti-ZIKV IgM were observed by age or sex. Our data suggest nearly all symptomatic ZIKV-infected individuals develop anti-ZIKV IgM antibody. However, as few pregnant women were included, it is unclear if these findings can be generalized. The observed estimates for the duration of detection of anti-ZIKV IgM antibody are consistent with current CDC testing recommendations.

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### CHARACTERISTICS OF RASH IN PATIENTS WITH ZIKA VIRUS INFECTION, PUERTO RICO, 2016

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Zika virus is a flavivirus that is spread most frequently by the bite of an infected *Aedes* species mosquito, and presents with symptoms such as rash, fever, arthralgia, and nonpurulent conjunctivitis. ZIKV is a cause of microcephaly and has been associated with other congenital birth defects if spread from a pregnant mother to its fetus. Since ZIKV was first identified in the Americas in 2015, there has been a paucity of data describing the characteristics of rash in infected individuals. In this cross-sectional study, we describe the characteristics of the rash of patients with ZIKV infection at the time of presentation to the Emergency Room. Patients with rash were enrolled from the Sentinel Enhanced Dengue Surveillance System (SEDSS), a surveillance system that recruits patients presenting to an emergency department or out-patient clinic with fever, history of fever for <7 days, rash and arthralgia, or rash and nonpurulent conjunctivitis, and follows them through their illness. Blood, urine, nasopharyngeal and oropharyngeal specimens are collected and tested by RT-PCR and immunodiagnostic methods as appropriate for Zika and other infectious agents. Fifteen out of the 24 recruited patients were confirmed to be PCR positive for ZIKV. Rash was the initial symptom in only 2 (13.3%) patients, while a headache was the most common initial symptom (5 of 15 [33.3%]). The number of days between the start of symptoms other than rash and rash was a median of 2 days. The body parts where the rash most commonly started were the face in 6 (40%) and chest in 6 (40%). All 15 (100%) patients had rashes that were erythematous, maculopapular, generalized, blanching, and confluent. Rash itchiness was present in 11 (73.3%) patients compared to 4 of 9 (44.4%) of the negative cases ( $p=0.212$ ). The most common patterns of progression of the rash were cephalocaudal 5 (33.3%) and centrifugal 5 (33.3%). Correctly identifying the rash of a ZIKV infection can be useful in differentiating it from other viral diseases with similar presentation.

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### IVERMECTIN INHIBITORY EFFECTS ON ZIKA VIRUS AND CHIKUNGUNYA VIRUS INFECTION

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Zika virus (ZIKV) and chikungunya virus (CHIKV) are co-circulating in many countries including Thailand. Both viruses are transmitted to humans through the bite of *Aedes* mosquitoes. Outbreaks of these viruses have sporadically occurred and there are no vaccines or antiviral treatments available for either of these potentially devastating diseases. Ivermectin is an anti-parasitic drug which is approved for use in humans at a concentration of 150-200 µg/Kg which has been shown in pharmacokinetic studies to correspond to serum concentrations in humans of 7-95 ng/ml. In this study, we investigated the potency of ivermectin to inhibit ZIKV (Asian genotype: SV127/14) and CHIKV (Vaccine strain clone 181) infections *in vitro*. Two mammalian cell lines (vero and LLC-MK2) and a mosquito cell line (C6/36) were treated with various concentrations of ivermectin and inoculated with ZIKV or CHIKV at MOI of 0.1. We found that ivermectin was able to reduce the number of plaques of ZIKV and CHIKV in both cell types. No impact on cytopathic effect in LLC-MK2 and C6/36 cell lines was observed but an inhibitory effect during virus penetration/replication was seen in both mammalian and mosquito cell lines. Importantly, the half maximal inhibitory concentration (IC50) of ivermectin for ZIKV (618 ng/ml) and CHIKV (2,360 ng/ml) infections in vero cells would fall within the range of serum concentrations previously deemed to be safe by the Food and Drug Administration for mass-drug administration for parasitic infections. These data suggest that ZIKV is more sensitive to ivermectin than CHIKV. The results from this study indicate a potential use for ivermectin as a treatment option for ZIKV and CHIKV.

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### PASSIVE IMMUNIZATION AND CHALLENGE IN AN AG129 MOUSE MODEL OF YELLOW FEVER VIRUS INFECTION

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Yellow fever virus (YFV) is a mosquito-borne Flavivirus that can cause hemorrhagic fever and liver disease. Despite the availability of an immunogenic and protective vaccine, YFV causes thousands of infections annually. Recent outbreaks have been exacerbated by vaccine shortages indicating the need for development of additional counter measures. Here we present a lethal mouse model of YFV infection that is suitable for testing both vaccine and therapeutic counter measures under BSL-2 containment. The fifty percent lethal dose of the attenuated YFV strain 17D-204 was determined in mice deficient in interferon  $\alpha/\beta$  and  $\gamma$  receptors (AG129). Groups of five mice received a single intraperitoneal (i.p.) inoculation of YFV-17D-204 ranging from 10 to 5x10<sup>5</sup> plaque forming units (PFU)/mouse and were observed daily for signs of morbidity. The majority of mice experienced drastic weight loss preceding euthanasia. Some mice developed neurological symptoms including weakness in limbs and limb paralysis. Overall, eight animals survived until the conclusion of the study; however, all had signs of infection including viral RNA in serum and tissues as detected by quantitative RT-PCR. To determine the immunogenicity of the World Health Organization's vaccine reference strain YFV 17D-168-73, groups of wild-type 129 mice were vaccinated by i.p. injection with 1,000 PFU. An additional group served as a naïve control. Serum collected following vaccination was used to determine

neutralizing antibody titers by plaque reduction neutralization test (PRNT). All mice vaccinated with YFV 17D-168-73 seroconverted to YFV. Serum with the five lowest PRNT50 titers were transferred into AG129 donor mice. The day after transfer, mice were challenged with a lethal dose of YFV 17D-204. Mice that received serum from unvaccinated animals developed lethal disease characterized by rapid weight loss and high viral loads in the brain. Mice that received serum from mice vaccinated with the YFV 17D-168-73 were protected from challenge. This model can be used for the evaluation of medical counter measures to YFV infection.

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### **DISTINGUISHING SECONDARY DENGUE VIRUS INFECTION FROM ZIKA VIRUS INFECTION WITH PREVIOUS DENGUE BY A COMBINATION OF THREE SEROLOGICAL TESTS**

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The explosive spread of Zika virus (ZIKV) and associated microcephaly present an urgent need for sensitive and specific serodiagnostic tests, particularly for pregnant women in dengue virus (DENV)-endemic regions. Recent reports of enhanced ZIKV replication by dengue-immune sera have raised concerns about the role of previous DENV infection on the risk and severity of microcephaly and other ZIKV complications. In this study, we established enzyme-linked immunosorbent assays (ELISAs) based on ZIKV and DENV non-structural protein 1 (NS1) to test convalescent-phase and post-convalescent-phase serum/plasma samples from reverse-transcription-polymerase chain reaction-confirmed-cases including 20 primary ZIKV, 20 ZIKV with previous dengue, 54 secondary DENV, and 16 primary DENV1 infections. ZIKV-NS1 IgM and IgG ELISAs combined can detect ZIKV infection with a sensitivity of 95% and specificity of 60%. ZIKV-NS1 IgG cross-reactivity by samples from cases with secondary DENV infection ranged from 67% to 28% (within 1 month to 1-2 years post-onset of symptoms, respectively). Addition of DENV1-NS1 IgG ELISA can distinguish primary ZIKV infection; the ratio of absorbance of ZIKV-NS1 to DENV1-NS1 IgG ELISA can distinguish ZIKV with previous DENV and secondary DENV infections with a sensitivity of 87% and specificity of 81.3%. An algorithm for ZIKV serodiagnosis based on three simple ELISAs is proposed to distinguish primary ZIKV, ZIKV with previous dengue, and secondary DENV infections; this could be applied to serological diagnosis for ZIKV vs DENV clinical infections, serosurveillance, monitoring ZIKV infection during pregnancy and in studies to understand the epidemiology, pathogenesis and complications of ZIKV in dengue endemic regions.

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### **EVALUATION OF ZIKA CASES IN ACTIVE DUTY U.S. MILITARY AND DEPENDENTS**

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The Naval Infectious Diseases Diagnostic Laboratory (NIDDL) is a DoD Clinical Laboratory Improvement Program (CLIP/CLIA) and College American Pathologist (CAP) accredited laboratory that serves as a reference clinical laboratory for the detection and identification of high-risk and emerging infectious diseases in support of joint services military treatment facilities (MTFs) across the globe. The NIDDL has been a major center for Zika virus (ZIKV) testing for the CONUS and OCONUS DoD healthcare facilities including the US Coast Guard since ZIKV was declared as a WHO emergency and began to impact military population in 2016. With the support from the CDC, the Triplex qRT-PCR that can detect ZIKV, dengue virus (DENV), and/or chikungunya virus (CHIKV) simultaneously was implemented to detect ZIKV in symptomatic cases. The CDC Zika IgM-capture ELISA and ZIKV PRNT were also applied to support ZIKV diagnosis. In addition to symptomatic cases, samples were also received from asymptomatic active duty members or their spouses who had traveled to endemic ZIKV areas or had unprotected sex. Of the 584 suspected ZIKV cases, 27 (4.6%) were confirmed ZIKV cases: 9.0% (15/167) symptomatic ZIKV cases, 1.9% (5/259) asymptomatic cases, and 4.4% (7/158) have no history provided. Four patients were pregnant at the time of diagnosis, one which was symptomatic and gave birth to a baby without microcephaly. Five of suspected ZIKV patients (3.0%) were confirmed to be DENV cases by Triplex qRT-PCR. The majority of ZIKV infections happened after the patients traveled to countries in Latin America, primarily Puerto Rico, Mexico, Jamaica, and Honduras. Two cases had previously traveled to Singapore. Phylogenetic analysis is in progress to characterize and compare the positive ZIKV cases. These findings present an initial assessment on the burden of Zika infections among DoD personnel and is the foundation for future studies to examine short and long-term clinical history in these cases.

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### **CLINICAL AND EPIDEMIOLOGICAL FACTORS ASSOCIATED WITH ZIKA VIRUS INFECTION IN LEON, NICARAGUA, 2016-2017**

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Zika virus (ZIKV) is a flavivirus related to dengue that usually causes mild symptoms but was unexpectedly shown to cause severe congenital malformations like microcephaly in some infants born to women infected during pregnancy. Like dengue, ZIKV is spread by *Aedes* mosquitoes, it can also be sexually transmitted. The ZIKV epidemic arrived in Nicaragua in mid-2016. We conducted a cohort study of patients presenting to a health center with fever, rash, or conjunctivitis in Leon, Nicaragua, October 2016-February 2017. Acute ZIKV infection was diagnosed by PCR in blood, urine, or saliva. All participants completed a questionnaire about demographic and medical characteristics at baseline. We recruited 55 patients and detected ZIKV in 35 (64%). Median age was 22 years (range 2-75 years); 39% were under 18 years of age, and 69% were female. There was no significant difference in risk of ZIKV infection by age or sex. All 6 pregnant women enrolled were ZIKV-infected. History of prior dengue or chikungunya infection was not associated with ZIKV infection. Though not significant, water storage was associated with increased risk (OR 2.8, 95% CI 0.72, 12.02). There was a trend towards a protective effect of community spraying (OR 0.20, 95%CI 0.004, 1.84) but not personal bed net or repellent use. Recent sexual activity was not associated

with ZIKV infection. Rash was strongly associated with ZIKV infection (OR 13.50, 95%CI 2.19, 138.87) but fever (OR 0.24, 95%CI 0.02, 1.35) and conjunctivitis (OR 2.60, 95%CI 0.60, 11.30) were not. Retro-orbital pain (OR 0.19, 95%CI 0.04, 0.77) and myalgia (OR 0.19, 95%CI 0.03, 0.84) were associated with not having ZIKV. Total white blood cell counts were higher in ZIKV-infected subjects ( $p=0.03$ ), with a greater proportion of lymphocytes ( $p=0.005$ ); other cell lines were unaffected. Our findings are congruent with reports from other countries, confirming that rash and lack of fever are characteristic signs of ZIKV infection in the general population. Additionally, community-wide vector control campaigns stressing appropriate water storage and judicious use of insecticide may have a role in arbovirus epidemic control.

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### DEVELOPMENT, CHARACTERIZATION, AND PRE-CLINICAL IMMUNOGENICITY AND EFFICACY OF A PURIFIED, INACTIVATED ZIKA VIRUS VACCINE (PIZV) CANDIDATE

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In 2015, clusters of Guillain-Barre Syndrome (GBS) in adults and microcephaly in newborns were temporally associated with Zika virus (ZIKAV) infection in Northern Brazil, prompting the World Health Organization (WHO) to declare ZIKAV a public health emergency of international concern (PHEIC). Takeda is responding to this global health emergency by developing a purified, inactivated vaccine for ZIKAV (PIZV). This report describes the generation of a ZIKAV pre-master virus seed (pre-MVS) and preclinical efficacy of PIZV derived from the pre-MVS. The pre-MVS was generated by amplifying a ZIKAV strain PRVABC59 isolate obtained from the CDC in Vero cells (P1), performing three rounds of plaque purification (P2-4), and twice amplifying in Vero cells (P5 and P6). Two pre-MVS clones were chosen for further pre-clinical development and analysis based on growth phenotype and genotype. Virus stocks amplified from the pre-MVS were purified and inactivated with formalin to generate the PIZV candidates. The PIZV candidates formulated with alum were immunogenic in CD1 mice. AG129 mice vaccinated with a low or high dose of PIZV candidates formulated with alum generated strong neutralizing antibodies against ZIKAV and were fully protected from lethal ZIKAV challenge. No weight loss or clinical signs of illness were observed in vaccinated mice, and none had detectable infectious viremia three days post challenge (PC). In contrast, challenge of all naïve mice resulted in high viremia on day 3 PC and morbidity/mortality between day 10 and 18 PC. Our results demonstrate that the PIZV candidates are highly efficacious against ZIKAV infection in the ZIKAV-susceptible AG129 mouse model.

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### EVIDENCE OF HUMAN INFECTION BY A NEW MAMMARENAVIRUSES ENDEMIC TO SOUTHEASTERN ASIA

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Southeast Asia is a recognised hotspot for emerging infectious diseases, many of which have an animal origin. Although known pathogens account for a large proportion of human infectious diseases in the region, a substantial number of febrile illnesses have an unknown aetiology. Mammarenavirus infections contribute significantly to the human disease burden in both Africa and the Americas, but to date only two mammarenaviruses, the widely spread lymphocytic choriomeningitis virus

and the Wenzhou virus recently described in Chinese rodents, are recorded from Asia. Here we report the presence of a novel mammarenavirus and of a genetic variant of the Wenzhou virus and provide evidence of mammarenavirus-associated human disease in Asia. The association of these viruses with widely distributed mammal species, commonly found in human dwellings and in peridomestic habitats, adds to the known aetiologies of infectious diseases for this region and illustrates the potential for widespread zoonotic transmission.

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### ZIKA VIRUS: A SYSTEMATIC REVIEW AND META-ANALYSIS IN EPIDEMIOLOGY, CLINICAL MANIFESTATIONS, AND OUTCOMES

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Zika virus is a mosquito-borne flavivirus that was recently declared as a global public health emergency due to its spreading around the world and its association with serious congenital malformations and neurological disorders. We aim to report the total Zika infected cases and the geographic distribution of those cases. We also intend to provide a thorough description of the epidemiological characteristics, signs and symptoms, biomarkers, outcomes, complications and route of transmission of Zika virus. We conducted a systematic review of the scientific literature, and meta-analyzed data that were sufficiently comparable. Data was obtained through an electronic search in 8 main databases, and the references lists of all included articles were also searched for relevant articles. Overall, this review included 258 articles. Most included articles were case reports and cross sectional studies, written in English language, and published between 2015 and 2016. The total number of confirmed, suspected, and probable cases of Zika virus infection was 54663. Confirmed cases span over 79 countries, with the highest frequency being in the region of the Americas. Excluding neonates with microcephaly, most patients were females and had a mean age of 35.8 years. Most common method of transmission was by mosquitoes, followed by sexual contact and blood transfusion. While most common clinical symptoms included skin rash, fever, arthralgia, myalgia, and headache, conjunctivitis and the most common lab abnormalities were leukopenia and thrombocytopenia. The medical literature reported 1018 cases of microcephaly, 288 Guillain-Barré Syndrome, and only one case of encephalitis in association with Zika virus infection. Death occurred in 17 cases with Zika virus infection, with a mean age of 50.5 years, and most of them had underlying diseases. Due to its serious complications, and since no cure or vaccine are available, Zika virus remains an important threat to the public health that necessitates a wide cooperation on a global scale in developing prevention and managing strategies.

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### SPATIO-TEMPORAL ANALYSIS OF EBOLA VIRUS DISEASE IN SIERRA LEONE, MAY 2014-SEPTEMBER 2015

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The 2014-2015 Ebola virus disease (EVD) outbreak was unprecedented, affecting ten countries, with the greatest burden of cases and deaths

occurring in Guinea, Sierra Leone, and Liberia. Over two years, Sierra Leone experienced 14,124 suspected and/or confirmed cases and 3,956 deaths. The timing and location of these cases, however, was not homogenous. We aim to measure the location and timing of EVD clustering using chiefdom-level data to shed light on the utility of geospatial tools in infectious disease surveillance. A total of 8,358 confirmed EVD cases were documented from May 23, 2014 to September 13, 2015 by the Sierra Leonean Ministry of Health and Sanitation. Spatial scan statistics were used to describe the trajectory of EVD clusters over space and time. Areas of high and low clustering were detected using cases aggregated into one- and two-week periods. Four clusters of high transmission were identified, the first of which was detected on June 20, 2014, three months prior to the deployment of the United Nations Mission for Emergency Ebola Response. Three regions of low transmission clustering were detected, beginning in January 2015 and persisting until the epidemic's end on September 13, 2015. The use of spatial scan statistics to identify clustering of EVD in Sierra Leone successfully described the timing and spread of the virus across the country. Disease surveillance that incorporates freely available geospatial tools to prospectively analyze routine data recorded by the health system can contribute to deploy early emergency responses, and therefore thwart the spread of infectious agents.

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### DEVELOPMENT OF HIGHLY SENSITIVE SEROLOGICAL TESTS FOR REOVIRUS SEROPREVALENCE

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Luciferase immunoprecipitation system (LIPS), offers the advantage of using easily produced crude cell lysate containing viral antigens that can be used directly for antibody detection in patient serum samples. This highly sensitive and quantitative serological assay is also an affordable and rapid alternative to conventional serology methods. We have developed the LIPS platform for two members of the pteropine reovirus family, the Melaka virus and Nelson Bay virus, to utilize for a seroprevalence study in Singapore, which is currently understudied and unknown. This study will help us to not only implement an efficient serology platform for use in the Singapore region, but also to enhance our capability in clinical research to differentially diagnose various diseases.

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### DEMOGRAPHIC DETERMINANTS OF ANTI-POLIOVIRUS TYPE 3 ANTIBODIES AMONG ORALLY IMMUNIZED INDIAN CHILDREN

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Oral vaccines perform poorly in developing countries. The oral polio vaccine (OPV) is less immunogenic in developing countries. In India, the per-dose vaccine efficacy of OPV has been estimated at 21% as compared to 65% in industrialized countries and hence more doses of OPV are necessary to protect children in India compared to children in developed countries. This poor performance is associated with various factors like maternal antibodies, age at vaccination, malnutrition and micronutrient deficiencies, persistent exposure to pathogens in the environment and tropical enteropathy. Immunogenicity is lowest for serotype 3 among the three poliovirus serotypes in children vaccinated with OPV. We examined the demographic determinants of anti-poliovirus type 3 antibodies among children of age 1-59 months in Vellore, Southern India. We pooled data from three studies that had documented neutralizing antibodies (NAb) titres to poliovirus type 3 (PV3) and basic demographic data of 9518 children residing in rural and urban parts of Vellore. Of 9518 children

analysed at a mean age of 9.2 (standard deviation [SD]-7.3) months, 86% (95% confidence interval (CI): 85.3% - 86.7%) had protective antibodies to PV3. The seroprevalence was 87.33% (86.36% - 88.24%) for males and 84.68% (83.61% - 85.70%) for females. The number of OPV doses received was the main determinant of seroprevalence; children were twice likely to be seropositive for one dose increase of OPV. In multivariable logistic regression analysis OPV, male sex and age were independently associated with seropositivity while place of residence was not (Odds Ratios (OR): 1.87(95% CI: 1.75 - 2.00) per dose of OPV, 1.23 (1.09 - 1.39), 0.95 (0.92 - 0.97) and 1.07 (0.93 - 1.23) respectively). Though immunisation coverage in India has improved, immunogenicity to oral polio vaccine remains lower than in industrialised countries. Understanding the causes that play a role in poor performance of oral vaccines remains important for developing nations.

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### ZIKA VIRUS INFECTION AND CHIKUNGUNYA FEVER OUTBREAKS IN RIO GRANDE DO SUL, BRAZIL, 2014-2016

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In the last years Brazil has experienced outbreaks of important arboviral diseases, including Yellow Fever, Dengue, Chikungunya and Zika. Of continental size and with a population of approximately 207 million people, with highly urbanized areas, the country's conditions favor the spreading of mosquitoes such as *Aedes aegypti* and *A. albopictus*, vectors of the above-mentioned viral diseases. The epidemiology of arboviral diseases varies throughout the country, with an increase in prevalence in all regions. Rio Grande do Sul (RS), the Southernmost state in Brazil, was free of such diseases until 2010, when the first autochthonous case of Dengue Fever was reported. Since then, not only DENV but also Chikungunya virus (CHIKV) and Zika virus (ZIKV) have been circulating in the state. This study reports the epidemiology of Chikungunya fever and Zika virus infection in RS from 2014 to 2016. The number of confirmed CHIKV cases increased from 7 to 79 in the period, most of them consisting of imported cases; autochthonous infections were reported only in 2016, with 5 cases. As for ZIKV, the first confirmed case in RS was in 2015 with a patient that had traveled to Recife, Northeast Brazil, where ZIKV had a high prevalence. In 2016, 86 cases of ZIKV were confirmed in RS, 44 of which were considered autochthonous infections. Furthermore, 2 cases of microcephaly due to Congenital Zika Syndrome were reported in the period in babies born from infected mothers. In conclusion, this study provides detailed information about the first cases of CHIKV and ZIKV in Rio Grande do Sul, contributing for arboviruses surveillance in Brazil and for disease control and prevention.

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### THE MOLECULAR EPIDEMIOLOGY AND SPATIAL DYNAMICS OF HUMAN PARAINFLUENZA SEROTYPE 3 IN PERU

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Human parainfluenza viruses (HPiVs) cause almost one third of lower respiratory tract infections and pose considerable burdens on the most vulnerable, who are infants, immunocompromised and chronically-ill

patients. The most prevalent serotype identified in these groups of people is HPIV-3. However, despite being frequently identified there is insufficient genomic data to clearly understand the evolution and temporo-spatial transmission dynamics of HPIV, particularly in low and middle-income countries such as Peru. Redressing this knowledge gap is critical for vaccine development and public health preparedness. We therefore undertook whole genome sequencing of 60 HPIV-3 positive respiratory specimens obtained from influenza-like illness surveillance between 2007 and 2012 in Lima (n =3), Piura (n=41) and Loreto (n =16), three ecologically and demographically distinct locations within Peru. These data were aligned with background sequence data from GenBank to yield hemagglutinin-neuraminidase gene (n = 545) and whole genome (n = 118) datasets. Recombinants were removed after screening with the RDP-4 software. Maximum-likelihood phylogenies were inferred using a GTR+I+G substitution model in the RAxML package. We found ample evidence of frequent viral diffusion in and out of Peru and extensive viral traffic between Latin America and other regions around the globe. There was some evidence of regional HPIV-3 circulation within South America, including viral traffic between Argentina and Peru. We also identified spatial clustering of HPIV-3 strains by location within Peru, indicating that Peruvian HPIV-3 epidemics are at least semi-localized. While the lack of data from Lima limited conclusions of fine-scale transmission dynamics between Peruvian locales, the mixing of data from the geographically and functionally distant locales of Piura and Loreto supported the rapid dispersal of this virus within a single country. Our findings indicate HPIV-3 is a spatially fluid virus in the tropics, with widespread diffusion on multiple spatial scales driven by human mobility.

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### ISOLATION AND CHARACTERIZATION OF BUKAKATA ORBIVIRUS, A NOVEL VIRUS FROM A UGANDAN BAT, AND ASSOCIATED PULMONARY PATHOLOGY IN EXPERIMENTALLY INFECTED JAMAICAN FRUIT BATS (*ARTIBEUS JAMAICENSIS*)

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In 2013, a novel orbivirus (*Reoviridae: Orbivirus*) was isolated from an Egyptian fruit bat (*Rousettus aegyptiacus*) in Uganda. Preliminarily named "Bukakata orbivirus" after the region where the infected bat was captured, this virus is the fourth identified orbivirus of bats. The genomes of all four bat orbiviruses (Bukakata, Ife, Fomede, and Japanaut viruses) were sequenced to assess their phylogenetic placement within the genus *Orbivirus*, and develop hypotheses regarding virus-vector associations. Whole genomes of all four viruses were sequenced using an Illumina platform and assembled *de novo*. Phylogenetic analysis placed Fomede and Bukakata orbiviruses in the tick-borne clade, and Japanaut and Ife in the mosquito/*Culicoides* clade. To begin studying the effect of infection with Bukakata orbivirus on a bat host, three male Jamaican fruit bats (*Artibeus jamaicensis*) were inoculated intraperitoneally with 5.3 log<sub>10</sub> pfu Bukakata orbivirus and monitored daily for signs of clinical disease. On day 12, all three bats were diffusely hyperemic and tachypneic and, thus, were humanely euthanized. Histopathologic lesions of perivascular inflammation, hemorrhage and edema were present in varying degree of severity in the liver, lung and kidney of all three bats. Additional lesions included meningeal hemorrhage in two of

the bats and evidence suggestive of early hepatic vasculitis in the other. Eosinophilic and suppurative gastroenteritis affected all bats with one containing intraluminal bacilli, suggesting secondary bacterial infection. Immunohistochemistry and qPCR will be performed to assess relative abundance of virus in various organ systems to optimize future analyses. Future experimental infections will be performed to monitor temperature, physiological and immune parameters, virus shedding and viremia throughout the course of infection. These preliminary data are critical in the assessment of the potential role of bats as reservoirs for arboviruses.

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### FACTORS INVOLVED IN HUMAN INFLUENZA VIRUS ISOLATION USING MDCK CELL CULTURE FROM SOUTH AND SOUTHEAST ASIAN SURVEILLANCE SPECIMENS

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Surveillance for influenza viruses is required for monitoring of influenza virus activity, preparedness for the upcoming season and evaluation of vaccine efficacy. USAMD-AFRIMS has conducted influenza surveillance in Bhutan, Nepal, Philippines, Maldives and Thailand since 2008. Real-time RT-PCR and virus isolation using Madin-Darby canine kidney (MDCK) cell culture are regularly used to detect and classify influenza virus subtypes. Here, we investigated potential factors that may affect the success of influenza virus isolation. Among 5,628 positive RT-PCR samples, 3,427 (60.9%) were successfully isolated and identified by hemagglutination inhibition assay. The positive virus isolation rate of influenza B was significantly higher than that of influenza A (68.3% versus 54.6%). 52.3% of RT-PCR positive specimens for influenza A were H1N1 and 47.4% were H3N2. The isolation rate for influenza A subtypes differed significantly, with an isolation rate of 65.5% for H1N1 and 53.8% for H3N2. Among influenza B isolates, B/Brisbane was the most common subtype (46.8%), then B/Massachusetts (31.3%). Rates of isolation increased with lower cycle-to-threshold values (Ct) from RT-PCR, which translates to higher viral load. The rates of influenza A virus isolation in high (Ct ≤ 20), moderate (20 < Ct < 25), low moderate (26 < Ct < 30) and low viral content (Ct ≥ 30) were 79.3%, 67.7%, 40.8% and 12.1%, respectively. For influenza B virus, isolation rates in high, moderate, low moderate and low viral content were 95.8%, 84.2%, 55.6% and 19.8%, respectively. One possible explanation for these results is that MDCK cells are more susceptible to influenza B than influenza A at all titers of viral inoculum. The relatively low isolation rate (60.9%) compared to RT-PCR might due to decreasing MDCK permissiveness with a high number of cell passages. Viral genetic differences may play an important role, as we have seen growth curve differences between subtypes at the same TCID<sub>50</sub>. Information from this study can be used to improve influenza virus isolation rates for influenza surveillance and vaccine development efforts.

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### AVIAN INFLUENZA EXPOSURE IN YOUNG THAI MALES FROM SUPHANBURI PROVINCE OF THAILAND

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Highly pathogenic avian influenza A H5N1 virus outbreaks in Thailand resulted in human deaths during 2004-2006. Suphanburi Province reported the highest cumulative number of H5N1 poultry outbreaks in the country. The high density of rice crops and large number of free-grazing ducks in the Province are recognized as risk factors for avian to human H5N1 transmission. In this study, we determined seroprevalence of H5N1 virus exposure among Royal Thai Army recruits whose place of

birth and residence was located in Suphanburi (high-risk area of H5N1 exposure) as compared to recruits from Bangkok where has no report of H5N1 outbreak (low-risk area of H5N1 exposure). A total of 1,200 recruit sera collected during 2001-2002, 2004-2005, and 2010-2011 were analyzed to represent periods superseding, during, and following H5N1 human-case outbreaks in Thailand. H5N1 seropositivity by enzyme-linked immunosorbent assay (ELISA) was not indicated for any period evaluated in either province. Weak reactivity to A/Duck/Hong Kong/820/80 (H5N3) and A/Hong Kong/156/97 (H5N1) strains was displayed in 4 of 1,200 sera (0.3%) by hemagglutination inhibition assay. All samples were also measured for IgG antibody specific to seasonal influenza A (H1N1 and H3N2) using a commercial ELISA kit and 1,149 of 1,200 sera (95.8%) were seropositive. The seropositivity was significantly associated with study area, time periods (2001-2002 to 2010-2011), and residence area ( $p$  value < 0.05). The time period was significantly associated with IgG seropositivity by multivariate analysis (OR 8.4; 95% CI, 3.1 - 22.7 for 2004-2005, OR 21.3; 95% CI, 4.9 - 92.7 for 2010-2011). The IgG antibody prevalence of seasonal influenza A increases with later time period. The detectable antibody concentration was slightly higher in Bangkok than in Suphanburi. A high prevalence of seasonal influenza A was exhibited in this study but there was no serologic evidence of H5N1 infection, demonstrating that there were no cross-reacting between seasonal and avian influenza antibodies among this population.

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### CHARACTERIZATION OF NOVEL NATURAL RNA VIRUSES OF THE AFRICAN MALARIA MOSQUITO, *ANOPHELES COLUZZII*

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Little is known about *Anopheles* mosquito interactions with arboviruses and other RNA viruses. Most research to date on *Anopheles* host-pathogen interactions and immunity has focused on *Plasmodium*, which has led to a relative lack of study of *Anopheles* and viruses. Only one arbovirus is known to be consistently transmitted by *Anopheles* mosquitoes, the alphavirus o'nyong-nyong. Culicine mosquitoes appear to be the major arbovirus vectors, although for unknown reasons because anthropophilic anophelines bite the same viremic hosts. In addition, mosquitoes are colonized by a largely undescribed natural virome with unknown potential to influence biology and immunity of mosquito vector populations. These observations pose the question of what it would require for other arboviruses to adapt to anopheline mosquitoes as transmission vectors, and highlight the importance of studying *Anopheles* antiviral immunity, but tractable experimental models are lacking. We recently discovered two novel viruses in *Anopheles coluzzii* by deep sequencing and de novo assembly of small RNAs. One is a dicistrovirus in the same family as Drosophila C virus (DCV), and was named *Anopheles C virus* (AnCV), the other is in the same family as the important livestock arbovirus, bluetongue virus, and was named *Anopheles cypovirus* (AnCPV). Prevalence of AnCV and AnCPV varies developmentally in mosquitoes, with significant differences between the larval and adult stages. Abundance of the two viruses is negatively correlated in individual mosquitoes. Functional analysis revealed the implication of mosquito immune signaling pathways on virus replication, in some cases with differential influence on the two viruses. An experimental model was developed for AnCPV infection of *Anopheles* by bloodmeal in order to study antiviral response during the primary midgut infection and subsequent disseminated infection. The interaction between AnCV/AnCPV and *Plasmodium* species in *Anopheles* could also result in environmentally friendly weapons against malaria transmission.

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### COMPARISON OF SAMPLE PREPARATION METHODS FOR NEXT GENERATION SEQUENCING OF INFLUENZA A VIRUSES

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Influenza virus (IFV) represents a major public health concern worldwide. Study of the IFV genome has led to a greater understanding of reassortment, variance, mutations associated with antiviral drug resistance and antigenicity changes. In this study, two methods of double-stranded (ds) cDNA synthesis, ds cDNA synthesis kit (Invitrogen) with random hexamer and Single-Reaction Genome Amplification (SRA) with Flu A universal primers (Uni12A, Uni12G and Uni13) were compared. In addition, two different methods of library preparation were also compared, the Illumina Nextera XT and QIAGEN QIAseq FX. Ten IFV Type A isolates were used in this study to include five pandemic 2009 isolates and five H3N2 isolates. Performance of the different methods was measured by comparing percent of reads mapped to the IFV reference genomes (A/California/07/2009(H1N1) and A/Brisbane/11/2010(H3N2)) and mean depth of coverage (DOC) of the mapping per million reads sequenced. Statistical comparisons between the different treatments were performed using the paired t-test. When using SRA, the percentage of mapped reads increased 87 times (95%CI: 65-109) and DOC increased 126 times (95%CI: 92-161) when compared to the ds cDNA synthesis with random hexamer method. Overall, QIAseq FX library preparation provided significantly higher DOC ( $p=0.018$ ) with a mean difference of 275 (95% CI: 50-500) despite a 2.7% (95%CI: 0.5%-4.9%) decrease in the number of reads generated ( $p=0.016$ ). Better coverage was seen when stratified by the ds cDNA synthesis method but not statistically significant ( $p=0.117$  for random hexamer,  $p=0.078$  for SRA) due to limited sample size. In summary, SRA followed by QIAseq FX library preparation provided the whole genome of IFV for each sample with high IFV sequence reads and coverage through each genome segment especially at the 5' and 3' regions.

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### PREVALENCE OF NOROVIRUS INFECTION IN HOSPITALIZED CONGOLESE CHILDREN IN BRAZZAVILLE, REPUBLIC OF CONGO

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In the Republic of Congo (RoC), diarrheal diseases are the third cause of consultation and hospitalization after malaria and acute respiratory infections. Parasites, bacteria and viruses are the main agents responsible for these severe diarrheas. Our institution was the first to characterize pathogens responsible for severe diarrheal infections in Congolese children. Therefore, epidemiological data on Rotaviruses and Adenoviruses infections were recently published. In the same hospitalized children, we determined the prevalence of Norovirus infections according to the rainy or dry season and age and identified co-infection with rota- and or adenoviruses. Stool samples used in this descriptive study were collected between June 2012 and June 2013 in 249 children aged under five years and hospitalized in the pediatric ward at Makélékélé hospital (Southern Brazzaville, capital of RoC) for severe diarrhea. Viral RNA was extracted from stool samples and the presence of norovirus was screened by duplex nested Reverse Transcription Polymerase Chain Reaction (RT-PCR) using specific primers for genogroup I and II. The prevalence of Noroviruses either of genogroup I or II was 27.7 % (69/249). Children between 7 and

12 months were the most infected (59.4 %). There was no significant difference according to sex however higher proportion of infected children was found during the dry season (June-September). We detected Rotavirus-norovirus, Norovirus-adenovirus and Rotavirus-norovirus-adenovirus co-infections in 3, 1%, 1% and 0, 9% respectively. This study shows that Norovirus is an etiological agent that must not be ignored among causes of severe diarrhea in Congolese children. This investigation should be extended to other parts of the country.

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### **RICKETTSIA PARKERI IN AMBLYOMMA MACULATUM (ACARI: IXODIDAE) COLLECTED FROM MULTIPLE LOCATIONS IN SOUTHERN ARIZONA**

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*Rickettsia parkeri* is an emerging human pathogen transmitted by *Amblyomma* ticks in predominately tropical and subtropical regions of the western hemisphere. In 2014 and 2015, 1 confirmed and 1 probable case of *Rickettsia parkeri* rickettsiosis were reported from the Pajarita Wilderness Area, a semi-arid mountainous region in southern Arizona. To examine more closely the potential public health risk of *R. parkeri* in this region, a study was initiated to investigate the pervasiveness of *Amblyomma* ticks in mountainous areas of southern Arizona as well as to ascertain the infection prevalence of *R. parkeri* in these tick populations. During July 2016, a total of 182 adult ticks were collected and evaluated from the Pajarita Wilderness Area in Santa Cruz County and 2 additional sites in Cochise and Santa Cruz counties in southern Arizona. DNA of *R. parkeri* was detected in 44 (24%) ticks. DNA of "*Candidatus Rickettsia andeanae*" and *Rickettsia rhipicephali* was detected in 3 (2%) and 1 (0.5%) of the samples, respectively. These observations corroborate previous collection records and indicate that established populations of *A. maculatum* exist in multiple foci in southern Arizona. The high incidence of *R. parkeri* in these tick populations suggests a public health risk as well as the need to increase education of *R. parkeri* rickettsiosis for those residing, working in, or visiting this area.

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### **ENTOMOLOGICAL SURVEILLANCE IDENTIFY PRESENCE OF LUTZOMYIA VERRUCARUM SANDFLY (DIPTERA: PSYCHODIDAE) IN LEISHMANIASIS ENDEMIC COMMUNITY IN MEXICO**

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Continuous endemicity and re-emergence of vector-borne disease in Mexico have call for new approaches to vector control programs including regular surveillance. Correct and accurate identification of vectors is essential for successful surveillance program. This study is using DNA barcoding under the Mexican Barcode of Life (MexBol) project to improve vector surveillance and accurately delineate arthropod vector diversities

including sandfly for leishmaniasis control in Mexico. In October and November, 2016, sandflies were collected from different regions in leishmaniasis endemic state of Quintana Roo, Mexico using CDC light and Shannon traps as part of the health ministry surveillance program. Samples collected were sorted by sex and female samples were pooled for PCR for pathogen examination. Thirty-three (33) male samples were morphologically identified as 2 species with *Lutzomyia cruciata*, 28 (84.8%) and *Lutzomyia deleoni* 5 (15.2%). However, molecular identification using the 658-bp fragment of mitochondrial cytochrome oxidase subunit 1 gene revealed earlier identified *L. deleoni* to be *L. verrucarum* with nucleotide sequence having 90-93% identity match with species described from Peru after analysis on NCBI Basic Local Alignment Search Tool. Phylogenetic analysis with the Neighbour-Joining (NJ) tree also showed these species to cluster 100% with *L. verrucarum* that was also isolated from leishmaniasis endemic community in Peru. Although, further sample collection has been planned at the geographical location where these species were collected to confirm presence of local population, the role of exotic *L. verrucarum* on local transmission of leishmaniasis is currently unknown as this species has not been previously reported in Mexico. There is the need for a detailed ecological study to fully understand the role of this species in the transmission dynamics of leishmaniasis in Mexico.

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### **PHLEBOTOMINE SAND FLIES (DIPTERA: PSYCHODIDAE) AS POTENTIAL VECTORS OF LEISHMANIA PARASITES IN TSATEE, A NEW ENDEMIC COMMUNITY, VOLTA REGION**

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Leishmaniasis is a disease caused by *Leishmania* parasites which are transmitted by infected female sand flies. In Ghana, cutaneous leishmaniasis (CL) continues to spread to previously non-endemic areas. Trapping methods employed in the Ho District have caught predominantly large numbers of *Sergentomyia* species and very few *Phlebotomus* species. Identified *Leishmania* parasites were *L. tropica* and *L. major*, found in pools of *Sergentomyia ingrami* and *S. hamoni* and none in any of the *Phlebotomus* species found there. It suggests that contrarily to published works, *Phlebotomus* species played no role in the transmission of the disease in the affected communities, a major role ascribed to *Sergentomyia* species. This study expanded the scope by conducting similar studies in Tsatee, a new endemic community in the adjoining South Dayi District. It aimed to identify the potential vector species and detect *Leishmania* parasites that are responsible for CL transmission in the study area. Sand flies were collected using CDC light traps and aspirators. The non-blood fed sand flies studied were 99.91% *Sergentomyia* (n=3272) and 0.09% (n=3) *Phlebotomus*. All the blood fed species were of the genus *Sergentomyia*. *Leishmania* infections were detected using primers which target the conserved region of *Leishmania* spp. minicircle DNA of the parasite kinetoplast. *Leishmania* infection rates were *S. africana* (0.16%), *S. ingrami* (1.92%), *S. schwetzi* (1.80%), *S. hamoni* (2.60%), *S. similima* (0.32%), *S. ghesquierei* (1.05%), *S. antennata* (0.63%), *S. durenii* (3.30%) and for the first time in Ghana, *P. rodhaini* (33.33%). With the individual blood fed sand flies, infections were detected at rates *S. africana* (12.12%), *S. ingrami* (50.50%), *S. schwetzi* (33.33%), *S. similima* (7.69%) and *S. antennata* (50.00%). Detecting infections in *Sergentomyia* species further support the notion that they may play a significant role in the transmission process. The findings suggest an efficient *P. rodhaini* vector (33.33%), thus a major role of *Phlebotomus* in disease transmission cannot be discounted. The PCR products will be sequenced to identify the *Leishmania* species.

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### IMPACT OF IRRADIATION ON REPRODUCTIVE PERFORMANCE OF WILD AND LABORATORY *ANOPHELES ARABIENSIS* MOSQUITOES

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Sterile Insect Technique (SIT) aims at suppressing or decreasing insect pest population by introducing irradiated mass-reared insects into wild populations. However, both mass rearing and radiations can affect life history traits of some insects making them less competitive than their wild counterparts. In the malaria mosquito *Anopheles arabiensis*, many progress have been done to improve mating competitiveness of mass-reared irradiated males. However, to date, no study has been done to decipher entangled negative effects of laboratory rearing and irradiation on important reproductive traits. Such data could help to target more precisely research efforts to improve current techniques. Here, we used two sources of *An. arabiensis* from the same locality; one reared in laboratory for 5 generations and the other collected at late larval instar in the field. Pupae were irradiated and adult insemination rate, fecundity, oviposition behavior, fertility and male survivorship were assessed. Results revealed different impacts of both mosquito origin and irradiation onto reproductive processes. First, insemination rate was more intensively affected by irradiation in laboratory-reared mosquitoes than in wild ones. Second, oviposition behavior was different between the two mosquito origins with a quick "adaptation" of laboratory-reared mosquitoes to artificial conditions. Third, a greater proportion of eggs hatched in irradiated field than in laboratory-reared mosquitoes, but conversely, a larger proportion of larvae survived to the first instar in laboratory-reared than in field mosquitoes. Finally, while irradiation had no effect on longevity, field males survived better than those from laboratory. Effects of both mass-rearing and irradiation must be taken into account when investigations attempt to improve insect quality used in SIT.

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### HIGH THROUGHPUT SCREENING OF THE MICROBIOTA ASSOCIATED WITH TWO MALARIA VECTORS OF COLOMBIA

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The number of deaths caused by malaria has decreased worldwide, however, the dramatic increase in insecticide-resistant *Anopheles mosquitoes* has accelerated the search for alternative strategies to diminish or eliminate malaria vector populations. Recent studies have shown that some bacteria of the mosquito microbiome have important negative effects on the parasite development within the mosquito midgut, as in the vector's survival. However, little is known about the microbiota of Latin American anopheline mosquitoes and its significance for parasite blocking. Therefore, the purpose of this study is to characterize the midgut microbiota composition of two main Latin American malaria vectors, *Anopheles darlingi* and *A. nuneztovari*, collected in two malaria-endemic regions of Colombia. We characterized the bacterial microbiome of 64 adult (A) mosquito midguts, 12 larvae (L) midguts and 7 breeding sites (BS). A total of 15,909,048 bacterial 16S rRNA reads were grouped

into 274,990 swarms, from which we identified 14,440 unique OTUs. Preliminary results showed that the composition of the microbiota differs between regions and groups but not between species or feeding status. A deeper examination of the metadata is currently in progress and will reveal the microbiome composition at a more detailed taxonomical level, which is essential to discover novel potential candidates for vector biocontrol strategies.

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### SPECIES COMPOSITION OF PHLEBOTOMINE SAND FLIES AND BIONOMICS OF *PHLEBOTOMUS ARGENTIPES* IN AN ENDEMIC FOCUS OF VISCERAL LEISHMANIASIS IN BIHAR STATE, INDIA

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Visceral leishmaniasis (VL) also known as Kala-azar in India is transmitted to man by *Phlebotomus argentipes*. In the Indian subcontinent, Bihar state is endemic to VL. A year-long study was initiated in twenty four villages, within two districts of Bihar, to examine the species composition of Phlebotomine sand flies. In each village twelve CDC light traps were installed in three locations which include houses, cattle dwellings, and vegetation. In each location, 4 traps were installed. Traps were activated every two weeks for 12 hours from 1800 to 0600 hours. Sand flies trapped were transferred to the lab in Patna, identified, and recorded as to sand fly species. Our trapping was initiated in February 2016 and continued to December 2016. Sand flies were identified morphologically and molecular techniques were used to confirm identification. Blood meals were identified from trapped blood fed sand flies. We trapped 126,394 *P. argentipes*, of which 76,554 were males and 49,840 were females. We also trapped 1,465 *P. papatasi* and 24,218 *Sergentomyia* species. Earlier studies suggested sand fly behavior is more exophilic now, contrary to previous reports. We also trapped a large proportion of sand flies outside of houses and cattle sheds in vegetation. This suggest a new sand fly management tool is required to control the vector in peri-domestic vegetation in order to achieve elimination of VL in India.

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### DENGUE VECTOR CONTROL: BUILDING THE EVIDENCE BASE

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Dengue is endemic in many parts of the world with an estimated 390 million infections annually. Vector control remains the primary method of prevention and control. Despite substantial investment, the evidence guiding such interventions is limited in its quality and scope and is widely disbursed among academic journals and within national control programmes. Here we summarise our efforts to consolidate and evaluate the existing evidence base for dengue vector control. We have conducted a series of systematic literature reviews covering both single vector control interventions, including peridomestic space spraying (Esu 2010), Temphos (George 2015), *Bacillus thuringiensis israelensis* (Boyce 2013), copepods (Lazaro 2015) and larvivorous fish (Han 2015), pyriproxifen (Moaz 2017, submitted) and indoor residual spraying (Samuel 2017, submitted), as well as outbreak response (Pilger 2010) and service delivery (Horstick 2010). Our analysis shows that 1) vector control can be effective, but implementation and sustainability remain issues 2) single interventions are probably not effective, but even combinations of interventions have mixed results, 3) Interventions in response to outbreaks have limited effectiveness and, 4) the quality of service delivery, rather than the choice

of intervention, is likely the most important determinant of effectiveness. Based on these findings, we recommend a combination of chemical methods such as indoor residual spraying and insecticide treated materials, together with biological methods, where possible. Both larvae and adult mosquitos should be targeted simultaneously, while addressing waste and environmental management may have an incremental benefit. Our results also show that there is an urgent need to develop rigorous guidance for vector control studies, ensuring the validity and comparability of results. Studies should include measurements of human transmission data, where and when possible.

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### RESPONSES OF *GLOSSINA PALLIDIPES* AND *G. MORSITANS MORSITANS* TSETSE FLIES TO ANALOGUES OF DELTA-OCTALACTONE AND SELECTED BLENDS

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Previous studies have shown that delta-octalactone is an important component of the tsetse-refractory waterbuck (*Kobus defassa*) repellent odour blend. In the present study, structure-activity comparison was undertaken to determine the effects of the length of the side chain and ring size of the lactone on adult *Glossina pallidipes* and *Glossina morsitans morsitans*. The responses of the flies to each compound were studied in a two-choice wind tunnel. Increasing the chain length from C3 (delta-octalactone) to C4 (delta-nonalactone) enhanced repellency to both species (*G. pallidipes* from 60.0 to 72.0%, and *G. m. morsitans* from 61.3 to 72.6%), while increasing the ring size from six (delta-octalactone) to seven members (delta-nonalactone) changed the activity from repellency to attraction that was comparable to that of the phenolic blend associated with fermented cow urine ( $p > 0.05$ ). Blending delta-nonalactone with 4-methylguaiaicol (known tsetse repellent) significantly ( $p < 0.05$ ) raised repellency to 86.7 and 91.7% against *G. pallidipes* and *G. m. morsitans* respectively. Follow-up Latin Square Designed field studies (Shimba hills in coastal areas in Kenya) with *G. pallidipes* populations confirmed the higher repellence of delta-nonalactone (with/without 4-methylguaiaicol) compared to delta-octalactone (also, with/without 4-methylguaiaicol). The results show that subtle structural changes of olfactory signals can significantly change their interactions with olfactory receptor neurons, and either shift their potency, or change their activity from repellence to attraction. Our results also lay down useful groundwork in the development of more effective control of tsetse by 'push', 'pull' and 'push-pull' tsetse control tactics.

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### POPULATION GENETICS ANALYSIS OF *PHLEBOTOMUS PAPTASI* SAND FLIES FROM NORTH AFRICA AND MIDDLE EAST REGIONS BASED ON MITOCHONDRIAL CYTOCHROME B HAPLOTYPES

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The *Phlebotomus papatasi* sand fly is a vector of cutaneous leishmaniasis (CL) over a widespread geographic range. Population genetics is a valuable tool in understanding the level of genetic variability present in vector populations, vector competence, and the development of novel control

strategies. This study elucidated the genetic differentiation between *P. papatasi* populations in Egypt and Jordan that inhabit distinct ecotopes and compared this structure to *P. papatasi* populations from a broader geographical range. The mtDNA cytochrome *b* gene was amplified and sequenced from 116 individual sand flies from Aswan and North Sinai, Egypt, as well as Swaymeh and Malka, Jordan. Haplotypes were identified and used to generate a median-joining network,  $F_{ST}$  values, and isolation-by-distance were evaluated. Additional sand fly individuals from Afghanistan, Iran, Israel, Jordan, Libya, Tunisia, and Turkey were included as well as previously published haplotypes to provide a robust genetic variation analysis. Thirteen haplotypes were identified with nine variant sites from the Egypt and Jordan. No unique haplotypes were identified from samples in North Sinai, Egypt, two were observed in Aswan, Egypt, four from Swaymeh, Jordan, and two in Malka, Jordan. The Jordan populations clustered separately from the Egypt populations and produced more unique haplotypes than Egypt. Pairwise  $F_{ST}$  values fall in the range 0.024-0.648 and indicate a strong differentiation between Egypt and Jordan populations, although this population structure is not due to isolation-by-distance. Other factors, such as environmental influences and the genetic variability in the circulating *L. major* parasites, may contribute significantly to this heterogeneity. The present study aligns with previous reports in that genetic differentiation exists between the populations of this widely dispersed species but overall, the species remains relatively homogeneous.

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### ALTERNATIVE USE OF INSECTICIDE TREATED BED NETS IN COMMUNITY BASED EDUCATION AND SERVICE CENTERS IN WESTERN KENYA

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In Kenya, the use of Insecticide treated bed nets for control of malaria is widespread especially in endemic areas. The western part of the country is one such area. Cross-sectional surveys were carried out in 20 Health Centres as part of the Community Based Education and Service programme of Moi University College of Health Sciences to determine the prevalence of mosquitoes, malaria and use of ITNs. Cluster sampling technique was used with each health centre as the sampling unit. In a random sample of 3, 326 households, the mosquito prevalence showed a range of 19% (Mautuma) to 100% (Sirisia) in the health centres sampled. The corresponding malaria prevalence was between 13% to 95% in children and 18% to 90% in adults. The malaria prevalence was assessed from the Health centre records. All the households reported use of insecticide treated bed nets. Cases of alternative use of the bed nets were also reported and observed. The results of this study show alternative use of ITNs to be rampant in COBES centres in Western Kenya. Efforts should be made to intensify Public Health Education to sensitize the Community on proper use of ITNs which would otherwise negate the malaria control programme in the country.

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### COMMUNITY OPERATED BLACK FLY TRAPS FOR ONCHOCERCIASIS SURVEILLANCE

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Entomological measures of transmission are key data necessary to document the suppression and interruption of transmission of *Onchocerca volvulus*, the causative agent of onchocerciasis. Black fly collection has relied upon human landing collections, which are inefficient and carry

the risk of promoting pathogen transmission. The Esperanza Window Trap (EWT) has shown promise as an alternative method for collection of *Simulium damnosum* sensu lato, the primary vector of *O. volvulus* in Africa. Here, we report the results of a community directed trial of the EWT in Northern Uganda. Traps operated by residents were compared to human landing collections in two communities over five months. EWTs collected numbers of *S. damnosum* that equaled or exceeded those obtained by a two-person human landing collection team. Three traps, when operated by a single village resident, collected over four times as many *S. damnosum* as did the two-man collection team. The results suggest that the EWT may be effectively operated by community residents and that the trap represents a viable alternative to human landing collections for entomological surveillance of *O. volvulus* transmission.

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### SITE-SPECIFIC OCCUPANCY AND SIMULATED EXPANSION DYNAMICS OF A SECONDARY VECTOR OF CHAGAS DISEASE: A THREE-YEAR FOLLOW-UP IN THE ARGENTINE CHACO

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Sylvatic triatomine species may adapt to domestic or peridomestic habitats (domestication) and become relevant for the transmission of *Trypanosoma cruzi*, the causal agent of Chagas disease. *Triatoma sordida* occasionally colonized human sleeping quarters in Paraguay, Bolivia and Brazil whereas the few studies conducted in Argentina found sylvatic and peridomestic colonies marginally infected with *T. cruzi*. Following a 12-year period free of vector control actions, over three years we surveyed site-specific triatomine infestations before and every 4-5 months after a community-wide house spraying with pyrethroids in a well-defined rural area of northern Argentina including 353 houses. Timed-manual searches found 78 of 2177 (peri)domestic sites occupied by *T. sordida* at baseline, with most (85.9%) insects in chicken nests, chicken coops and chicken trees (suitable habitats). A large fraction of suitable habitats were empty despite their proximity to occupied sites. An incidence function metapopulation model (including distances between sites and maximum local catch across the 3-year period as a proxy of vector carrying capacity) was fitted to the baseline pattern of occupancy. Simulation over 500 time steps predicted a steep increase up to an equilibrium of 250 occupied patches, almost fourfold the observed occupancy. Simulations using R were unable to recover the data fitted by the model, indicating that the observed occupancy pattern represented a transient state (not a quasi-stationary equilibrium) despite the absence of major disturbances (deforestation, insecticide). The prevalence of house infestation with *T. sordida* decreased from 19.0% at baseline to a minimum (8.1%) at 8 months post-spraying (MPS), and then increased gradually to a mean of 14.2% over 22-32 MPS. Only 1% of *T. sordida* bugs (mainly invading adults) were collected in human sleeping quarters. Our study shows that *T. sordida* thrived in peridomestic habitats, and occasionally invaded domestic quarters but failed to colonize them. Its expansion at a mesoscale was likely limited by the instability of peridomestic habitats and its low dispersal ability between them.

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### EFFECTIVENESS OF TWO TYPES OF LONG LASTING INSECTICIDAL NETS AFTER TWO YEARS OF USE FOR MALARIA VECTOR CONTROL IN AN AREA OF HIGH PYRETHROID RESISTANCE, MULEBA -TANZANIA

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Long Lasting Insecticidal Nets (LLIN) provide a physical and chemical barrier to mosquitoes, but in areas with high pyrethroid resistance, when net become holed and insecticide efficacy decline with net age. As reported previously, resistant mosquitoes will penetrate easily through holes and survived. Of key concern is whether an LLIN that is subject to wear and tear under everyday household conditions fail to protect LLIN users. Prospective cross-sectional survey were carried in four villages, two with Olyset nets and two with Olyset Plus nets, Muleba district 2016/2017. The houses were selected randomly by using random table number in each village. Total of 40 houses (10 from each village) were selected with total 80 study nets. Physical integrity, number of mosquito found resting inside nets, and insecticidal activity was quantified. Physical integrity was categorized by proportionate hole index (pHI). LLIN were assigned based on pHI value following WHO category. The field mosquitoes were used to test for susceptibility status of Olyset Plus nets in comparison to standard Olyset nets. In all villages 87.5% of nets were more than 20 month of use, and had at least one hole of any size. The totals of 189 blood fed mosquitoes were collected inside standard Olyset nets, no mosquitoes was collected inside Olyset Plus nets regardless of holes size. In Standard Olyset nets, the number of resting mosquitoes were increasing with hole size with proportional hole index >643. Adopted WHO resistance assay, field mosquitoes tested for 0 month net were 7%, 13% and 60%, 63% (*An.gambiae*, *An.funestus*) resistant to Olyset Plus and standard Olyset nets. After 20 months of net use 25, 60% and 69, 90% (*An.gambiae*, *An.funestus*) were resistant to Olyset Plus and for standard olyset. In area with high pyrethroid resistance mosquitoes, Olyset Plus nets, treated with pyrethroid in additional to PBO provide protection to an individual's regardless of age and holes size. All type of nets develops large holes after one and half year of use...

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### LANDSCAPE GENETICS OF INSECTICIDE RESISTANCE IN ANOPHELES ANOPHELES SINENSIS

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*Anopheles sinensis* is one of the most abundant vectors of malaria and other diseases in Asia. Vector control through the use of insecticides is the front line control method of vector-borne diseases. However, the extensive use of insecticides has imposed strong selection pressure on mosquito populations for resistance. Despite the importance of kdr mutations in pyrethroid resistance, the evolutionary origin of the kdr mutations is unknown. We examined the evolutionary genetics of kdr mutations in relation to spatial population genetic structure of *An. sinensis* China. We found three non-synonymous mutations at the kdr codon L1014 of paratype sodium channel gene. A patchy distribution of kdr mutation allele frequencies from southern to central China was found. Near fixation of kdr mutation was detected in populations from central China, but no kdr mutations were found in populations from southwestern China. More than eight independent mutation events were detected in the three kdr alleles, and at least one of them evolved multiple times subsequent to their first divergence. Significant and large genetic differentiation was detected

between populations from southwestern China and central China. The patchy distribution of *kdr* mutation frequencies is likely a consequence of geographic isolation in the mosquito populations and the long-term insecticide selection. Our results indicate multiple origins of the *kdr* insecticide-resistant alleles in *An. sinensis* from southern and central China. Local selection related to intense and prolonged use of insecticide for agricultural purposes, as well as frequent migrations among populations are likely the explanations for the patchy distribution of *kdr* mutations in China. On the contrary, the lack of *kdr* mutations in Yunnan and Sichuan is likely a consequence of genetic isolation and absence of strong selection pressure. The present study demonstrated the combined impact of demographic and selection factors on population structure.

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### QUANTIFYING THE INTENSITY OF PERMETHRIN INSECTICIDE RESISTANCE IN *ANOPHELES* MOSQUITOES IN WESTERN KENYA

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Resistance among malaria vectors to classes of insecticides used in long lasting insecticidal nets and indoor residual spraying poses a challenge to malaria vector control. Methods for evaluating insecticide resistance in malaria vectors include WHO tube and CDC bottle assays. Upgrade to CDC bottle assay using different insecticide dosages has proved applicable in ascertaining intensity of resistance. We determined prevalence and intensity of permethrin resistance and investigated efficacy of commonly used long lasting insecticidal nets (LLINs) against 3 day old *Anopheles* mosquitoes from four sub counties in western Kenya (Nyando, Bondo, Rachuonyo and Teso). *Anopheles* larvae were reared to 3 day old adults for exposure to; WHO tube, CDC bottle and cone assay. For resistance intensity, mosquitoes were exposed to 21.5µg/ml, 43µg/ml, 107.5µg/ml and 215µg/ml doses of permethrin using CDC bottle assays. Mortality after 24h was recorded for WHO assays, time of knockdown for bottle assays while both were used for cone assay. Mortality to 0.75% permethrin ranged from 23.5-96.1%. Intensity of permethrin resistance was highest in Barkanyango Bondo with 84% knockdown at 30 minutes diagnostic time when exposed to 215µg/ml dose. Mortality for nets was (0-39) %, (12-88) % and (26-89) % for Olyset, PermaNet 2.0 and PermaNet 3.0 respectively. Efficacy of nets was reduced in Bondo and Teso. Study results shows that there was confirmed resistance in all sites, however, intensity assays were able to differentiate Bondo and Teso as sites with highest levels of resistance, which coincidentally were two sites with reduced net efficacy. This portrayed that at certain resistance intensities, vector control using LLINs may be compromised. Therefore it is necessary to incorporate intensity assays in order to determine the extent of threat that resistance poses to malaria control.

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### UNDERSTANDING THE COMMUNITY CONTEXT OF *Aedes aegypti* MOSQUITO BREEDING IN COASTAL KENYA: IMPLICATIONS FOR CONTROL

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Community engagement is crucial for controlling *Aedes aegypti* mosquito-borne diseases since these mosquitoes primarily breed in water storage containers around people's homes. Mosquito source reduction often provides the greatest benefit at a minimal cost; however, only an

estimated 15% of coastal Kenyans engage in source reduction due to lack of awareness and motivation. This study in Kwale County, Kenya had three objectives: i) to identify target containers, ii) to understand current perspectives on source reduction, and iii) to explore how to encourage parents and children to adopt source reduction practices. Entomological surveys were conducted in 500 households between May and July 2016 to locate mosquito habitats and measure abundances of mosquito larvae. Based on these surveys, 40 houses were selected for semi-structured in-depth interviews to determine perspectives about mosquitoes and source reduction. Finally, educational materials and behavioral recommendations were developed during a stakeholder workshop and pilot tested in 3 villages. The entomological surveys identified 893 mosquito habitats. Tires, discarded plastic bottles, trash, buckets, jerry cans, and animal drinking troughs contained the greatest number of mosquito larvae. The in-depth interviews revealed common misconceptions, including a fear that drinking mosquito larvae could transmit malaria or cause diarrhea. Therefore, many people considered it important to cover containers used for drinking or cooking, but did not consider water for laundry or sanitation a concern. Based on the container types and perceptions, we pilot tested a curriculum with children and parents emphasizing mosquito types and diseases, life cycle, breeding sites, and recommendations for source reduction. Parents and children understood the new information well. Children practiced source reduction by collecting unwanted plastic containers for re-use, while parents practiced various types of source reduction but still prioritized covering water containers for drinking and cooking water. Changing behavior requires continuous effort and community engagement.

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### INSECTICIDE SUSCEPTIBILITY IN *ANOPHELES GAMBIAE* S.L.: A NATIONWIDE SURVEY PRIOR TO A MASS DISTRIBUTION OF LONG LASTING INSECTICIDE TREATED NETS IN TOGO

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A nationwide survey of insecticide susceptibility tests was conducted between June and July 2016 to inform the decision on the type of LLIN to be distributed during the universal coverage campaign in Togo. Susceptibility tests were performed on 4,932 unfed adult female *Anopheles gambiae* s.l. aged 2 to 5 days. These female mosquitoes were obtained from larvae collected from 6 NMCP sentinel sites. Overall 8 insecticides were used: 4% DDT, pyrethroids and 0.05% deltamethrin, 0.75% Permethrin and 0.05% lambda-cyhalothrin, carbamates and 0.1% bendiocarb and 0.1% propoxur and organophosphates and 0.1% malathion and 0.1% fenitrothion. The Kisumu strain was used as reference. The knock-down and 50 and 90% values were calculated and the 24 hours post-exposure mortality rates were recorded. Based on WHO criteria, results showed very high knock-down values for pyrethroids while it was impossible to determine those of 4% DDT. The mortality rates recorded for 4% DDT were less than 5% in most of the sites. All the mosquitoes were resistant to pyrethroids. The highest mortality rate was recorded with 0.05% deltamethrin and 44% and 5% malathion led to a 100% mortality rate in the Plateau region. This study showed that insecticide resistance in *An. gambiae* s.l. is a matter of great concern and there is an urgent need for a countrywide insecticide resistance management.

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### EVIDENCE OF MULTIPLE DDT RESISTANCE MECHANISMS IN THE MALARIA VECTOR *ANOPHELES GAMBIAE* FROM DAR ES SALAAM, TANZANIA

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Significant reductions in malaria transmission have been achieved over the last 16 years. However, increasing drug and insecticide resistance threatens these gains. *Anopheles gambiae*, which is a major vector of the malaria parasite *Plasmodium falciparum* in Africa, has over the years developed resistance to insecticides including dieldrin, 1,1-bis(*p*-chlorophenyl)-2,2,2-trichloroethane (DDT), and pyrethroids. The objective of this study was therefore to determine the mechanisms contributing to DDT resistance in *Anopheles gambiae* s.s. from Dar es Salaam, Eastern Tanzania. Female mosquitoes of standard age, reared from larvae sampled across varieties of natural breeding sites, were used in the study. Members of the *An. gambiae* complex were PCR-identified and screened for *Vgsc-1014S* and *Vgsc-1014F* mutations. A DDT-resistant population of *An. gambiae* s.s. with two controls (sympatric and allopatric controls) were screened for GSTe2 and P450 genes-expression profiles using real-time quantitative polymerase chain reaction (qPCR). We found significantly higher allelic frequencies of the *Vgsc-L1014S* mutation in DDT-resistant *An. gambiae* s.s. than in the controls ( $p < 0.001$ ). Cytochrome P450 genes: *Cyp6m2*, *Cyp6p3*, *Cyp6z3* and *Cyp6z1* were significantly over-expressed in DDT-resistant *An. gambiae* s.s. compared with the control populations. We report the increased expression of multiple DDT-associated resistance mechanisms in the primary African malaria vector, *An. gambiae* s.s. from Dar es Salaam. The presence of multiple resistance mechanisms in *An. gambiae* that are common in both DDT and pyrethroids may have confounding effect in resistance-management strategies. However, the geographical extent of the insecticide resistance mechanisms observed in this study need to be investigated.

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### FITNESS EFFECTS OF VSSC MUTATIONS S989P+V1016G IN A PYRETHROID RESISTANT STRAIN OF THE YELLOW FEVER MOSQUITO, *Aedes aegypti*

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*Aedes aegypti*, an important vector of many human diseases, is a serious threat to human health due to its wide geographic distribution and preference for living near humans. Pyrethroid insecticides are commonly used to control *Ae. aegypti* in endemic areas, especially during disease outbreaks. Insecticides are still the only way to control *Ae. aegypti* in dengue endemic areas, which has led to extensive use of pyrethroids in the past decades. Consequently, *Ae. aegypti* resistance against pyrethroids is now found worldwide and directly impacts our capacity to control the diseases this species transmits. Resistance alleles often have a cost to the individuals carrying when there is no insecticide present. Understanding the fitness costs and evolution of resistance is critical in integrated resistance management practices for it helps to determine how quickly resistance will be lost under field conditions after pesticide application has ceased. An important mechanism of resistance to pyrethroids is due to mutation in the voltage-sensitive sodium channel (*Vssc*). A common resistance allele contains the S989P+V1016G mutations (*kdr*). I developed a congenic strain of *Ae. aegypti* that contains a voltage-sensitive sodium channel (*Vssc*) allele, S989P+V1016G (IsoKDR), but has the same genetic background as the laboratory susceptible ROCK strain. The goal of

this study was to estimate the fitness cost of this resistance allele by quantifying the susceptible (S) and resistant (R) allele frequencies in a laboratory cage experiment. This was accomplished by crossing ROCK and IsoKDR and then rearing the progeny for 9 generations while evaluating the change in the *kdr* frequency. Our results support our hypothesis that these two *Vssc* mutations have a fitness cost. Further studies are in progress to determine how these mutations impact fitness by looking at changes in fertility, fecundity, and development of IsoKDR relative to ROCK. The implications of these results to the control of *Ae. aegypti* will be discussed.

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### INSECTICIDE RESISTANCE IN *ANOPHELES GAMBIAE* S.L. (DIPTERA: CULICIDAE) FROM ETHIOPIA (2012-2016): A NATIONWIDE STUDY FOR INSECTICIDE RESISTANCE MONITORING

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Indoor residual spraying (IRS) and long-lasting insecticidal nets (LLINs) remain the cornerstones of malaria vector control. However, the development of widespread insecticide resistance and its implications for operational failure of preventative strategies are of increasing concern. We characterized contemporary nationwide insecticide resistance among *Anopheles gambiae* s.l. populations in Ethiopia and describe temporal changes in resistance between 2012 and 2016. Mosquito larvae were sampled from a range of breeding sites in seven regions annually during the long rainy season, and susceptibility levels to organochlorines, pyrethroids, organophosphates, and carbamates, assessed using WHO susceptibility tests and CDC bottle bioassays. In select sites, specimens were identified to species-level and underlying resistance mechanisms investigated using CDC synergist assays based on pre-exposing mosquitoes to piperonyl butoxide (PBO), and by PCR screening for knockdown resistance (*kdr*). Intense resistance to DDT and pyrethroids was pervasive across Ethiopia and over time, consistent with historic use of DDT for IRS and concomitant increases in insecticide-treated net coverage over the last 15 years. Temporal changes in resistance to malathion, bendiocarb, propoxur and pirimiphos-methyl corresponded to shifts in the national insecticide policy. By 2016, resistance to the latter two insecticides had emerged, with the potential to jeopardize future long-term effectiveness of vector control activities in these areas. Restoration of mosquito susceptibility to pyrethroids following pre-exposure to PBO, a lack of association between *kdr* allele frequency and vector mortality rate, and the absence of cross-resistance between insecticides belonging to the same chemical class, indicates the existence of metabolic mechanisms conferring insecticide-specific resistance. If inter- and intra-class rotation of different insecticides could be exploited to reduce selection pressures, this may have the potential to safeguard continued efficacy of IRS and other malaria control strategies in Ethiopia.

### MICROBIAL LARVICIDES FOR MOSQUITO CONTROL: IMPACT OF LONG LASTING FORMULATION OF *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS* AND *BACILLUS SPHAERICUS* ON NON TARGET ORGANISMS IN WESTERN KENYA HIGHLANDS

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Microbial larvicides *Bacillus thuringiensis* var. *israelensis* and *Bacillus sphaericus* have been used extensively globally for mosquito control. These larvicides have been found to be effective and safe to non target organisms co-habiting with target vectors. Recent advent of long lasting microbial larvicides, though evade the previous challenge of short duration of activity, increase the risk of persistence of crystal toxins in the treated larval habitats. The current study monitored the impact long lasting microbial larvicides FourStar® and LL3 on non target organisms co-habiting with mosquito larvae in an operational study to control malaria vector in western Kenya highlands. A total of 300 larval habitats were selected from three highland villages in western Kenya. The larval habitats were monitored for five weeks to collection baseline information on non target organisms co-habiting with mosquito larvae and then randomized into two treatments (respective FourStar® and LL3) and control arms. Following treatments, non target organisms were sampled weekly for five months to assess the impact of long lasting microbial larvicides intervention. Before treatment, the mean density of all non target organisms combined in the control, LL3 and FourStar® habitats was 1.42, 1.39 and 1.49 individuals per habitat per sampling occasion, respectively. Following treatment, the mean density of all non-target organisms remained fairly unchanged for 21 weeks in which the mean density at that point was 1.82, 2.11 and 2.05 for the respective control, LL3 and FourStar® habitats. Statistical analysis revealed that the two microbial larvicides did not significantly alter abundance, richness and diversity of 11 taxa studied, when compared the intervention and control larval habitats. However, both FourStar® and LL3 significantly reduced the density of malaria vector. In conclusion, one round of label application of long lasting microbial larvicide FourStar® or LL3 in natural larval habitats did not alter richness, abundance and diversity of aquatic non-target organisms co-habiting with mosquito larvae to an ecologically significant level.

### INSECTICIDE RESISTANCE STATUS OF THREE MALARIA VECTORS: *AN.OPHELES GAMBIAE* S.L., *AN. FUNESTUS* AND *AN. MASCARENSIS* FROM THE SOUTH, CENTRAL HIGHLANDS AND EAST COASTS OF MADAGASCAR

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Insecticide-based vector control is the key method to malaria control in Madagascar. This study investigated the resistance status of malaria vectors in Madagascar to various insecticides. Adult female *Anopheles gambiae* s.l. mosquitoes reared from field-collected larvae and pupae were tested

for their resistance to DDT, permethrin, deltamethrin, alpha-cypermethrin, lambda-cyhalothrin, bendiocarb and pirimiphos-methyl. Resting *An. funestus* and *An. mascarensis* female mosquitoes collected from unsprayed surfaces were tested against permethrin, deltamethrin and pirimiphos-methyl. The effect on insecticide resistance of pre-exposure to the synergists piperonyl-butoxide (PBO) and S,S,S-tributyl phosphorothioate (DEF) also was assessed. Molecular analyses were done to identify species and determine the presence of knock-down resistance (*kdr*) and acetylcholinesterase resistance (*ace-1<sup>R</sup>*) gene mutations. *An. funestus* and *An. mascarensis* were fully susceptible to permethrin, deltamethrin and pirimiphos-methyl. *An. gambiae* s.l. was fully susceptible to bendiocarb and pirimiphos-methyl. Among the 17 *An. gambiae* s.l. populations tested for deltamethrin, no confirmed resistance was recorded, but suspected resistance was observed in two sites. *An. gambiae* s.l. was resistant to permethrin in four sites (mortality 68-89%) and to alpha-cypermethrin (89% mortality) and lambda-cyhalothrin (80% and 85%) in one site. Pre-exposure to PBO restored full susceptibility to all pyrethroids tested except in one site where only partial restoration to permethrin was observed. DEF fully suppressed resistance to deltamethrin and alpha-cypermethrin, while it partially restored susceptibility to permethrin in two sites. Molecular analysis suggests absence of *kdr* and *ace-1<sup>R</sup>* gene mutations. This study suggests involvement of detoxifying enzymes in the phenotypic resistance of *An. gambiae* s.l. to pyrethroids. The absence of resistance in *An. funestus* and *An. mascarensis* to pirimiphos-methyl and pyrethroids and in *An. gambiae* s.l. to carbamates and organophosphates presents greater opportunity for managing resistance in Madagascar.

### MULTIPLE KNOCKDOWN RESISTANCE (KDR) MUTATIONS IN INDIAN *Aedes Aegypti*

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*Aedes Aegypti* is a primary vector for several arboviral infections like dengue, chikungunya, Zika virus and yellow fever. In absence of specific drug or vaccine against these infections vector-control is the only option to control these infections. Development of insecticide resistance in this vector poses serious threat to control these infections. Knockdown resistance (*kdr*) mutations in the voltage gated sodium channel (VGSC)—target site of action for DDT and pyrethroids, is one of the mechanisms of resistance against these two insecticide groups. Several such *kdr* mutations are reported in *Aedes aegypti* globally, however in India, we earlier reported presence of two *kdr* mutations (F1534C and a novel mutation T1520I) in domain III-S6 of VGSC. Here we report presence of multiple *kdr* mutations in Bengaluru viz., S989P and V1016G present in domain IIS6 and F1534C and F1534L present in domain IIIS6. Among these F1534L is not reported earlier from anywhere in this species. Other novel mutation T1520I reported in Delhi (India) is absent in this population. Among these mutations S989P and V1016G were found tightly linked. In addition S989P+V1016G were always linked to F1534. We developed PCR based methods for identification of all *kdr* mutations reported in India (S989P, V1016G, F1534C, F1534L and T1520I).

### ADAPTATION OF THE CDC BOTTLE BIOASSAY FOR NEONICOTINOIDS AND BUTENOLIDES

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The CDC bottle bioassay is an established tool for mosquito susceptibility monitoring. Prolonged discriminating time or an increase of the discrimination dose concentration can detect upcoming resistance of wild caught mosquitoes at an early stage. Therefore such a monitoring tool is of tremendous importance for effective vector control programs with existing products as well as for new active ingredients with no or

low cross-resistance entering the market. Clothianidin is a promising new compound being developed by Bayer as a combination product for indoor residual spraying against malaria vectors. The methodology of the standard bottle assay recommends acetone as practical solvent. However, clothianidin and the active ingredients imidacloprid, thiamethoxam and flupyradifurone show incompatibility for this assay if acetone as single solvent is used. We determined that the addition of Mero® (81% Rapeseed oil methyl ester) has shown to improve efficacy of the named compounds in the described bottle assay. The combination of Mero® and acetone reduces crystallization effects, keep the active ingredient for a longer period of time in a dissolved state and probably improves the uptake of the compound. This study highlights the practical benefits of adding Mero® to the acetone solvent, if used in the CDC bottle bioassay with selected neonicotinoids and butenolides. More than 2000 mosquitoes of the susceptible *Anopheles gambiae* Kisumu strain and 1000 of the RSPH strain were used in several bottle bioassays to identify the discriminating dosage and time for clothianidin, imidacloprid, thiamethoxam and flupyradifurone. 90µg of the named neonicotinoids solved in an acetone/Mero® mixture revealed 100% knockdown to the test insects within 45min. For flupyradifurone a higher active ingredient concentration of 200µg per bottle was necessary. This study highlights a simple adaptation to an existing method which will allow determination of susceptibility of mosquitoes in-field to these new compounds.

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### IDENTIFYING HEME IMPORTERS AND EXPORTERS THROUGH RNA SEQ ANALYSIS IN *Aedes aegypti*

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After a bloodmeal, mosquitoes import heme into the midgut epithelium. Heme acts as an essential signal for oogenesis in *Aedes aegypti*. However, the mechanisms behind heme import in *Ae. aegypti* are largely unexplored. In this study, RNA sequencing data from 4 different *Ae. aegypti* cell culture experiments where cells were exposed to an overabundance or deficiency of heme was examined to identify heme-responsive genes. Zinc mesoporphyrin (ZnMP), a heme fluorescent analog, was used to measure changes in heme uptake prior to mRNA sequencing. A soft cluster analysis was performed to identify genes encoding potential membrane bound importers and exporters based on expression profiles across the samples for each experiment. Stronger candidates were obtained by comparing genes in each dataset to each other. In total, 168 potential importers were found to be heme-regulated in only 2 datasets, 36 were heme-regulated in 3 datasets and 1 was heme-regulated in all 4 datasets. In contrast, 105 potential exporters were common to only 2 datasets, with just 2 present in 3 of the 4 datasets. Future work will focus on generating an RNA seq dataset from adult midgut exposed to overabundance or deficiency of heme and comparing those results to current cell culture results to find those differentially expressed transcripts common amongst all or most of the datasets which should lead to stronger predictions of possible heme transporters. Finally reverse genetic analysis will be performed on the top candidate genes to confirm their status as importers or exporters in *Aedes aegypti*.

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### EVOLUTIONARY HISTORY OF THE MACULIPENNIS GROUP OF MALARIA MOSQUITOES REVEALED BY TRANSCRIPTOME AND CHROMOSOME-REARRANGEMENT ANALYSES

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The Maculipennis group of malaria mosquitoes is subdivided into two North American and one Eurasian subgroups. Although previous studies considered the Nearctic subgroups as ancestral, the details of the malaria mosquitoes' migration from North America to Eurasia remains a subject of debate. To reconstruct historic relationships between the North American to Eurasian mosquitoes, we conducted a multigene phylogenetic analysis of 7 Palearctic and 2 Nearctic species based on their transcriptomes. Maximum likelihood trees were generated using 2267 orthologous genes for each autosomal arm and the X chromosome separately. The transcriptome analysis indicated a closer relationship of *An. beklemishevi* with other Palearctic members rather than with the Nearctic members of the group. Also, *An. beklemishevi* clusters more closely with *An. freeborni* that occupies the Western United States rather than with *An. quadrimaculatus*, a species from the Eastern United States. Our time-calibrated tree strongly suggests a single migration of the mosquitoes of the Maculipennis group from North America to Eurasia through the Bering Bridge Land about 11 million years ago and their further divergence into the Asian and European clades. In addition, we constructed a rearrangement-based phylogeny using 21 genes located at a 1 Mb distance from each other on chromosome X. Positions of these genes were determined on X chromosomes of seven Palearctic species using fluorescent *in situ* hybridization. The mapping demonstrated that all species differ from each other by several fixed inversions on the X chromosome. The obtained inversion-based dendrogram supports a more basal position of *An. beklemishevi* among other Palearctic species in agreement with the molecular phylogeny. The research was supported by the Russian Science Foundation grant 15-14-20011.

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### GENETIC STRUCTURE AND PHENOTYPIC VARIATION OF *ANOPHELES DARLINGI* AT THE MICROGEOGRAPHIC LEVEL IN AN IMPORTANT MALARIA ENDEMIC REGION OF COLOMBIA

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Various studies have demonstrated that ecological heterogeneity derived from human activities promotes ecological adaptation and evolutionary divergence of mosquito vectors. This study, conducted at the microgeographic level, evaluated the influence of environmental heterogeneity on *Anopheles darlingi* genetic and morphometric traits. Specimens of *An. darlingi* collected from multiple municipalities in the Colombian malaria endemic region Urabá-Bajo Cauca and Alto Sinú (UCS) were analyzed using 13 microsatellite *loci*. We estimated spatial genetic structure and population variation among and within populations and wing geometric morphometrics analysis was performed. The microsatellite results showed low genetic differentiation and high gene flow among populations. Additionally, four highly admixed subpopulations were detected with no particular association among them and the

municipalities. Wing geometric morphometrics analysis showed a subtle but significant difference in wing shape for only El Bagre vs Mutata populations, possibly influenced by geographical distance. Discrimination among populations in the morphospace, defined by the canonical variance axes, showed a slight separation of the Tierralta population. There was no significant correlation between the genetic and geographic or genetic and environmental distances. We hypothesize that environmental heterogeneity in the UCS region is not enough to promote *An. darlingi* population structure. It remains to be ascertained which local factors govern phenotypic variation among these populations and how these may impact mosquito physiological and bio-ecological traits

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## 20-HYDROXYECDYSONE (20E) INDUCES PRIMING OF MOSQUITO IMMUNITY AND LIMITS MALARIA PARASITE INFECTION IN *ANOPHELES GAMBIAE*

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The act of acquiring a blood meal provides reproductive and physiological advantages for mosquitoes, however little is known about the impacts of blood feeding on mosquito immunity. With a critical role in determining vector competence, understanding the influence of mosquito physiology on pathogen development is important for the identification of potential targets to reduce transmission of mosquito-borne diseases. Given this importance, we are interested in the influence of hormones, specifically 20-hydroxyecdysone (20E), which significantly increases 18-24 hours post blood feeding. Coinciding with the timing of pathogen challenge, emerging evidence suggests that 20E may also influence mosquito immunity, arguing that targeting 20E synthesis may function as a potential tool to interfere with pathogen development in the mosquito host. Here we report the effects of 20E on mosquito physiology and innate immune responses through *in vitro* and *in vivo* experiments, highlighting the roles of 20E on mosquito immune regulation. We found that the injection of 20E, as well the topical application of Halofenozide, a 20E-mimicking insecticide, lead to an increase in 20E-regulated gene expression in *An. gambiae* and a significant decrease in malaria parasite numbers. Additionally, topical applications of Halofenozide increased mosquito survival following bacterial challenge. Together, this suggests that 20E primes the mosquito immune system for pathogen challenge. Experiments are currently underway to extend these studies to other mosquito vectors to investigate the effects of Halofenozide application on arbovirus infections. With direct public health applications, we believe repurposing hormone mimicking insecticides will be an important new tool to reduce the transmission of mosquito-borne disease.

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## NOVEL INSIGHTS INTO *ANOPHELES* IMMUNE FACTORS WITH PRO/ANTI-*PLASMODIUM* MELANIZATION FUNCTIONS IN DIVERSE VECTOR-PARASITE SPECIES COMBINATIONS

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*Anopheles-Plasmodium* interactions have been the subject of intense study, given the importance of the mosquito vector for malaria transmission. The functions of the *Anopheles* immune system receptors C-type lectins CTL4 and CTLMA2, and leucine-rich repeat protein LRIM1, were first described based on the *A. gambiae-P. berghei* laboratory model, and field *A. gambiae-P. falciparum*. The two C-type lectins were identified as *P. berghei* agonists, that inhibit parasite melanization, and LRIM1 as an antagonist involved in killing this parasite. None of these factors appeared to have an effect on the human *P. falciparum* parasite. The functions of these genes in mosquito immunity to *Plasmodium* were not further explored. Here we show that the implication of the *A. gambiae* C-type lectins, in protecting parasites from melanization, is infection intensity

dependent, and not *Plasmodium* species-specific as previously thought. We then explored the influences of these genes on *Plasmodium* infection in the New World vector *A. albimanus*. Our findings revealed that silencing the predicted orthologues of CTL4 and CTLMA2 in *A. albimanus* had the opposite effect than that in *A. gambiae*, while LRIM1 had a conserved function, for both parasite species, suggesting that the C-type lectin genes have diverged their function in different mosquito species. Only a moderate degree of identity was observed between the *A. gambiae* CTL4 and its *A. albimanus* predicted orthologue, suggesting that it may not be a true orthologue. Interestingly, *Plasmodium* infection in *A. albimanus* results in a variety of oocyst/sporozyte phenotypes depending on the silenced gene. Moreover, our data suggests that the functions of the C-type lectins in regulating infection intensity and melanization, are unlinked.

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## CHARACTERIZE THE FUNCTION OF HOST IMMUNITY MIRNAS TO BLOCK *PLASMODIUM FALCIPARUM* INFECTION BY MOSQUITO TRANSGENESIS IN *ANOPHELES GAMBIAE*

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Malaria, caused by the protozoan parasite *Plasmodium falciparum* and transmitted by *Anopheles gambiae* mosquitoes, is a major threat to human health, responsible for up to half of million deaths worldwide in 2016. *Plasmodium* invasion of the *Anopheles* tissues represents a critical step in the parasite-transmission cycle. Recent studies showed that insect innate immunity plays an important role in blocking *Plasmodium* transmission in *Anopheles*. Overexpression of immune effector gene (*Relish 2*) in transgenic *Anopheles* mosquitoes have been shown to significantly block parasite transmission. MicroRNAs (miRNA) are small regulatory noncoding RNAs and sized from 21 to 25 nt, has been shown to play a central role in the regulation of gene expression. miRNAs target the 3' untranslated regions (3'-UTR) of mRNAs, thereby repressing their translation. Previously, we reported that aga-miR-989 and aga-miR-305 exhibited elevated abundance of expression in midguts of the mosquito infected by *Plasmodium*, and artificial aga-miR-305 mimics increased susceptibility to *P. falciparum* infection and resulted in expansion of midgut microbiota, suggesting that aga-miR-305 acts as a *P. falciparum* and gut microbiota agonist by negatively regulating the mosquito immune response. Here, we report an approach to characterize the function of aga-miR-305 and other selected miRNAs with regards to their influence on *Plasmodium* infection in *A. gambiae* using mosquito transgenesis by overexpressing miRNA sponges in female mosquitoes and thus eliminating miRNA function, thereby increasing mosquito immunity, and reveal the possible miRNA targets by transcriptome analysis in transgenic mosquitoes.

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## GENETIC MANIPULATION OF MOSQUITO NERVOUS SYSTEM

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Although mosquitoes transmit pathogens responsible for some of the most devastating diseases, relatively little is known about their neurobiology or behavior. Investigating the neurobiological basis of fundamental mosquito behaviors could facilitate modification of these behaviors for vector and disease control. To study and eventually manipulate neural control of a particular behavior, tools are needed to identify neurons involved in the behavior, determine how activity of the neurons influences the behavior, and explore how the neurons form a functional circuit. While such tools are available for analysis of genetic model organisms, mosquito neurobiologists lack such sophisticated genetic tools for analysis of the neural basis of behaviors. The binary expression system of Gal4-UAS, is routinely utilized for neurobiological studies in model organisms. Although the Gal4-UAS system has been introduced

in mosquitoes, very few Gal4 and UAS strains have been developed. To enhance the genetic tractability of *Aedes aegypti*, the principle mosquito vector for many arboviruses like Zika & dengue we performed a FAIRE-seq open chromatin profiling study that permitted genome-wide discovery of >121,000 putative cis-regulatory elements (Behura et al., 2016). GFP reporter assays were used in a screen to identify regulatory elements that drive gene expression in the central and sensory nervous systems. The screen performed in *Drosophila* using putative regulatory elements from *A. aegypti* was designed to enhance for the selection of elements that drive neural gene expression in *A. aegypti*. Select elements from the screen have been cloned into a Gal4 transgenic vector that is compatible with multiple modes of transformation in *A. aegypti* and other insect species. In the present study, an *A. aegypti* Gal4 ofactory driver line was identified and characterized. Additional driver lines, including one that generated sex-specific neural gene expression in reporter assays, are presently being generated. Future studies will focus on generation of UAS responder lines that will allow us to study and manipulate the mosquito nervous system.

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### MAPPING QTLs IN *CULEX QUINQUEFASCIATUS* THAT CONTROL THE DENSITY OF BACTERIAL SYMBIONT *WOLBACHIA PIPIENTIS*

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The unique characteristics of *Wolbachia* are being exploited to develop *Wolbachia* infection of vector mosquitoes as an approach for interrupting the transmission cycle of viral disease pathogens. This approach hinges on the ability of *Wolbachia* infection to increase host resistance to viral pathogens, determined, in part, by the density of *Wolbachia* in host tissues. Little is known, however, about how *Wolbachia* density is regulated in native or heterologous hosts. We measured the broad-sense heritability of *Wolbachia* density between families in field populations of the mosquito *Culex pipiens*, and found that densities in ovary and non-gonadal tissues of females in the same family are not correlated, suggesting that *Wolbachia* density is determined by distinct mechanisms in the two tissues. Using introgression analysis between two different strains of the closely-related species *Culex quinquefasciatus*, we found that *Wolbachia* densities in ovary tissues are determined by cytoplasmic genotype, while densities in non-gonadal tissues are determined by both cytoplasmic and nuclear genotypes and their epistatic interactions. Quantitative-trait locus mapping using a high-density SNP-based genetic map of the *Cx. quinquefasciatus* genome identified two major-effect quantitative-trait loci explaining a combined 23% of variance in *Wolbachia* density specifically in non-gonadal tissues. A better understanding of how *Wolbachia* density is regulated will provide insights into how *Wolbachia* density can vary spatiotemporally in mosquito populations, leading to changes in *Wolbachia*-mediated phenotypes such as viral pathogen resistance.

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### IMPACTS OF ELIMINATION AND EXOGENOUS TRANSFECTION OF *WOLBACHIA* ON GUT MICROBIOTA AND TRANSCRIPTOME OF *AEDES ALBOPICTUS*

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*Aedes albopictus* is an important vector of dengue, Zika and Chikungunya worldwide and a sole vector for dengue in southern China. Intracellular bacteria *Wolbachia* is known to cause cytoplasmic incompatibility, recent

studies also demonstrated that it plays an important role in limiting infectivity of arboviruses in the mosquito vectors. Novel *Wolbachia* strains are being introduced to mosquito populations to modify vector competence and block arbovirus transmission. However the mechanisms of immuno-modulation of *Wolbachia* on the mosquito vectors are not well understood, one potential mechanism is that novel *Wolbachia* strain introduction modify mosquito gut microbiota and subsequently enhance mosquito immune response to arboviruses. The objective of this study was to examine the impact of elimination and exogenous transfection of *Wolbachia* on gut microbiota and transcriptome of *Aedes albopictus* mosquitoes. The assemblages of bacterial microbiota in *Ae. albopictus* wildtype strain, *Wolbachia*-free strain and a strain transfected by new types of *Wolbachia* in egg, larvae and adult (day 1, 7, 14, 21, blood feeding and non-blood feeding) stages were examined using 16S rRNA MiSeq amplicon-sequencing on Illumina. We found that removal of natural *Wolbachia* from *Aedes albopictus* populations by tetracycline reshaped the structure of bacterial microbiota and significantly increased gut bacterial diversity. Similarly, transfection of exogenous *Wolbachia* strain also significantly increased diversity of the bacterial microbiota. Such impacts are most pronounced at the egg stage. We are currently using RNA-seq technique to determine the impact of elimination and exogenous transfection of *Wolbachia* on transcriptome of immune genes. The information obtained from this study would help to establish a metagenomic foundation for better understanding the impact of bacterial microbials on vector development and disease transmission.

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### GENOMIC BASIS OF BLOODFEEDING BEHAVIOR IN *ANOPHELES MINIMUS*, THE PRIMARY MALARIA VECTOR IN SOUTHEAST ASIA

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*Anopheles minimus* represents one of the most important malaria vectors in Southeast Asia. The adult behavior of the species was reported to be highly diverse with regards to blood meal source preferences, as well as biting and resting behavior. Understanding the genomic basis of host feeding preference and biting activity of vector species is valuable to identify their potential to adapt to new environments and malaria control interventions, and the information helps to develop appropriate strategies of vector control management. The aim of the study is to examine the genomic basis of host bloodfeeding preference in *An. minimus* by next-generation whole genome sequencing. Bloodfed mosquitoes were collected using CDC light traps in a malaria endemic area of the China-Myanmar border region. Molecular identification of *An. minimus* species and host blood meal was conducted by PCR using DNA from the abdomen. Head and legs of 24 mosquitoes with human blood meal (H-group) and 24 mosquitoes with animal blood meal (A-group) were used to extract DNA individually for whole genome sequencing by Illumina. A total of 336,451 variants were identified in the 48 mosquito genomes (median coverage =22.4x). Among them, 310,254 and 279,699 genomic variants were found in H-group and A-group, respectively. A total of 1,268 differential genomic variants were detected between H-group and A-group, including SNPs in genes of odorant receptor protein, cuticular protein, hormone-related protein and other proteins. The results suggested that mosquito host preference is associated with genetic variation in a large number of genes. Further analysis is being conducted to identify the key genes for mosquito bloodfeeding preference. Such information is valuable to understand the evolution of mosquito host preference in response to vector control measures.

### ANOPHELES FUNESTUS IN CENTRAL AND SOUTHERN AFRICA: ANALYSIS OF MITOCHONDRIAL DIVERSITY

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*Anopheles funestus* s.s. is a primary vector of malaria in sub-Saharan Africa. Despite its important role in malaria transmission, knowledge of *An. funestus* s.s. evolutionary history and population differentiation in southern Africa remains understudied. In this study, we examined the phylogenetic relationships and demographic history of *An. funestus* s.s. populations throughout southern Africa using mitogenome sequences. We deep sequenced and analyzed the complete mitochondrial genome of 43 *An. funestus* s.s. from 3 sites in Zambia, Democratic Republic of Congo and Tanzania. We found a high genetic diversity of *An. funestus* s.s. with 41 unique haplotypes out of 43 mitogenome sequences, and 355 parsimony informative sites. Phylogenetic reconstruction using the novel mitogenomes confirmed the existence of two divergent *An. funestus* mitochondrial lineages, I and II, in northern Zambia and revealed new records for lineage II in Tanzania. Statistical parsimony network analysis revealed two major groups within lineage I, and no general structure for those samples in lineage II, likely due to under sampling within this population. Studies are ongoing with regard to the biological relevance of these mitochondrial lineages within *An. funestus* s.s., which may have implications for vector control.

### IMPACT OF OPTIMALLY AND SUB-OPTIMALLY CLOSED EAVES ON THE HOUSE ENTRY BEHAVIOR OF MALARIA VECTORS

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A surge in malaria transmission is feared due to the increasing resistance of malaria vectors to insecticides. Structural house improvement (e.g. closed or screened eaves; screened windows) is a promising complimentary tool to other malaria interventions such as insecticide treated nets. House improvement covers and protects all individuals in a house equally. When implemented on a large scale, house improvement may not be employed wholly in 100% of houses; and therefore it's critical to assess whether sub-optimal (partial) house improvement will have any effect on mosquito house entry. We investigated the effect of partial house improvement on the house entry behaviour of malaria vectors and other mosquitoes in southern Malawi. Houses were modified according to five treatments: fully closed eaves, three different levels of partially closed eaves, and completely open eaves. All houses had fully screened windows and closed doors. We sampled host seeking mosquitoes inside these houses using Centers for Disease Control (CDC) light traps. The effect of house improvement level on the number of mosquitoes caught was tested using non-parametric tests (Kruskal-Wallis and Mann-Whitney U tests). Fully improved houses had significantly fewer *Anopheles* mosquitoes than houses that were partially improved. Likewise, the catches of *Culicine* mosquitoes were significantly different in houses that were fully and partially improved. The results highlight the importance of quality control measures when implementing structural house improvements as a malaria intervention.

### THE REAPPEARANCE OF ANOPHELES FUNESTUS AS A MAJOR MALARIA VECTOR IN THE ETHIOPIAN RIFT VALLEY AFTER 40 YEARS

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Construction of dams and irrigation schemes is critical to increase agricultural productivity and boost economy in Africa. With this regard, many dams are currently under construction in Ethiopia, with primarily purposes of irrigation and hydroelectricity generation. While these water development schemes are of paramount importance for economic growth, their impact on malaria transmission has been a public health challenge. This study aims to elucidate malaria transmission linked to Kesem dam and irrigation scheme in central Ethiopian Rift Valley. Larval and adult mosquito was collected monthly from two villages close (<1 km) from the dam and another two further away (>8 km; control villages) between September 2014 and October 2015. Determination of blood meal sources and detection of *Plasmodium falciparum* sporozoites was done using enzyme-linked immunosorbent assay (ELISA). Five years of monthly malaria case data (2010-2014) were also collected from health centers in the study villages. *Anopheles funestus* s.l. was believed to have been disappeared from the Ethiopian Rift Valley after introduction of DDT in 1970s. However, our sampling detected both larval and adult stages in the study area. Mean monthly malaria incidence was three times higher in the dam villages than the non-dam villages. Larvae of *An. funestus* s.l. and *An. arabiensis* were largely collected from the shoreline of the dam and irrigation canals. CDC light trap captures showed that *An. arabiensis* was predominant indoors while *An. funestus* s.l. was equally found both indoors and outdoors. For the first time in 40 years, this study confirmed the role of *Anopheles funestus* s.l. as a primary vector of malaria as evidenced by its high human blood index (0.81) and entomological inoculation rate (157 infective bites per person per year). The findings of this study indicate the reappearance of *An. funestus* s.l. and its role in malaria transmission in the newly irrigated areas of the Ethiopian Rift Valley. Proper water resource management that incorporates malaria intervention strategies is therefore required to mitigate malaria related to water resources development.

### BITING BEHAVIOR OF ANOPHELES DARLINGI IN FOUR COMMUNITIES IN THE MAZAN DISTRICT OF THE PERUVIAN AMAZON

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*Anopheles darlingi* is considered the main vector of malaria in the Loreto Region, where its seasonal abundance is strongly influenced by river levels and rainfall. In the Mazan district northwest of Iquitos, the highest levels of the Napo and Mazan rivers and the greatest anopheline densities occur during the rainy season, when malaria transmission is also greatest. Despite control measures undertaken by the Ministry of Health, between 2011-2015 (IRS, LLINs and Active Case Detection) malaria cases increased substantially, possibly the result of increased residual transmission (exophagic behavior of *An. darlingi*). Previous studies in 2008-2009 reported 98.6% (of 8,246) *An. darlingi*, with a Human Biting Rate (HBR) ranging 0.102-41.13 bites/person/hour. To characterize the vector biology behavior in the area, in the present study, mosquito surveys were carried out in 4 endemic malaria communities located near the confluences of the

Napo and Mazan rivers 35 km from the city of Iquitos. In each community, indoor and outdoor collections were performed two nights in March, June, September, and November 2016, each night from 6PM-6AM. Two barrier screens, to collect those mosquitoes seeking for blood meals, were set up one night/month and twelve hours collection (6PM-6AM). In total, 5,787 mosquitoes were collected by HLC and 746 by barrier screens. *An. darlingi* was the most abundant species (5,520, 95.39%) in each community and every survey. HBR varied among localities and by season (0-341 bites/night/person) reaching the highest in Visto Bueno and Libertad (both in Mazan river) in Jun. Overall, a high degree of exophagy (79%) was observed during the four collections. Similar densities of mosquitoes, most of which were *An. darlingi* (89.68%) were captured by barrier screens located near the forest (408) and near the river (388). In conclusion, communities located in Mazan river reported a higher abundance and HBRs of *Anopheles darlingi*, compared to communities in the Napo River. In addition, malaria transmission may be maintained due to the preference of these mosquito populations to bite outdoors, when people are not under malaria protective measures.

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### STUDIES ON THE BITING BEHAVIOR OF AEADES MOSQUITOES' IN SOME SELECTED COMMUNITIES IN NORTHERN GHANA

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The mosquito *Aedes aegypti* maintains close association with human populations and it is the principal vector of yellow fever and dengue fever, as well as chikungunya virus (CHIKV) which is responsible for high disease burden and the recent Zika virus. In Ghana *Aedes* mosquitoes are the main vectors of yellow fever and are known to have locale-specific distribution. There have been an increase in the incidence of arboviral disease, most of which are transmitted mainly by *Aedes* species, it has become imperative to study the biting behavior of this important vector species in order to prepare for any future outbreaks and also to implement appropriate vector control programmes. There is also the need to update the existing information on biting patterns of *Aedes* mosquitoes since very little information available in Ghana is scanty and scattered in few reference journals. We studied the comparative biting patterns of *Aedes* in (Damongo) Northern Region, (Bolgatanga), Upper East Region and (Nadowli), Upper West region of Ghana for 16 months, from 2015 to 2016. Female adult mosquitoes were collected both indoor and outdoor between the hours of 06:00 and 18:00 hours GMT from all the study areas using human landing collections. All mosquitoes were sorted and identified morphologically using appropriate taxonomic keys. In all an average of 1196 mosquitoes were collected, 61 % which were *Aedes aegypti*, 1 % were *Aedes vittatus*, 10 % were *Anopheles* and 28 % were *Culex*. *Aedes aegypti* was the predominant biting species in Bolgatanga (43.4%), followed by Damongo (35.6%), with the least number being 21% in Nadowli. *Aedes vittatus* was found biting only in Nadowli and more in the rainy season of 2015 than in the rainy season of 2016. *Aedes aegypti* had a bimodal biting behavior peaking at 07:00 - 08:00 and 16:00 - 17:00 hours GMT. The information on the biting behavior is important for planning and implementing effective vector control programmes in the three regions of the North and in Ghana as a whole.

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### PRESENCE AND ABUNDANCE OF AEADES AEGYPTI ADULT MOSQUITOES IN RURAL AND URBAN AREAS OF WESTERN AND COASTAL KENYA: A POTENTIAL RISK FOR THE OCCURRENCE AND TRANSMISSION OF ARBOVIRAL DISEASES

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*Aedes aegypti* is the main vector for yellow fever, dengue fever, chikungunya and Zika viruses. Presence and abundance of this adult vectors is associated with the risk for the occurrence and transmission of these arboviruses and potential introduction of new viruses. **Methods:** Presence and abundance of *Ae. aegypti* adult mosquitoes were determined indoors and outdoors in two western (urban Kisumu and rural Chulaimbo) and two coastal (urban Ukunda and rural Msambweni) sites in Kenya. Sampling was performed using human landing catches, Prokopack automated aspirators and Biogents-sentinel traps. A total of 2,247 *Ae. aegypti* mosquitoes were collected: 785 by human landing catches, 459 by Prokopack and 1003 by Biogents-sentinel traps. About three times as many *Ae. aegypti* mosquitoes were collected in urban than rural sites (1665 versus 582). Comparable numbers of them were collected in western (1201) and coastal (1046) sites. Over 80% of them (999/1244) were collected outdoors through human landing catches and Prokopack aspiration. The probability of collecting *Ae. aegypti* mosquitoes by human landing catches was significantly higher in the evening than morning hours ( $P=0.002$ ), outdoors than indoors ( $P<0.001$ ) and in Ukunda than in Chulaimbo ( $P<0.001$ ). Significantly more *Ae. aegypti* mosquitoes were collected using Prokopack aspiration outdoors than indoors ( $P<0.001$ ), in Ukunda than in Chulaimbo ( $P<0.001$ ) and in Kisumu than in Chulaimbo ( $P=0.005$ ). Significantly more mosquitoes were also collected using Biogents-sentinel traps in Kisumu than in Chulaimbo ( $P<0.001$ ). In conclusion, *Aedes aegypti* mosquitoes were found in all the study sites with the probability of exposure to their bites being highest outdoors, in urban areas and in evening hours.

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### COMBINING AREA-WIDE MOSQUITO REPELLENTS AND LONG-RANGE ATTRACTANTS TO CREATE A "PUSH-PULL" SYSTEM THAT PROTECTS AGAINST DISEASE TRANSMITTING MOSQUITOES IN TANZANIA

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Despite high coverage of indoor interventions such as insecticide-treated nets, mosquito-borne infections persist partly because of outdoor-biting, early-biting and insecticide-resistant vectors. This study assessed protective efficacy of a push-pull system against outdoor-biting and indoor-biting pyrethroid resistant mosquitoes in a Tanzanian village. A partially randomized cross-over design was used to test the efficacy of push-pull for 32 nights in four experimental huts and 16 nights in four local houses, in an area with high pyrethroid resistance. The push-pull system consisted of repellent dispensers which actively dispensing transfluthrin vapors and one lure-and-kill device the odour-baited mosquito landing box situated outside two huts and households. Two other huts and households without push-pull were used as controls. Mosquitoes were collected

indoors hourly using exit traps in huts and outdoor by HLC. *Anopheles gambiae* complex and *An. funestus* group were caught during huts and household experiments were distinguished between sibling species by using PCR. *Plasmodium falciparum* antigens in mosquito salivary glands were detected by ELISA method. Primary outcomes were, 1) number of mosquitoes caught outdoors and indoors, 2) sibling species composition and 3) number of *P. falciparum* infected mosquitoes. Push-pull offered marginal protection against host-seeking mosquitoes, in experimental huts there was a significant 30% reduction in outdoor-biting for *An. arabiensis* ( $P < 0.001$ ), and 41.5% for *Ma. uniformis* ( $P < 0.014$ ). There were no significant biting reductions for other mosquito species. Number of mosquitoes caught inside exit traps between treated and control huts were statistically similar. All *An. gambiae* analyzed by r-DNA PCR were identified as *An. arabiensis*, but for *An. funestus* group, 86.9% were *An. funestus* s.s., 9% *An. rivulorum* and 3.9% *An. lesoni*. No *Plasmodium* infected *Anopheles* were detected. Push-pull provided modest protection against early-biting and outdoor-biting mosquitoes. This approach could possibly contribute to reducing transmission of mosquito-borne infections, if optimized.

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### EFFICACY AND PERSISTENCE OF LL3 AND FOURSTAR MICROBIAL LARVICIDES AGAINST DIFFERENT LARVAL STAGES OF MALARIA VECTORS IN WESTERN KENYA HIGHLANDS

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The chemical based malaria vector control interventions are threatened by development of insecticide resistance and change in behavior of the vectors calling for the need for alternative control methods. Bacterial larvicides have the potential to target insecticide resistant and outdoor biting mosquitoes and safe to the environment. The currently available microbial larvicide formulations have short duration of activity requiring frequent re-applications which increase cost of interventions. This study was designed to evaluate efficacy and duration of activity of two (LL3 and FourStar®) long-lasting formulations of *Bacillus thuringiensis israelensis* (Bti) and *Bacillus sphaericus* (Bs) under field conditions in western Kenya highlands. Three sites were selected for this study in the highlands of western Kenya. In each site, one hundred anopheline larval habitats were selected and randomized into three arms, namely: 1) LL3; 2) FourStar® and 3) untreated control larval habitats. The habitats were sampled for mosquito larvae by using standard dipping technique and larvae found were recorded according to the larval stages of the different *Anopheles* species. The larvicides were applied at manufacturers recommended dosage of 1 briquette per 100 square feet. Both treatment and control habitats were sampled for mosquito larvae just before treatment (day 0), and then 24hrs, 3 days and weekly post treatment for 5 months. Results show that after intervention with the larvicides, larval density in the treatment habitats was significantly reduced as compared with the control habitats. Both larvicides showed a higher impact in larval stages of *Anopheles gambiae* s.l. than in *An. funestus*. A higher reduction in *Anopheles* larval density was observed in the abandoned goldmines, pond and abandoned fish ponds with the least reduction observed in drainage channels. The LL3 larvicide had a relatively higher percent reduction than FourStar though not significantly different. This study showed that both LL3 and FourStar® long lasting microbial larvicides were effective in reducing larval of *An. gambiae* complex and *An. funestus* group mosquitoes for three months.

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### DYNAMICS OF MALARIA TRANSMISSION, PREVALENCE AND INCIDENCE RATES IN KORHOGO AREA, NORTHERN CÔTE D'IVOIRE

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Despite the striking successes achieved last decade against malaria, there is a compelling need to find alternative methods for maintaining the effectiveness of mosquito vector control. REACT project aims to assess novel malaria vector control strategies in combination with the community-wide coverage of Long-Lasting Insecticidal Nets (LLINs) in 30 villages of Korhogo, northern Côte d'Ivoire. Epidemiological studies in this malaria endemic area need to be updated since the last data was published more than 10 years ago. In an attempt to fill this gap and get baseline data for the REACT randomized control trial, an entomological and epidemiological surveys were carried out in six randomly selected villages. Clinical malaria cases were collected retrospectively in the local health centers records from July 2016 to January 2017. During the rainy and dry seasons, mosquitoes were collected in each village using human landing catch method on volunteers from 5:00 pm to 09:00 am. *Anopheles* species were morphologically identified and a sub-sample dissected for parity determination based on the tracheolar skeins. Polymerase chain reaction (PCR) analysis were used to ascertain the species of the *An. gambiae* complex, *Plasmodium.sp* sporozoite infection and insecticide resistance mechanisms. Furthermore, malaria active case detection was performed on all individuals aged from 6 months to 14 years in the rainy and dry seasons. Blood samples were collected for malaria detection using Rapid Diagnostic Test (RDT) and positive samples were confirmed by examination of Giemsa-stained thick blood films. We will therefore present the dynamics of malaria transmission, prevalence and incidence in sentinel villages in the North of Côte d'Ivoire.

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### SEASONAL VARIATION IN ABUNDANCE AND BITING BEHAVIOR OF MALARIA VECTORS, ANOPHELES GAMBIAE S.L. AND AN. FUNESTUS USING CLIMATE DATA IN RURAL TANZANIA

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Malaria prevalence can be highly influenced by climatic conditions that drive the abundance and seasonal dynamics of *Anopheles* vectors. In particular, rainfall influences the breeding habitat while microclimatic conditions determine the survival and biting behaviours of adult *Anopheles* mosquitoes. Household data on mosquito abundance and microclimatic conditions from South-east Tanzania were analysed to quantify its effect on *Anopheles* abundance and biting behaviour. Mosquitoes were sampled using human landing catches (HLC) both inside houses outdoor area for 616 nights trap. Climatic information (temperature and humidity) and mosquito were simultaneously collected. Daily rainfalls were aggregated in three time bands 1-2, 2-3 and 3-4 weeks before sampling day. Aggregated and current rainfall records were used to explain mosquito abundance. Generalized additive mixed models (GAMMs) and linear mixed models (GLMMs) were used to estimate the seasonal abundance and proportion of outdoor biting in *Anopheles* species respectively. *An. gambiae* s.l. was observed to be more abundant during the high rain season, while *An.*

funestus were observed to be more abundant during the month of little/no rainfall. An increase in mean temperature of 1°C caused an increase in *An. arabiensis* abundance by ~11% and *An. funestus* by 66%. *An. gambiae* s.l. abundance was positively influenced by rainfall 2-3 and 3-4 weeks before while, *An. funestus* abundance was positively influenced by rainfall 3-4 weeks before. An increase in mean temperature of 10C indoors relative to outdoors resulted in high proportional of *An. gambiae* s.l. biting outdoors while *An. funestus* ability to rest outdoor was not significantly influenced by an increase in indoor temperature relative to outdoor ( $p=0.962$ ). We show that not only the abundance of *Anopheles* vectors, but also their relative propensity to bite outdoors vs. indoor changes in response to seasonally varying microclimatic conditions. With these results it is possible to say that, the effectiveness of the vector control currently in place varies throughout the year with changes in microclimatic condition

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### ESTIMATING THE POPULATION AT RISK OF ZIKA IN THE ASIAN REGION

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On November 18, 2016, the World Health Organization ended its designation of Zika virus (ZIKV) as a Public Health Emergency of International Concern (PHEIC). At the same time, ZIKV transmission continues in Asia, with the number of Asian countries reporting Zika cases increasing over the last two years. Applying a method that combines epidemiological theory with data on epidemic size and drivers of transmission, we characterized the population at risk of ZIKV infection in 15 countries in Asia. Projections made under the assumption of no pre-existing immunity suggest that up to 785 (range: 730-992) million people in Asia could be at risk of ZIKV infection under that scenario. Assuming that 20% of ZIKV infections are symptomatic, this implies an upper limit of 146-198 million for the population at risk of a clinical episode of Zika. Due to limited information about pre-existing immunity to ZIKV in the region, we were unable to make specific numerical projections under a more realistic assumption about pre-existing immunity. Even so, combining numerical projections under an assumption of no pre-existing immunity together with theoretical insights about the extent to which pre-existing immunity may lower epidemic size, our results suggest that the population at risk of ZIKV infection in Asia could be substantially larger than in the Americas. As a result, we conclude that the WHO's removal of the PHEIC designation for Zika virus should not be interpreted as an indication that the threat has subsided.

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### THE IMPACT OF AVIAN MALARIA ON VECTOR COMPETENCE AND WEST NILE VIRUS TRANSMISSION INTENSITY

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Empirical evidence demonstrates that variability in epidemiological metrics, such as host competence, vector competence, and parasite virulence, contribute to transmission heterogeneity across space and time. Accounting for these heterogeneities can improve disease control programs by targeting biological mechanisms that disproportionately impact pathogen transmission at the population-level. Polyparasitism, the simultaneous infection with multiple parasitic species, is a widespread phenomenon in nature known to drive transmission heterogeneity. *Culex* mosquitoes, the main vectors of West Nile virus (WNV), may ingest a variety of viral, protozoan, and macro-parasitic organisms found among avian and mammalian hosts. However, the downstream transmission consequences of mosquitoes ingesting these organisms are unknown. Here, we use experimental infections to assess how co-circulating plasmodium parasites impact West Nile virus (WNV) competence in *Culex quinquefasciatus* mosquitoes. Vector competence metrics, such as survivorship, midgut infection, dissemination, salivary gland infection, and

transmission, were recorded daily to parameterize mathematical models. Vectorial capacity,  $R_0$ , and global sensitivity analyses were used to evaluate how WNV-*Plasmodium* co-infection of *Culex* mosquitoes changes the intensity of WNV transmission.

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### COULD THE RECENT ZIKA EPIDEMIC HAVE BEEN PREDICTED?

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The recent zika epidemic probably could not have been predicted. Without the prior knowledge of the zika virus being already present in South America, and given the lack of understanding of key epidemiological processes and long-term records of zika cases in the continent, the best related prediction was for potential risk of an Aedes-borne disease epidemic. Here we use a recently published two-vector capacity model to assess the predictability of the conditions conducive to epidemics of diseases like zika, chikungunya or dengue, transmitted by the independent or concurrent presence of *Aedes aegypti* and *Ae. albopictus*. We compare the potential risk of transmission forcing the model with the observed climate and with state-of-the-art operational forecasts from the North American Multi Model Ensemble (NMME, 116 realizations in total), finding that the predictive skill of this new seasonal forecast system is highest for multiples countries in Latin America and the Caribbean during the December-February and March-May seasons, and slightly lower -but still of potential use to decision-makers- for the rest of the year. In particular, we find that suitable conditions for the occurrence of the zika epidemic at the beginning of 2015 could have been successfully predicted in several zika hotspots. Nonetheless, the initiation and spread of an epidemic depends on the confounding effect of multiple factors beyond climate conditions, and thus this type of approach must be considered as a guide and not as a formal predictive tool of vector-borne epidemics.

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### THE ROLE OF ANOPHELES GAMBIAE, AN. ARABIENSIS AND AN. COUSTANI IN INDOOR AND OUTDOOR MALARIA TRANSMISSION IN FIVE ECOLOGICAL ZONES OF NIGERIA

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Long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) against indoor biting and resting malaria vectors have reduced the burden of malaria in Africa. However significant transmission may be

ongoing outdoors. We evaluated indoor and outdoor malaria transmission dynamics of *Anopheles* mosquitoes in Nigeria. Malaria vectors were collected using Human-baited Centre for Disease Prevention and Control Light Trap (CDC LT) and Pyrethrum Spray Sheet Collections (PSC) in five ecozones of Nigeria. Specimens were identified morphologically with subsequent identification of sibling species using PCR. The proportions of mosquitoes harbouring sporozoite (sporozoite rates) were determined by ELISA. Entomological inoculation rates (EIR) were calculated using standard methods. A total of 2794 *An. gambiae* sensu lato mosquitoes and 500 *An. coustani* were analysed by ELISA. The highest indoor sporozoite rate of 7.4% was recorded in *An. gambiae* s.s. in Akwa Ibom in the mangrove forest collected using CDC LT, followed by 7.3% in Nasarawa using PSC method. A sporozoite rate of 4.2% was recorded for *An. arabiensis* in Bauchi and 3.1% in Sokoto, both from the PSC collections. For outdoor transmission, the highest sporozoite rate of 6.7% was recorded in *An. arabiensis* in Nasarawa in the Guinea Savannah using CDC LT whereas among *An. gambiae* s.s. outdoor collections, sporozoite rate of 2.9% was reported in Akwa Ibom. For the first time in Nigeria, sporozoite positive *An. coustani* was found both indoors and outdoors (sporozoite rate of 1.8% and 0.8% respectively) from the CDC LT in Nasarawa. EIRs ranged from 50.95 infectious bites per person per year (ib/p/yr) for *An. gambiae* s.s., 18.0 ib/p/yr for *An. arabiensis* recorded in Nasarawa, to 9.0 ib/p/yr for *An. arabiensis* in Ebonyi. Malaria transmission is occurring both indoors and outdoors, the main vectors being *An. gambiae* s.s. and *An. arabiensis*, with *An. coustani* playing a role as secondary vector both indoors and outdoors in Nasarawa. Effective malaria control programs may require new tools that target both indoor and outdoor transmission.

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### INCREASING MALE MOSQUITO CATCH WITHIN VECTOR SAMPLING COLLECTIONS MALE MOSQUITO CATCH WITHIN VECTOR SAMPLING COLLECTIONS

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Sample collection is essential to the study of disease vectors, and various methods have been developed for this. However, many malaria vector sampling tools/methods end up collecting mostly female mosquitoes. Traditional collection methods such as Human Landing Catch, indoor Pyrethroid Spray Catch and CO<sub>2</sub>-baited traps bias collections towards females that are either seeking blood meals or resting indoors, yet balanced vector sampling of both males and females would provide more comprehensive study parameter estimates for understanding vector dynamics. We have tested several additional methods thought to target male mosquito populations. We present the results of these collections, carried out in Uganda, with an aim to generate discussion on effective male mosquito collection methods. We have used aspirations (ASP) in inhabited buildings and in the eaves of inhabited houses, Clay Pot Traps (CPT), Bush aspiration, resting bucket traps (RBT) and swarm sampling (SWN). The use of additional male targeted methods increased the percentage of males caught to more than 30% compared to less than 10% in our previous sampling. ASP in the eaves of inhabited houses appears most productive and caught the highest number of males. However, SWN which is effective in other African regions, has been challenging and unpredictable at our study sites. Mosquitoes have been observed swarming low over larval habitats making it difficult to sweep samples. When swarming over bare ground, swarms were not more than 1m off the ground. An additional complication was mosquitoes swarming within lake fly swarms when lake flies are abundant. It is likely that SWN is not reaching its full potential. With new and innovative vector-targeted control methods such as sterile male technique and genetic modification of mosquitoes being developed, there is an increasing need for an effective male mosquito collection method for use in East Africa. Possible options to improve current traps could include adding lures to target males. We are hoping to promote an open dialogue on the methods that other experienced mosquito biologists may have found promising.

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### ENTOMOLOGICAL SURVEILLANCE OF MALARIA IN BURUNDI: AN IMPORTANT STEP FOR THE SUCCESS OF VECTOR CONTROL STRATEGIES

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Malaria remains among the most important public health problems in Burundi and constituted 48% of all causes of death. Since 2011, the Government of Burundi and USAID have worked on increasing the national capacity to use entomological data to inform the country's malaria prevention and control program. This study was conducted from March 2014 to February 2017 in 8 sentinel sites to assess vector behavior, infectivity and susceptibility to insecticides. Human landing catches, pyrethrum spray catches and CDC light trap collection methods were used to collect mosquitoes from sentinel sites. The study showed that *Anopheles gambiae* s.s. and *Anopheles funestus* s.s. are predominant (84%) vectors of malaria. The human biting activity of malaria vectors varied significantly ( $p=0.009$ ) among the sentinel sites with an average human biting rate of 9.89 [9.65-10.14] bites/man/night for *An. gambiae* s.l. and 1.91 [1.81-2.02] bites/man/night for *An. funestus*. The average indoor biting rate of *An. gambiae* s.l. was 9.76 [9.38-10.15] bites/man/night versus 10.33 [9.94-10.74] bites/man/night outdoors. For *An. funestus*, the indoor biting rate was 2.19 [2.01-2.38] bites/man/night versus 1.41 [1.26-1.56] bites/man/night outdoors. The average parous rate was 76.94% [71.64-82.53] for *An. gambiae* s.l. versus 72.26% [58.73-87.97] for *An. funestus*. Infectivity of *Plasmodium falciparum* was around 2% for both species. Estimated entomological inoculation rates were 5 infectious bites/person/month for *An. gambiae* s.l. and 1.14 infectious bites/person/month for *An. funestus*. Resistance of *An. gambiae* s.l. to pyrethroids and DDT was observed in 7 sentinel sites, while susceptibility to organophosphates and carbamates was observed in 6 sites with no confirmed resistance to pirimiphos-methyl. The *kdr*-East allele was only detected in *An. gambiae* s.s. populations with frequencies ranging from 96-100%. These results represent an important source of information to guide the implementation of vector control in Burundi. It will be important to consolidate entomological data and extend surveillance activities to the majority of regions where malaria epidemics occur.

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### JOB SATISFACTION OF BRIGADISTAS IN NICARAGUA: A CRITICAL ASPECT FOR TASK-SHIFTING

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*Brigadistas* are the community health workers (CHWs) in Nicaragua, and are responsible for classifying and diagnosing common childhood illnesses, counseling mothers and care givers, administering treatment, encouraging referral of serious cases and making follow-up home visits. Current trends in task-shifting and integrated program delivery require CHWs (e.g., *brigadistas*) to deliver several essential services, including maternal, newborn and child health, and family planning, and thereby to expand the scope of their responsibilities. In 2014, we conducted a cross-sectional study, among *brigadistas* who were assigned in hard to reach

areas ('C' category communities) in five *municipios* in the departments of Matagalpa and Jinotega in north central Nicaragua, with an aim to assess their job satisfaction, prior to adding newborn care to their responsibilities. Following stratified random sampling strategy, 15 *brigadistas* were selected from each of the following four strata: each of four strata: male vs. female and age <25 vs >30 years. We completed in-person interviews for 45 eligible and available *brigadistas* (75% of planned 60 interviews). Measured on a 0-5 Likert scale, *brigadistas'* job satisfaction was high (mean: 4.2, range: 2.5 - 5.0; 1 rated as 2.5, 16 rated as 4.0 and 25 rated as 5.0). Facilitators of job satisfaction included training (53%) and "doing good for the community" (31%). The main barrier was lack of time (36%) due to family, work, or study commitments. These results can be useful for policy makers and program managers for making an informed decision before expanding the scope of work for *brigadistas* in Nicaragua.

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### VALIDATION AND USABILITY OF MEDSINC - AN INTEGRATED MOBILE HEALTH (MHEALTH) SOFTWARE PLATFORM FOR CLINICAL ASSESSMENT OF COMMON CHILDHOOD ILLNESSES

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A lack of access to healthcare professionals and limited healthcare infrastructure are the major causes for the high mortality and morbidity in children under five (U5) years of age in low and middle income countries (LMICs). THINKMD and strategic partners have performed validation, usability and acceptability field studies of an integrated community health worker (CHW) specific mobile health (mHealth) clinical assessment platform, MEDSINC®, for children U5, to determine if utilization of the platform could address the shortage of healthcare professionals and limited health care infrastructure in these regions. MEDSINC® is a simple to use, self-teaching next-generation integrated clinical assessment software platform, which enables minimally skilled users to quickly determine how sick a child is by simultaneously determining an integrated clinical severity assessment (none/mild; moderate; severe) for respiratory distress, dehydration, infection risk (sepsis-SIRS), and malnutrition. MEDSINC® also screens for possible malaria, meningitis, anemia, urinary tract infection, skin infection, measles, ear infection and dysentery. CHWs were trained on the MEDSINC platform using a training of trainer strategy. Total training time was between 1.5-2.5 hours. Children within the 2-59 months of age range presenting to or residing in testing sites were eligible to be enrolled in this study. Upon receiving informed written consent from respective caregivers/parents, CHWs generated integrated clinical assessments using the MEDSINC® platform on children which were correlated with blinded local physician's clinical assessments of the same child (not by using the MEDSINC® platform). MEDSINC® has validation data from 995 patient assessments by minimally skilled workers using the MEDSINC® platform. To date, in each region tested, independent assessments by minimally skilled users using MEDSINC® compared with local health professionals have consistently shown between 80% to above 95% integrated clinical assessment correlation, demonstrating the ability of CHW using MEDSINC® to increase healthcare capacity in LMICs.

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### HEALTH BELIEFS OF YOUNG RURAL CHILDREN AT HIGH RISK FOR PODOCONIOSIS: A QUALITATIVE STUDY IN SOUTHERN ETHIOPIA

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Though investigation of health beliefs among children is one of important condition for primary prevention of disease, little effort has been made to understand these in the context of podoconiosis. This study aimed to explore the health beliefs of rural children at high risk for the disease. A cross sectional qualitative study was conducted in March 2016 in Wolaita Zone, Southern Ethiopia. Data were collected through in-depth individual interviews and focus group discussions, with a total of one hundred seventeen 9 to 15-year-old children recruited from podoconiosis affected families. The study revealed various misconceptions regarding risk factors for podoconiosis. Most children believed barefoot exposure to dew, worms, snake bite, frog urine, other forms of poison, and contact with affected people to be major causes of the disease. Their knowledge about the role of heredity and that of long term barefoot exposure to irritant mineral particles was also poor. Though most participants correctly appraised their susceptibility to podoconiosis in relation to regular use of footwear and foot hygiene, others based their risk perceptions on factors they think beyond their control. They described several barriers to preventive behaviors, including uncomfortable footwear, shortage and poor adaptability of footwear for farm activities and sports, and shortage of soap for washing. Children also perceived low self-efficacy to practice preventive behaviors in spite of the barriers. Health education interventions may improve children's knowledge and risk perceptions, while family-based socioeconomic empowerment programs may help overcome practical challenges that children perceived as barriers and boost their confidence to engage sustainably in podoconiosis preventive behaviors.

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### CHOICE OF UNDERGRADUATE COURSES OF SICKLE CELL DISEASE PATIENTS - BAILOUT OR FRANKENSTEIN?

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In recent times, attention has been drawn to a Global disease which stands awkwardly alone between the traditional tropical communicable disease and non-communicable diseases. With worldwide distribution and highest burden in the tropics - amongst the impoverished population, serious morbidity and mortality, comorbidity for other life-threatening diseases, relatively simple diagnosis, indeed sickle cell disease (SCD) meets the criteria to be tagged an NTD. However, it is quite different from the other tropical diseases in that it has genetic basis of inheritance. Various studies have evaluated its cognitive and systemic dilapidating consequences. However, no study has studied the pattern of undergraduate course choices among this cohort. The study was conducted at the Olabisi Onabanjo University, Nigeria, among 1st year undergraduate students who were routinely screened before University admission. SCD was defined as HbSS, HbSC and HbCC disease. A total of 6,661 students were screened. The prevalence of SCD among the screened students was 0.92% (n=61, male:female=1.03:1, mean age=18+2.8years) with HbSS, HbSC, and HbCC having prevalence of 0.42%, 0.46% and 0.03% respectively. Among the SCD cohort the highest prevalence was found in the HbSC group (50.8%), then HbSS group (45.9%) and least by HbCC group (3.3%). Most of the students with SCD were in the social sciences (n=20), Basic medical sciences (n=12) and General sciences (n=9). The least cohort were in the clinical sciences, law, and Pharmacy (n=1 respectively). The rest were distributed across other faculties, mostly in the Arts. The above clearly shows the preference of these cohort for less physically and academically tasking courses perhaps to reduce the frequency SCD crisis - bailout. It could also be in keeping with previous workers that suggest cognitive decline and performance among patients hence their cut off points couldn't meet up with the "prestigious courses" in the University -

Frankenstein. There is need for proper counselling and follow up among these patients from early school years till University by a multidisciplinary team, to improve academic outcomes.

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### VACCINATION AND SOCIOECONOMIC RISK FACTORS FOR CHOLERA IN AN ENDEMIC SETTING OF BANGLADESH

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Cholera remains a public health threat globally. Oral cholera vaccines (OCV) are considered an important preventive tool, with global uptake on the rise. However, the protection offered by OCVs is sub-optimal. This study is to identify socioeconomic factors affecting OCV performance in urban population in Dhaka, Bangladesh. A large feasibility study on OCV included 30 clusters in each of three arms: vaccine, vaccine plus behavioural change, and a non-intervention arm. A structured questionnaire used for Socio-economic characteristics of the study population. Vaccination records and hospitalization case reports were documented. The data were analysed for the three populations: A. recipient of two-doses of OCV in the intervention arm (vaccine and vaccine plus behavioural change arms), B. non-dose recipients in the intervention arm and C. all persons in the non-intervention arm. A generalized estimating equation was used to assess the socioeconomic association with cholera among these different populations adjusting for household level correlation in the data. A total of 4295 diarrhoeal cases in 268,896 participants were observed in the two-year follow-up. Of which, 528 cholera cases and 226 severely dehydrated cholera cases were identified. Among vaccinated individuals, those in lower age groups had marginally higher risk for any cholera episode (RR=0.98, 95% CI=0.97-1.00), and those living in a household having more than 4 family members were at significantly higher risk of a severe cholera episode (RR=0.55, 95% CI=0.32-0.96). Among non-recipients living in the intervention areas, having diarrhoea at baseline census was found to be a risk factor for cholera. Among these individuals, females or individuals having diarrhoea at baseline were more likely to have severe cholera. In contrast, individuals living in a house having poor floor construction material, living in a high density area or living a long distance to the icddr hospital were at significantly higher risk of cholera and severe dehydrated cholera indicating that Cholera vaccination reduces the risk of cholera due to socioeconomic disparities in a high burden setting.

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### UNDERSTANDING PERCEPTIONS AND EXPERIENCES OF TRACHOMA AMONG MAASAI IN TANZANIA

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The World Health Organization set a target for elimination of blinding trachoma by 2020. Despite decades of achievements in trachoma control worldwide, challenges remain in endemic communities including social issues. The aim of this study was to explore socio-cultural factors that may impact this disease among hyperendemic Maasai communities. This study was situated in a larger ethnographic study of trachoma among Maasai in Northern Tanzania. We used semi-structured interviews for an in-depth examination of the knowledge and understanding of the nature of trachoma. Stratified random sampling was used. Interviews

were conducted and recorded in Maa, by a native Maa speaking trained interviewer. Transcripts were translated into English to conduct a framework analysis. Results show a poor understanding of trachoma. The causes of trachoma were attributed to pollen, dust, and smoke. Although water was recognized as beneficial for trachoma, it was seen as treatment and not prevention. Traditional medicines from plants and roots were most often used for treating inflammation. The most common was a rough leaf used to scratch the inside of the eyelid until it bleeds. Veterinary medicines for cattle were also used to treat inflammation in children. Knowledge of mass drug administration (MDA) was inconsistent. Although many thought it helped the community, they perceived it as only for children and the sick. Many reported not taking the drug and some had no recollection of the previous MDA, six months earlier. There was little connection between childhood infection, trichiasis and related blindness. Trichiasis was often seen as a problem of old women, and treated locally by epilation. Although blindness was considered a burden to members of the boma, it was mostly attributed to old age, God and a curse from others. These findings can help guide approaches to SAFE in Maasai communities. Despite limited knowledge, the community recognized trachoma as a public health problem. A targeted education program would be an important step in effective control programs. These challenges are likely to exist in other marginalized populations in trachoma endemic areas.

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### SCIENTIFIC AUTHORSHIP AND COLLABORATION NETWORK ANALYSIS ON MALARIA RESEARCH IN BENIN: PAPERS INDEXED IN THE WEB OF SCIENCE (1996-2016)

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To sustain critical progress in the national malaria control program in Benin, prioritization and a multidisciplinary approach to malaria research remain important. To document the structure of the malaria collaborative research in Benin, we analyze authorship of the scientific documents published on malaria from Benin from January 1996 to December 2016. We generated a multigraph co-authorship network with authors representing nodes. Edges are drawn between two authors when they co-author a paper. We compute vertex degree, betweenness, closeness, and eigenvectors among other metrics to identify prolific authors (hubs). We further assess the articulation points and how information flows in the network. Finally, we perform hierarchical clustering analysis and Monte-Carlo simulations to test significance of the metrics. Overall, 427 publications are included in this study. The generated network contains 1792 authors with 116,388 parallel edges. This network is converted into a weighted graph of 1792 nodes and 95,787 edges. Descriptive analyses suggest that prolific authors with higher degrees tend to collaborate. The hierarchical clustering reveal 23 clusters, 7 of which form a giant component containing 94% of all the nodes in the network. Closer attention to this giant component show characteristics of small-world network: a small shortest path distance of 3 between pairs, a diameter of 10 and a high clustering coefficient of 0.964. Though, Monte-Carlo simulations suggest our observed network is an unusual type of small-world network. A cut vertex analysis of the network reveals the presence of 16 articulation points. The malaria research collaboration network in Benin is a complex, with some small-world network characteristics. This research reveals the presence of closed research groups where collaborative research likely happens only between members. Interdisciplinary collaboration appears to occur mostly between prolific researchers. Advanced analyses involving statistical modeling are needed to better explain the malaria co-authorship network in Benin.

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**GLOBAL HEALTH JOBS ANALYSIS PROJECT: CHARACTERIZING THE GLOBAL HEALTH AND DEVELOPMENT EMPLOYMENT MARKET**

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Global health has become an integral component of discussions of policy, economics, and global security. As a result, graduate programs in global health have flourished and public health graduates interested in global health and development are entering the job market in ever-growing numbers. However, the job market remains difficult to navigate for graduates and young professionals, and there is growing concern that the training and experience provided by these programs do not equip graduates with the skills and experience needed to succeed in the field. This session will present the final results of the Global Health Jobs Analysis, which collected data on publicly posted global health job vacancies with the aim of providing insight into the current global health job market. Data on position, location, education level required, years of experience required, regional and technical area of focus, language skills and international work experience required were analyzed for 1,007 global health job vacancies open to U.S. citizens and employment-eligible residents by 127 private and non-profit companies and six U.S. government agencies over a six-month period from November 2015 to May 2016. The quantitative analysis will include descriptive statistics and associations between variables. The qualitative analysis will focus on trends in the vacancy descriptions, with a focus on positions requirements and preferred qualifications. The presentation will discuss key insights from the mixed-methods analysis, examining the amount of work experience and the most common skills and technical competencies required for the global health profession. We will examine how able current global health graduates are to meet the needs of the profession and steps they can take to prepare to enter the market.

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**MAKING HEALTH MARKETS WORK FOR LOW-INCOME POPULATIONS IN KENYA AND GHANA: HOW CHANGES IN NATIONAL HEALTH INSURANCE FINANCING AFFECT PRIVATE PROVIDERS**

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The cost of healthcare is often a barrier to access for low-income populations. In Kenya and in Ghana, National Health Insurance (NHI) schemes have expanded coverage beyond civil servants, and both countries have programs that aim to expand NHI to people with low income. African Health Markets for Equity (AHME) is a multi-organization partnership that aims to increase coverage of health interventions amongst the poor through engagement with private providers in Kenya, Ghana, and Nigeria. Since 2013, AHME has worked with NHI officials to increase private provider accreditation rates and connect newly enrolled NHI patients with quality care in AHME-affiliated clinics. A qualitative evaluation was conducted in Kenya and Ghana to assess the effect of AHME on attitudes and practices of patients, providers, and government regulators. Between July 2013 and March 2017, we interviewed 214 private providers, 74 AHME partners, and 9 government officials about their experiences: (1) reaching low-income populations; (2) incorporating private-sector clinics into the NHI schemes; (3) views of collaboration between government and private provider networks. Analysis combined

the findings and trends from both data sources. Our findings highlight the need for NHI programs to balance supply and demand actions: programs to enroll poor populations have shown some success and are scaling up. Facility accreditation programs have been effective in Ghana, and are slowly becoming more efficient in Kenya. AHME experiences highlight the need to intensify outreach to low-income communities to connect them with accredited providers.

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**STRENGTHENING THE PUBLIC HEALTH WORKFORCE IN BANGLADESH THROUGH SCIENTIFIC TRAINING AND MENTORSHIP: A LESSON FOR LOW INCOME COUNTRIES**

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Developing independent scientists who rigorously research public health questions and publish findings contributes to a strong public health workforce able to respond to global threats. Fostering high-quality scientific skills requires a long-term commitment to training and mentorship. Since 2009, the Global Disease Detection center (GDD) in Bangladesh has allocated resources to promote an approach to public health research that guides junior scientists through analyzing and publishing research. The key elements used by GDD-Bangladesh for building critical-thinking and writing skills includes committed mentorship by senior scientists, classroom trainings, data collection assistance, support for statistical analysis, English language writing, and a writing guide. Senior scientists, statisticians, and writing coaches use a structured approach and timeline for providing actionable feedback; re-writing manuscript text by senior authors is explicitly discouraged. Between 2009 and 2016, GDD-Bangladesh provided training and mentorship to 75 junior Bangladeshi scientists through group training and one-on-one mentoring; 34 of them became first-time first authors in peer-reviewed journals. Approximately 95% of abstracts submitted to international conferences by junior researchers have been accepted. Twenty-six researchers trained at GDD-Bangladesh have gone on to pursue PhD degrees and continue to contribute to the public health science base. Field Epidemiology Training Programs in Bangladesh, Kenya, Zambia and South Africa have used this mentee-centered approach to build scientific skills; for example, 10 of 13 (77%) residents of the 2015 South Africa cohort graduated from an accredited university requiring a manuscript ready for publication. This systematic approach to scientific research and writing, with clearly outlined roles and responsibilities for first authors and mentors, has set out a pathway to publication that supports junior researchers to become first authors. This approach can be further leveraged to enhance surveillance and research skills throughout the government and academic health sectors.

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**PERSISTENT STUNTING FROM EARLY CHILDHOOD IMPAIRS COGNITIVE PERFORMANCE IN LATE CHILDHOOD: RESULTS FROM A BIRTH COHORT IN A SEMI-URBAN SLUM IN SOUTH INDIA**

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Deprivation of developmental potential of children in the developing countries plays a key role in the intergenerational transmission of poverty. Exposures to genetic and environmental factors result in growth faltering in early childhood and further deprive the developmental goals in late

childhood. This study assessed the effects of early childhood risk factors like linear malnutrition, socio-economic status, burden of gastrointestinal infections and early morbidity on the cognitive development of children during late childhood. The study recruited 303 children from a birth cohort study in urban slums of Vellore at 12th year of life. The cognitive performance (Malin's Intelligence Scale for Indian Children) and anthropometry of the children were assessed. Children were classified into persistent, caught-up, less stunted and never stunted based on stunting at 2nd, 3rd and 12th-year assessment. Multiple linear regression analysis was performed to study associations between the prevalence of stunting, early childhood infection, illness and socio-demographic variables like maternal education and maternal occupation on the cognitive performance (IQ scores) of children in late childhood (12th year). The mean (SD) intelligence quotient (IQ) of the cohort was 87.4 (7.7). Persistently stunted children had impaired cognitive performance in the late childhood (3.90, 95%CI 1.12 to 5.67) when compared to the children who were never stunted, whereas "caught up" children had lesser impairment in cognitive performance (3.18, 95%CI 0.87 to 5.49). After adjusting for the maternal education, early childhood respiratory illness, and infections like *giardia* and *helminths*, persistent stunting was significantly associated with reduced cognitive performance of the children during late childhood. Children who exhibited catch up growth after early childhood had an advantage in their cognitive performance over those persistently stunted. Targeted interventions are needed during both early and late childhood in order to alleviate the burden of malnutrition and its long-term consequences.

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### COMMUNITY PARTICIPATION IN MOSQUITO BREEDING SITE CONTROL: A MULTIDISCIPLINARY MIXED METHODS STUDY IN CURAÇAO

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As the arboviral diseases dengue, chikungunya and Zika emerge in the Americas, so does the need for sustainable vector control policies. To successfully achieve mosquito control, joint efforts of both communities and governments are essential. This study investigates this important but by-and-large neglected topic. In June and July 2015, a cross-sectional mixed method study applying a survey questionnaire (n=339), in-depth interviews (n=20) and focus group discussions (FDGs)(n=7) was performed in Curaçao. The study was designed based on an integrated theoretical framework of the Health Belief Model and the Theory of Planned Behaviour. Participants showed a good knowledge of, and a high-level performance of mosquito breeding site control (MBSC) practices. Personal protection against mosquitoes was perceived as less valuable than MBSC practices and was consequently applied to a lesser extent. The study highlights three possible ways of improving community participation in MBSC. First, it highlights the need for ongoing media coverage, based on communities' lived realities. Second, it shows a two-directional influence of governments' policies on communities' actions, which should be addressed in campaigns. Third, the presence of key persons in communities, which could be engaged in mosquito control policies to improve MBC in neighbourhoods, is described. In conclusion, this study reveals gaps between policy and communities' lived realities. These gaps

might be overcome with the proposed interventions, resulting in a higher performance of MBC of the community in Curaçao. Furthermore, this study shows how interdisciplinary mixed methods research can provide comprehensive and in-depth insights for mosquito control policies.

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### TASK SHIFTING THE IDENTIFICATION, EMERGENCY MANAGEMENT AND REFERRAL OF WOMEN WITH PRE-ECLAMPSIA IN MOZAMBIQUE, AND FACILITY CAPACITY TO RESPOND

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Maternal mortality is an important public health problem in low income countries. In Mozambique, maternal mortality in 2013 was still high on 480 deaths per 100,000 live births with eclampsia counting for the third obstetric cause. Delays in reaching health facilities and insufficient health professionals calls for community-level interventions. The aim of this study was to describe the feasibility of task-shifting the initial screening and initiation of obstetric emergency care for pre-eclampsia/eclampsia by community health workers in Mozambique and to document facility readiness to respond to this task-shift. The study took place in Maputo and Gaza Provinces in Mozambique using mixed methods. The qualitative data was collected through focus group discussions and in-depth interviews with various community groups, health care providers, and policy makers. All discussions were audio-recorded and transcribed verbatim prior to thematic analysis using QSR NVivo 10 software. Quantitative data was collected through self-administered questionnaires to community health workers and health facility assessment survey, analysed using Stata version 13. Community health workers in Mozambique are skilled to identify the basic danger signs of pregnancy; however, they are not able to manage these emergencies, or refer to the facility. Nurses at primary facilities were trained to manage eclampsia before referral. The necessary equipment for obstetric emergency care was not available in most primary facilities. MgSO<sub>4</sub> was available in most primary facilities (83%). Most health facilities (96%) had an ambulance for referrals. Although community health workers and patients favoured task-shifting, other healthcare providers highlighted the need to first address current barriers at health facilities including lack of equipment, shortage supervisors, and drugs stock out. This study showed that task-shifting initial screening and pre-referral management of pre-eclampsia/eclampsia is acceptable by the community, but an effort should be in place to address health system readiness to appropriately manage the emergency cases.

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### COMMUNITY ENGAGEMENT FOR EXCEPTIONALLY DANGEROUS PATHOGENS CONTAINMENT: THE POTENTIAL OF SENTINEL COMMUNITY SURVEILLANCE

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Sustaining Ebola virus disease (EVD) containment is a journey, not a step. That reality provides the justification for sentinel community surveillance (SCS). Consider if the Ebola index case in Nigeria was a Nigerian trader who contracted the virus outside Nigeria, returned to his rural community family by road and fell ill within a few days, his most likely point of reporting illness may have been a faith-based setting, a primary health

care centre, a one-doctor private clinic, or a general hospital. The chain of contacts and exposure, and the consequences would have been different from the trajectory consequent to that of Mr. Sawyer, the Liberian identified as Nigeria's Ebola index case. Nigeria and other West African and east African countries need sentinel community surveillance for active community engagement and mobilization to sustain their EVD containment and to drive proactive measures against other quick-kill infections that may emerge<sup>7</sup>. SCS would reinforce inclusiveness of the national preparedness and response effort, if developed as an integral part of the country's primary health care system.

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### VALIDATION OF MATERNAL REPORTS FOR LOW BIRTHWEIGHT AND PRETERM BIRTH INDICATORS IN RURAL NEPAL

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Tracking progress towards global newborn health targets depends largely on maternally reported data from nationally representative surveys. We evaluated the validity, across recall periods (1 to 24 months post-delivery), of maternal reports of birthweight, birth size and length of pregnancy. We compared maternal reports to 'gold' standards of birthweight measured within 72 hours of delivery and gestational age generated from reported first day of the last menstrual period (LMP) prospectively collected in a population-based study (n=1513). We calculated sensitivity, specificity, area the under the receiver operating curve (AUC) as a measure of individual-level accuracy, and the inflation factor (IF) to quantify population-level bias for each indicator. We assessed whether recall period modified accuracy by stratifying measurements across time bins and using a modified Poisson regression with robust error variance to estimate the relative risk (RR) of correctly classifying newborns as low birthweight (LBW) or preterm (PTB), adjusting for child sex, place of delivery, maternal age, maternal education, parity, and ethnicity. LBW using maternally reported birthweight in grams had low individual-level accuracy (AUC=0.69) and high population-level bias (IF=0.62). The LBW indicator using maternally reported birth size and the PTB indicator had lower individual-level accuracy (AUC=0.58, 0.56, respectively) and higher population-level bias (IF=0.28, 0.35, respectively) up to 24 months following birth. Accuracy did not generally change with recall time; however, it statistically significantly improved for the PTB indicator at 20 months post-delivery and on. The use of maternal reports may underestimate and bias LBW and PTB indicators. In settings with high prevalence of LBW and PTB, indicators from maternal reports may be subject to misclassification. In populations where a high proportion of births occur at home or where weight is not routinely measured, mothers perhaps place less importance on remembering birth weight. Further work is needed to see whether these conclusions hold in other similar rural and low-income settings.

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### THE PREVALENCE AND CONTRIBUTING FACTORS OF EARLY CHILDHOOD STUNTING IN RURAL COASTAL KENYA

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This study measured prevalence of early childhood stunting in rural coastal Kenya as associated with maternal parasitic infection status, socioeconomic status (SES), and nutritional intake. Pregnant mothers were

enrolled and tested for parasitic infections during their prenatal period and at delivery through stool, urine and blood testing. In a 6-week sub-study conducted in 2016, 244 of the resulting children, 6-37 months of age, who had been followed biannually since birth with parasitic infection and anthropometric testing, were assessed for current stunted growth using height-for-age Z-scores (HAZ). An environment index that included water, sanitation, assets, maternal education, and income (WAMI) was used to evaluate SES, while nutritional intake was assessed by questionnaire. Linear regression was used to examine contributions of maternal parasitic infection status, SES, and nutritional intake on current HAZ. At the 2016 visit, 25% of the children were stunted (HAZ < -2) and retrospective analysis showed that 54% were stunted during at least one prior biannual visit. Of the currently stunted children, 73% had mothers with at least one parasitic infection during the prenatal period, and 45% had mothers with at least one parasitic infection at delivery. There was no correlation between maternal prenatal or delivery parasitic infection status and current HAZ. WAMI was not significantly correlated with current HAZ (p = 0.31). Analysis of components of the WAMI showed that household ownership of a table (p = <0.001) or a bank account (p = 0.02) was significantly associated with higher current HAZ, while there was no association found for water, sanitation, maternal education, or income. Consumption of leafy vegetables was associated with higher HAZ (p = 0.02) while consumption of legumes was associated with lower HAZ (p = 0.03). These results demonstrate the high prevalence of early childhood stunting in rural coastal Kenya. Stunting was not predictable by WAMI, suggesting that factors beyond SES must be considered when evaluating the risk of childhood stunting and identifying ways of preventing this serious threat to global child health.

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### EXAMINING PERCEPTIONS AND ACCEPTABILITY OF FULL DIAGNOSTIC AUTOPSY IN KILIMANJARO REGION, TANZANIA

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Uncertainty about the causes of death in low- and middle-income countries (LMIC) has been recognized as a constraint to global health and development. While full diagnostic autopsy (FDA) is the best way to assess the cause of death, it is uncommon in LMICs due to low investment priority and assumptions about acceptability. Social science research was conducted from May 2016 through March 2017 to examine issues related to acceptability of FDAs in northern Tanzania where autopsy is being offered in two referral hospitals to assess the cause of death from febrile illness. Initial formative research entailed 29 key informant interviews, six observations of burial practices, and four group discussions. Subsequently, in-depth interviews were conducted with families of deceased, including 10 families that accepted and 10 families that refused FDA. The formative research illuminated beliefs that FDAs are conducted to obtain organs, make medicine, and train medical students, raising skepticism about hospital motives. Barriers to acceptance included perceptions that FDAs will not provide valuable information, disfigure the body, inflict pain, and delay burial, while facilitators involved learning how to protect family members from illness and discovering explanations for sudden death. Findings were used to develop educational approaches and make authorization culturally appropriate prior to initiation of autopsy procedures. Preliminary FDA acceptance is 39%. Families of deceased cited the desire to gain information about the cause, including if witchcraft contributed, so that similar conditions do not afflict family members in the future. Refusals mentioned the cause of death was already known, stigma associated with illness, and objections to the consent process. Findings highlight commonalities and differences in the formative research compared to actual reasons families accept or refuse autopsy. Results show

that FDAs can be acceptable in settings where traditional disease models prevail, highlighting the need to identify local understandings and beliefs and present information about procedures in a culturally relevant fashion.

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### PERFORMANCE EVALUATION OF THE AMAZON MALARIA INITIATIVE ON THE MALARIA PREVENTION AND CONTROL IN THE AMERICAS

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The Amazon Malaria Initiative (AMI) supported by USAID since 2001, aims to reduce the burden of mortality and morbidity due to malaria in the region of the Americas, through a collaborative malaria decision-making model implemented in six countries from the Amazon Basin (Brazil, Colombia, Ecuador, Guyana, Peru, Surinam) and five from Central America (Belize, Guatemala, Honduras, Nicaragua and Panama). A performance evaluation was conducted in partner countries in 2016 to: (1) identify the contribution of AMI to the capabilities of the National Malaria Control Programs (NMCP) between 2001 to 2015; (2) evaluate the capabilities of partner countries to address the changing scenarios of malaria transmission; and (3) identify the main challenges in addressing such changing scenarios (challenges at both NMCPs and international cooperation agencies). A mixed-methods approach was used, with emphasis on gender and populations in vulnerable conditions. The qualitative component included in-depth interviews with key informants from all partner countries, content analysis of selected documents, and case studies of four partner countries. We also analyzed national and regional trends of malaria epidemiology. AMI partner countries have shown a substantial but heterogeneous reduction of malaria cases since 2001, with some setbacks and slower recent progress, especially in the last four years. Partner countries have achieved significant progress and developed essential capabilities in all of AMI's six lines of work. AMI supported capacity development that addressed country-specific malaria control needs and scenarios. Furthermore, AMI has been a platform for inter-country collaboration, introducing innovations and strengthening information systems through national communication strategies and alliances. Key capacities for malaria control have been built and enhanced, allowing for an appropriate response to changing epidemiological scenarios. However, needs for improvement remain to consolidate the progress achieved, prevent reintroduction in near-elimination scenarios, and advance safely to malaria control in the region.

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### WHOSE CAPACITY? COLLABORATION THROUGH CAPACITY BUILDING

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This paper examines collaboration in transnational medical research from the viewpoint of African scientists working in partnerships with 'Northern' counterparts. It draws on ethnographic fieldwork in an HIV laboratory of an East African state university, with additional data from interviews with scientists working in related research institutions. 'Collaboration' is the preferred framework for the mechanisms by which Northern institutions support research in the South. The concept signals a shift away from the legacy of unequal (post-) colonial power relations, although, amidst persisting inequalities, the rhetorical emphasis on equality might hinder critical engagement with conflicts of interest and injustice. In order to 'collaborate', African scientists engage various strategies: they establish a qualified but flexible, non-permanent workforce, diversify collaborators

and research areas, source complementary funding to assemble infrastructures, and maintain prospective research populations to attract transnational clinical trials. Through this labour of collaboration, they sustain their institutions under prevailing conditions of scarcity.

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### THE EFFECTIVENESS OF STRATEGIES TO IMPROVE HEALTH WORKER KNOWLEDGE IN LOW- AND MIDDLE-INCOME COUNTRIES AND THE ASSOCIATION BETWEEN KNOWLEDGE AND CLINICAL PRACTICE: A SYSTEMATIC REVIEW

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Improving health worker (HW) performance in low- and middle-income countries (LMIC) is a priority. Strategies often aim to increase HW knowledge, which is assumed to be on the causal path to improved clinical practice and better patient outcomes; and compared to HW practices, knowledge is easier to measure. To characterize the effectiveness of strategies to improve HW knowledge in LMIC and the association between knowledge and practice, we conducted a systematic review of 15 electronic databases, 30 document inventories, and bibliographies of 510 articles. We included studies meeting accepted criteria for methodological adequacy (e.g., controlled trials) of any strategy to improve HW performance on any health topic in any language, published or not. This analysis focuses on studies that measured HW knowledge and clinical practice outcomes expressed as a percentage. Effect sizes were calculated as adjusted risk differences. We screened 105,299 citations, and 824 reports were included. Overall, 27 studies measured HW knowledge outcomes, 16 of which also assessed HW practices. These studies tested 14 strategies, usually with multiple intervention components, and most tested by only one study. Among 25 studies of facility-based HWs, short in-service training courses without other intervention components increased HW knowledge by a median of 13% (interquartile range [IQR]: 12, 29), and strategies that included both training and supervision increased HW knowledge by a median of 23% (IQR: 9, 31). Only providing printed information to HWs had essentially no effect. For lay HWs, strategies that included training increased HW knowledge by a median of 11%. The median change in clinical practice outcomes in these studies was 16%; however, we found no association between baseline HW knowledge and baseline practice (Pearson's  $r = 0.18$ ,  $p = 0.47$ ) or change in knowledge and practice improvement ( $r = 0.13$ ,  $p = 0.62$ ). Contextual and methodological heterogeneity made comparisons difficult. These results, which support a focus on improving HW clinical practices rather than only knowledge, should inform decision-making on improving HW performance in LMIC.

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### THE ROLE OF COMMUNITY ENGAGEMENT IN THE SUCCESS OF THE FIRST CLINICAL MALARIA VACCINE TRIALS IN EQUATORIAL GUINEA

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In 2015, the Equatorial Guinea Malaria Vaccine Initiative (EGMVI) conducted the first ever clinical trial in Equatorial Guinea (EG) to evaluate the safety and immunogenicity of Sanaria PfSPZ Vaccine® in healthy young adults (18-35 years). This paved the way for the second PfSPZ Vaccine® trial to evaluate safety and immunogenicity in participants aged 6 months to 65 years, and a head-to-head comparison of safety, immunogenicity and efficacy between PfSPZ Vaccine® and PfSPZ-CVac® in young adults (18-35 years) (Sanaria, Inc.). Because these were the first clinical trials ever conducted in EG, community engagement played a critical role in gaining the trust of local government officials, health workers, and community members. Tapping into existing local social systems facilitated access to study participants living in the targeted communities. During the community engagement, it was important to distinguish between existing, traditional malaria control interventions, e.g. bed nets and indoor spraying, and the research of investigational products such as the candidate malaria vaccines. Several challenges were encountered during the recruitment of study participants, particularly stemming from the influence that political factors and donors had on community perceptions. Understanding the purpose, procedures and potential risks and benefits of the study through sensitization meetings increased community involvement and willingness to participate in the trials. Building on the lessons learned from the first trial, community engagement resulted in high acceptance and positive feedback from the community in the second trial, in which 135 volunteers including infants were successfully recruited within 28 weeks. An effective community engagement strategy enabled the EGMVI to successfully recruit study subjects for the first malaria vaccine trials in the history of the country, and has enabled the nation to contribute to scientific research on the whole sporozoite malaria vaccines. Key points will include trust building, community-based health education and communication, and community engagement strategy models.

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### IMPACT OF MATERNAL INTERVENTIONS: TREND OF COVERAGE AND PROJECTIONS FOR 2030 OF ADDITIONAL LIVES SAVED IN MOZAMBIQUE

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Globally, maternal mortality ratio declined by only 2.3 % per year between 1990 and 2015. Mozambique has made important progress on maternal mortality (MNCH), reducing its mortality ratio by over 63 per cent by the year 2013. Nevertheless, there is a lot more to be done in order to accelerate maternal mortality reduction. We explored the use of projections, to see the potential of each intervention to contribute

on reducing preventable deaths. This study was undertaken determine which interventions will contribute more to prevent maternal deaths in Mozambique until 2030. We used historical coverage data in a Bayesian model to fit the trend of the interventions linked to maternal deaths (labor and delivery management, clean birth practices, active management of third stage of labor, MgSO<sub>4</sub> management pre-eclampsia/eclampsia, antibiotics for pPRoM, intermittent preventive treatment of malaria during pregnancy, tetanus toxoid vaccination, hypertensive disorder case and malaria case management) coverage up to the year 2030. We used the estimates produced in the model to generate the mortality changes and the addition lives saved by interventions using Lives Saved Tool (LiST; *Spectrum* version 5.27) in order to see the impact of each intervention across the time. The top 10 lifesaving interventions were scaled up to expose the missed opportunities that exist for intensify even more the mortality reduction. Clean postnatal practices is expected to be the intervention with the fastest coverage growth, observing 53.14% of absolute percentage increase, rising from 26.70 % in 2011 to 79.84% in 2030. Interventions related to malaria for prevention and treatment will also observe important coverage growth, with artemisinin compounds for malaria estimated to grow 27.06 % starting from 35.60% in 2015 to 62.66% in 2030. Labour and delivery management is expected to be the higher contributor in lives saving, being responsible for 23.91% of the lives saved. Preventive interventions such as facility delivery will be the top contributor on averting deaths with an aggregated number of 2524 extra additional lives saved.

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### STRENGTHENING SURVEILLANCE IN THE RETAIL PRIVATE SECTOR THROUGH MOBILE REPORTING IN TANZANIA

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Tanzania's national malaria strategy calls for targets of 80% confirmatory diagnosis of suspected malaria patients and appropriate treatment with artemisinin combination therapy (ACTs) for 80% of confirmed malaria patients by 2020. Given that 26% of patients with a fever seek care among drug shops in the retail private sector, it is imperative that the malaria program track case management practices in this sector. The National Malaria Control Program (NMCP) rolled out a system for drug shops to report aggregate monthly data using an SMS-based mobile platform. This platform was implemented in Morogoro Region, where 451 drug shops (75%) received case management training in July 2016 and paper registers to record individual case information and aggregated sums. Drug shops receive 2 reminders per month to report aggregated data for 8 key indicators on case management. Data reported between August and December 2016 were descriptively analyzed for reporting rate (% trained shops sending reports each month), completeness (% expected questions answered), data quality (consistency and logic of figures), test uptake (% fever cases receiving a malaria rapid test (RDT)) and adherence to test results (ACTs provided for positive RDTs, no ACTs provided for negative RDTs). Monthly reporting rates were lower than 50%, peaking at 47% in December 2016. Reporting was inconsistent: 10% reported for the full 5 months. Of the 887 reports received, 813 (92%) were complete. Approximately 30% of reports each month had quality errors. More than 90% of reported fever cases received an RDT, and 98% and 3% of positive and negative tests respectively received ACTs across the 5 months. Monthly reports from the retail private sector indicate strong case management practices, but there remain challenges with reporting rates and data quality. Limitations contributing to low reporting rates may include SMS formatting challenges, technical bottlenecks with the central

database, and difficulty with aggregated data entry and reconciliation. Upcoming focus group discussions with shops will provide insights on potential improvements to the system.

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### **BUILDING THE INFECTIOUS DISEASE DIAGNOSTIC CAPACITY OF A DEVELOPING NATION: EXPERIENCE FROM THE INDONESIA RESEARCH PARTNERSHIP ON INFECTIOUS DISEASE (INA-RESPOND)**

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The laboratory diagnostic capacity of developing countries is often insufficient to identify even the most common causes of illness. In Indonesia, this limited capacity forces many clinicians to rely solely on empirical diagnoses when prescribing treatments. Without known etiological agents, the risks of misdiagnosis and treatment failure are high, particularly when diseases share difficult-to-differentiate signs such as fever and rash. INA-RESPOND, established in 2011, has strengthened the infectious disease diagnostic capacity of the country using an applicable approach. A centralized reference laboratory was established so that diagnostic tests are developed, evaluated, and perfected before field implementation. Limited resources were optimally used by prioritizing assay development by: (1) pathogens generally known as the primary causes of fever-associated illness in Indonesia (e.g. Dengue, *Salmonellae*); (2) pathogens with circulating hosts/vectors but no known cases (e.g. *Rickettsiae*, Hantavirus); (3) pathogens likely to cause severe outbreaks (e.g. anthrax, H5N1); and (4) pathogens with known cases but poor awareness among clinicians (e.g. influenza, RSV, HHV-6). Molecular and serological tests were developed concurrently to ensure accurate laboratory diagnoses. The capacity was validated during two observational studies of hospitalized patients from 7 cities across Indonesia between July 2015 and March 2017. From 1566 subjects, 52 pathogens were identified by molecular tests and 16 by serology, leading to diagnoses for 66% of patients. Interestingly, 34% of diagnoses determined by field sites were found to be incorrect by the reference laboratory, which was later able to improve local capacity in a targeted manner. The expanded diagnostic capacity also revealed a previously unknown *Rickettsia spp.* burden, with 103 cases, and generated baseline prevalence data on diseases such as Chikungunya, Leptospirosis, and HHV-6. As the diagnostic capacity of Indonesia continues to grow, lessons from INA-RESPOND's success can inform the disease treatment and control efforts of other developing nations.

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### **A QUALITATIVE ASSESSMENT OF THE CONTRIBUTIONS OF THE AMAZON MALARIA INITIATIVE TO THE CAPACITIES OF NATIONAL MALARIA CONTROL PROGRAMS IN THE AMERICAS**

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Launched in 2011 with support of USAID, the Amazon Malaria Initiative (AMI) is a regional program implemented in 11 countries: Brazil, Colombia, Ecuador, Guyana, Peru, Suriname, Belize, Guatemala, Honduras, Panama and Nicaragua. AMI aims to reduce the morbidity and mortality of malaria in the Americas by working in six specific lines of action (LoA): antimalarial efficacy monitoring and prevention of emergence of resistance to antimalarials (LoA1); access to quality diagnosis and treatment (LoA2); quality assurance and control of antimalarial drugs and supplies

(LoA3); vector surveillance and integrated vector management (LoA4); epidemiological surveillance (LoA5); networking and systems strengthening (LoA6). In 2016, we did a performance evaluation of AMI, including a qualitative exploration of the stakeholders' perceptions about the AMI's contribution to national capacities by LoA, and factors that enabled AMI's success and barriers along the process. A total 38 in-depth interviews with leaders of National Malaria Control Programs (NMCP) in partner countries were done. The most recognized contributions were the generation of evidence via antimalarial resistance and efficacy studies (LoA1); training of health workers and generation of treatment/diagnosis guidelines (LoA2); and, strengthening of management systems of antimalarial drugs and supplies at NMCP (LoA3). According to all informants, AMI provided a platform that facilitated the exchange of resources and ideas between participating countries, strategic partners and institutions within the countries (LoA6). Contributions in LoA 4 and 5 were less perceived and variable, including support to the surveillance system and training in vector control strategies. Informants agreed that the political will towards malaria control and the participation of partner countries in the selection of AMI's strategies and priorities were key components in its success. Bureaucracy and personnel turnaround were identified as the main barriers for AMI's success. In summary, qualitative results suggest a positive impact of AMI and potentially replicable results in other regions.

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### **REGIONAL DIVIDE ON PERCEPTION AND MEANING OF HEALTH AND WELL-BEING OF KENYANS: AN ECOLOGICAL PERSPECTIVE**

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The past three decades have seen the growth in prominence of the concept of wellbeing in academia and the world over. As such, attempts to conceptualize and develop benchmarks for assessing the wellbeing of individuals, communities, cities and nations have been made, especially in the global north. This study explored contextual and place-based meaning and perception of health and wellbeing in low-middle income countries (LMICs), using Kenya as a case study. This was an ecological study where qualitative methods such as focus groups (FGs), key informant interviews and policy document reviews were conducted with in four representative provinces; Nyanza, Nairobi, Eastern and Central provinces of Kenya. FGs with homogenous groups of lay people male and female of the ages 15-24 years, 25-49 years and 50 years and above were conducted. Key informant interviews with male female and NGO representatives and member of county assemblies were also conducted. The results indicate a regional divide on perception and meaning of health and wellbeing. Preliminary analysis reveal that the indicators that matter to Kenyans are place based. For instance, in Eastern province, the most valuable indicator of health and wellbeing is water and food availability, whereas in the informal settlements in Nairobi, water and sanitation was given the first priority. These findings are important in providing indices relevant for policy making and to highlighting gaps in the measurement of the health and wellbeing of Kenyans which has always been based on morbidity and mortality statistics such as infant mortality.

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### **POSTPARTUM MANAGEMENT EVALUATION OF NEWBORNS WITH LOW BIRTH WEIGHT FROM A MALARIA ENDEMIC AREA IN COLOMBIA**

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Nearly three hundred million pregnancies occur in endemic areas of malaria in Africa every year, which poses a high risk for pregnant women and their fetuses. Yet, malaria is not an African issue only. Despite several Latin American countries show unstable transmission for malaria, epidemiological surveillance during pregnancy does not exist. Twenty

million children with low birth weight are born every year in the world and, in endemic areas, malaria during pregnancy as well as placental malaria are the main causes of low birth weight. Infants who are not diagnosed by the thin smear test for neonatal malaria are treated as cases of neonatal sepsis and the underlying problem is not solved. In order to tackling low weight, the over-feeding is a common practice and this in turn leads to early development of metabolic diseases such as diabetes and metabolic syndrome in the middle and long term. The aim of this research is to determine if the newborns diagnosed with low birth weight had an appropriate management and if the obstetrics-gynecology staff established a potential relation between this condition and placental malaria. This is a retrospective and descriptive study based on the review of 2014-2016 medical files of women who attended to antenatal care and had their deliveries at the Antonio Roldan Betancur Hospital in Apartadó, all of whom also had a postpartum follow-up note. Apartadó is an area endemic for malaria where a small percentage of newborns with low birth weight have access to medical follow-up. This research reveals that obstetrics-gynecology services in endemic areas of malaria in Colombia do not apply an appropriate epidemiological surveillance. In newborns with low birth weight, placental malaria was not thought of as a possible etiology in any case. The implementation of a surveillance protocol that includes placental studies and anthropometric measures of fetuses will promote an adequate management of newborns diagnosed with low birth weight and will decrease early neonatal death.

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### CREATING COLLABORATIONS FOR ARBOVIRAL RESEARCH IN COLOMBIA

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Arboviral infections are important issues in Colombia representing major causes of morbidity. Zika virus (ZIKV), a mosquito-borne RNA flavivirus, has caused a major outbreak in the Americas since it was first reported in Brasil in early 2015. Colombia alone reported more than 100,000 suspected cases of Zika virus infection between September 2015-November 2016, including nearly 20,000 in pregnant women. As part of a large multicountry study of pregnant women in areas affected by ZIKV, we have begun to evaluate the magnitude of health risks that Zika virus infection poses to pregnant women and their developing fetuses and infants in Colombia. To avoid some of the pitfalls of traditional international research set in resource-limited countries, we seek to build independent research capacity. In this report, we discuss the biggest challenges to the implementation of such a model of collaboration, as well as strategies for scaling up Colombia's participation in international arboviral research using examples from ongoing ZIKV studies. In our experience, a number of findings are relevant for improving collaborative efforts: 1) Language barriers still impose big challenges; although this has been slowly decreasing in basic research, it's still an enormous hurdle in clinical research still, particularly considering the regulatory burden of international rules and regulations for conducting research in human subjects. Regulatory agencies need to provide better access to training material in Spanish for conducting clinical research, because knowledge of the English language among local clinicians is low. 2) Besides the limitations in resources and laboratory space, international researchers need to consider the impact that bureaucracy, cumbersome regulations, and lengthy delays in obtaining supplies and reagents locally, will have in the logistics of their experimental work and plan accordingly. 3) Differences in the timeframes for regulatory approval between US-based and local institutions also require careful advance planning.

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### FIELD SEROSURVEY LOGISTICS FOR VACCINE PREVENTABLE DISEASES IN RESOURCE LIMITED SETTINGS: CHALLENGES AND OUTCOMES IN THE DEMOCRATIC REPUBLIC OF THE CONGO

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Infectious diseases represent the main factor of morbidity and mortality in developing countries, including the DRC. The DRC, the largest sub-Saharan country, has many inaccessible regions due to geographical obstacles, lack of communication, and other logistical issues. To accomplish their goals, all field and laboratory activities must successfully overcome many challenges. Therefore, it is useful to produce, set up, and carry out a consistent and coordinated protocol for all foreseen activities in the field in various conditions. Our team aimed to set up a functional laboratory in which viable samples could be collected and handled through any kind of obstacle while maintaining the cold chain until sample analysis in Kinshasa. From July to August 2016, we conducted a serosurvey of children 6-59 months in seven health zones of the Kabondo-Dianda Antenne, Haut-Lomami Province. Among the many logistical challenges the project faced were: mountainous and lacustrine regions; impassable roads and solid bridges, lack of transport or communication options, lack of electricity to run and charge electronic devices, sanitation issues and moving with a group of over 30 persons. Despite very challenging conditions, we were able to set up the laboratory (3 portable freezers, a centrifuge, a generator, 15 tablets, 2 batteries and chargers, stabilizers and all materials) in 5 different health zones covering an area of 33,361 Km<sup>2</sup>. We collected questionnaire data and viable samples including serum, plasma, and buffy coat from 1,900 children. This work was completed in close collaboration with local and traditional authorities; this was key and critical for successful field deployment and data collection, and was incorporated at each stage. Research and public health activities can noticeably reduce morbidity and mortality in resource-limited settings such as DRC. Although DRC's size and geographical obstacles do not allow for easy field deployment, it is possible to deploy high quality field laboratories by creating a consistent and sustained plan. Using these protocols, valuable samples and data can be produced despite suboptimal work conditions.

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### SMALL SCALE MIGRATION ALONG THE INTEROCEANIC HIGHWAY IN MADRE DE DIOS, PERU: AN EXPLORATION OF COMMUNITY PERCEPTIONS AND DYNAMICS DUE TO MIGRATION

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Madre de Dios, a southern state in the Peruvian Amazon basin, has experienced rapid development as well as an influx of migrants since the

construction of the interoceanic highway (IOH). This highway connecting Brazil, Bolivia, and the Peruvian coast, was completed in 2011. This study explores perceptions of migration and development in 8 communities along the IOH in Madre de Dios following construction of the highway. This was a multiple methods study involving interviews with key informants (KIs), focus group (FG) discussions, and application of a survey in the selected communities. A total of 12 FGs and 34 KI interviews were conducted in 2014 and 2015. Surveys were applied to 522 participants and included questions on demographics, financial, personal, social, human, and physical capital. Comparing migrants (self-reported moving after construction of the IOH) and non-migrants, we found no difference in food security or access to health services. Additionally, over 60% of respondents from both groups reported that illness was their primary threat to well-being. There were significant differences in land ownership and access to water. On average, non-migrants owned more land than migrants ( $p=0.041$ ). Non-migrants were also more likely to have piped water directly in their home ( $p=0.027$ ). Looking at perceptions about migrants, KIs and FGs discussed both positive perceptions of migrants – increased cultural exchange and new technology – and negative perceptions – increased drugs and alcohol in their communities and a lack of investment in the community. Both migrants and non-migrants reported trusting the local government more than the national government. Although we hypothesized that migrants would have decreased access to food, water, health services, and land, the only significant differences were in land ownership and water access. Even within these categories, the majority of migrants had access to water and owned some amount of land suggesting there is a level of community integration. Efforts to improve community infrastructure should be carried out at the local level and focus on improving issues reported by both groups.

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### EMOTIONAL PERCEPTIONS DURING PREGNANCY WITH POTENTIAL EXPOSURE TO ZIKA VIRUS: A PILOT STUDY

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Common mood disorders are common during pregnancy. The development of gestational depression has been linked to low socioeconomic status and lack of social support. High-risk pregnancies, accounting for 22% of pregnancies worldwide, are associated with higher levels of stress than uncomplicated pregnancies. Pregnant women living within the host range of the Zika virus (ZIKV) likely experience additional traumatic stress. This project is part of a larger community study on pre-eclampsia, the leading cause of maternal mortality in developing nations. A recently published meta-analysis indicates that chronic infection is also a risk factor for this condition. We are now exploring the risk of chronic viral infection during pregnancy, specifically Zika virus and other emerging flaviviruses. We expected that experiencing a high-risk pregnancy in addition to possible ZIKV exposure would result in a multi-layered trauma and significantly higher rates of emotional distress. A cohort of women was surveyed at the Maternidade Escola Janeiro Cicco as well as community centers within Natal. Patients were recruited between January-March 2017. The survey included qualitative questions about family planning, exposure to insect bites, and knowledge about Zika virus infection during pregnancy. The Self Reporting Questionnaire (SRQ-20) developed by the World Health Organization (WHO) was also administered. This screening questionnaire was developed by the WHO to screen for psychiatric disturbance and is validated for use in Brazil. 100 patients were enrolled. Significant emotional disturbances were identified in a majority of patients. The majority of pregnancies were unplanned, although most women said they would use contraception after this pregnancy. Zika virus affected women's concerns about their current pregnancies. Few women routinely used insect repellent. No women surveyed had received insect repellent from the national government

or other organizations. Zika virus continues to be of great concern to pregnant women, and a number of interventions could potentially reduce concern about and transmission of the virus.

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### STRENGTHENING NATIONAL INTEGRATED COMMUNITY CASE MANAGEMENT (ICCM) PROGRAMS: AN EVALUATION OF RAPID ACCESS EXPANSION (RACE) CONTRIBUTIONS IN DRC, MALAWI, MOZAMBIQUE, NIGER AND NIGERIA

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Over the last decade integrated community case management (iCCM) emerged as a strategy to expand child health services in hard to reach communities in sub-Saharan Africa. This strategy of recruiting and training community health workers to diagnose, treat, and refer children under five for malaria, pneumonia, and diarrhea had great momentum at global and national levels in 2013. Harnessing this momentum, in 2013 WHO launched the Rapid Access Expansion (RACE) Program in the Democratic Republic of Congo, Malawi, Mozambique, Niger, and Nigeria (Abia and Niger States). The goal of the program was to increase coverage of diagnostic, treatment, and referral services for malaria, pneumonia and diarrhea and to decrease mortality and the number of severe cases among children under five. At the same time there was a need to understand how to best measure success and build country ownership and capacity to sustain iCCM interventions. ICF provided monitoring and evaluation support to RACE to assist with measuring progress and strengthening data quality. ICF is also conducting a final evaluation of each RACE project site. The final evaluation uses a comprehensive and innovative mixed method approach that will: 1) assess changes in outcome and coverage indicators through analysis of baseline and endline household survey data and trend analysis of routine project monitoring data; 2) assess potential gender differentials in careseeking and iCCM coverage through analysis of baseline and endline household survey data; 3) estimate change in child mortality and number of lives saved using the lives saved tool (LiST) model; 4) document project contextual and external factors that may have influenced child health in project areas via document review, interviews, and trend analyses; 5) conduct an equity analysis; and 6) make a plausibility argument to demonstrate the plausible contribution of RACE to changes in careseeking, assessment and treatment coverage and estimated mortality change. Final evaluations will be completed in August 2017 and results will be available to present at the ASTMH conference. The presentation will include key findings from each country.

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### STRATEGIES TO IMPROVE COVERAGE OF COMMUNITY-BASED DISTRIBUTION PROGRAMS: A SYSTEMATIC REVIEW OF THE LITERATURE

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Community-based public health services aim to provide public health interventions at scale. However, many of these programs, including those targeting soil-transmitted helminths (STH), vitamin A supplementation, and child immunization, often fail to achieve World Health Organization (WHO) coverage targets. The purpose of this systematic literature review was to identify successful interventions to increase coverage of community-based distribution and to quantify the magnitude of observed change in coverage associated with each strategy. We systematically searched PubMed, Embase, and CAB Direct for studies that compared coverage estimates of community-based distribution of drugs, vaccines, or other public health services in the presence and absence of an

intervention intended to increase distribution coverage. We identified 2,561 studies from which 119 full text articles were evaluated for possible inclusion. After review, twelve studies describing 14 interventions met inclusion criteria. Data abstracted from articles included location and design of study, strategy used to increase coverage, baseline coverage, and coverage obtained with the intervention. Local Ministries of Health funded eight of the interventions described, while six interventions were implemented using supplemental donor funding. Strategies identified to increase coverage included community involvement in the design and implementation of distribution (n=3, range of reported differences in coverage: +9%, +23%), increased distribution of health education and awareness materials (n=5, range: -4%, +26%), distributor incentives (n=1, +20%), converting from school- to community-based distribution (n=2, range: -2%, +82%), management by a non-governmental organization (n=1, +9%), and integrated distribution through Child Health Days (CHDs) (n=2, range: -31%, +72%). Several specific interventions to improve coverage of community-based public health service distribution have demonstrated impact. These results may be useful in the design of future community-based public health intervention programs.

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### POLICY REVIEW ON THE MANAGEMENT OF PRE-ECLAMPSIA AND ECLAMPSIA BY COMMUNITY HEALTH WORKERS IN MOZAMBIQUE

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Maternal mortality ratio still high in Mozambique, being 480 per 100,000 live births in 2015. Pre-eclampsia is one of the leading causes of maternal death within the country. Limited access to health care facilities and a lack of skilled health professionals contribute to the high maternal morbidity and mortality rates and indicate a need for community-level interventions. The aim of this review was to describe the health policies related to the role of community health workers in the management of Pre-eclampsia and Eclampsia in Mozambique. A review of policy documents regarding the community health workers programme in Mozambique was performed through desk review of relevant documents from Mozambique's Ministry of Health, literature review and contact with key informants in the field of health policy in Mozambique. These documents included peer reviewed articles, government and institutional policies and relevant official documents. After independence in 1975, Mozambique introduced new policies to strengthen and extend primary health care. In 1978 the community health worker programme was established, with the primary goal of promoting health and preventing disease in remote and rural populations. In the late 80's and early 90's, this programme was scaled down due to several factors including a prolonged civil war; however, the decision to revitalize the programme was made in 1995. In 2010, the reformed programme expanded to include management of common childhood illnesses including malaria, diarrhoea and acute respiratory infections. According to their curriculum, community health workers have a limited role in maternal health which includes simple health promotion, detection of warning signs in pregnancy, and referrals. Their role does not include the management of Pre-eclampsia or Eclampsia. To strengthen community-level care and reduce maternal mortality, there is a need to

design policies to promote task-sharing to community health workers. The provision of basic maternal health services should be broadened to include the detection and pre-referral management of Pre-eclampsia and Eclampsia.

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### RELUCTANCE TO SUBMIT MANUSCRIPTS TO SCIENTIFIC JOURNALS: AS EXPERIENCED BY RESEARCHERS IN DIFFERENT DISCIPLINES IN THAILAND

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It is axiomatic among the scientific community that even the most brilliant research is useless unless it is reported. At the same time, it can be a struggle for researchers to deal with the constant pressure to "publish or perish". Peer review and publication are major concerns among scientific and other researchers. The objective of this study was to explore the self-reported experiences of researchers regarding the most important factors influencing reluctance to submit their manuscripts. An anonymous online questionnaire was sent to researchers in different disciplines in a variety of academic and scientific research institutes in Thailand, during March 2017. Based on the level of problematic issues reported using a Likert rating scale, the highest rating for each item was considered potentially significant. The respondents comprised 45 (55%) researchers in basic science or laboratory-based fields, 17 (21%) researchers involved in clinical research, and 10 (24%) researchers in the fields of behavioral or public health. The problematic ratings were mainly at the "to some extent" and "to large extent" levels. The percentages of problematic issues varied across the three groups. The ratings for highly problematic issues for researchers in basic science, clinical science and behavioral science, were as follows: deciding on the journal for publication (35%, 7%, 50%); identifying the "selling point" of the paper (38, 38%, 53%); authorship-related issues (4%, 25%, 5%); submission process (16%, 19%, 30%); conflict of interest (9%, 13%, 20%); data sharing (16%, 6%, 16%); likelihood of acceptance after revision (25%, 38%, 45%); and experience of rejection (9%, 25%, 25%). Significant differences were found for journal selection and authorship issues. The study results, however, revealed different weightings for the problematic issues faced by researchers in submitting their manuscripts. Identifying "reluctance to submit" issues can inform the planning of research and publication supports for researchers.

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### A NEW APPROACH TO ASSURING THE QUALITY OF COMMUNITY-BASED HEALTH SERVICES IN MADAGASCAR

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In Madagascar, community health volunteers (CHVs) play a critical role in providing primary health care, especially to rural populations who live more than five km from a health facility. CHVs are trained and supported by the nearest health center and the USAID Mikolo project to provide integrated community case management (iCCM) treatment (diarrhea, malaria, and pneumonia); short-acting family planning (FP) methods; maternal health care (MH); and health promotional activities. However, assuring the quality of CHV services has been a significant challenge. The absence of effective and sustainable quality control to improve CHV performance is not only demotivating for CHVs, but potentially harmful for women and children receiving poor-quality services. To address these quality issues, the Project created a new approach hypothesized to assure, improve, and sustain the quality of community-based health services. The Project's quality improvement approach includes formal training or refresher training every year, on-the-job coaching and mentorship every

two months, monthly performance monitoring and group supervision at the health facility, standards-based competency evaluation every quarter, and annual certification or re-certification. From October 2014 to April 2015, the project introduced this performance improvement package in 375 communes. After the first six months, the project assessed compliance with national standards among 2,154 CHVs offering MH/FP and 2,909 CHVs offering iCCM. Among the CHVs offering MH/FP, 1,462 (68%) achieved a compliance score of 80% or higher for FP, compared to 49% at baseline ( $p < 0.002$ ). Among iCCM CHVs, 1,979 (68%) achieved a compliance score of 80% or higher for iCCM, compared to 60% at baseline ( $p < 0.02$ ). All CHVs who achieved at least 80% were cross-trained to become polyvalent. Ultimately, these results demonstrate that this approach has actively improved the performance of CHVs. As such, the USAID Mikolo Project quality assurance and improvement approach is a critical component of sustainable health service availability and uptake and to achieving universal health coverage in Madagascar.

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### COMPETENCY TEST FOR VISUAL INSPECTION OF CERVICAL CANCER LESIONS WITH ACETIC ACID IN EQUATORIAL GUINEA

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As part of MCDI-Equatorial Guinea Cervical Cancer Screening and Treatment Project, a training on Visual Inspection with Acetic Acid was held in March 2017 in Malabo. Twenty six gynecologists, doctors, midwives and nurses, as well as relevant Ministry of Health managers, were trained. To assess participants' competency 36 printed images from JHPiEGO were used. The images were inspected prior to the training to ensure they were not pixelated or had poor resolution. Response sheets were entered into an Excel template that automatically assigned a score to each of the answers, aggregated for a general score for 4 competencies: identify cancer, diagnosis of VIA positive, identify Squamocolumnar Junction and identify correct management plan. The individual and occupational category scores were mainly affected by the fourth competency, management of the case. Possibly due to selection bias (i.e., due to recruitment being based on the recommendations of knowledgeable clinician very familiar with their prior work experience), the three auxiliaries showed a competence comparable to the rest. The 4 clinicians with largely managerial roles tended to perform better than the other participants, who saw patients in public facilities. At the group level, the highest competency corresponded to identification of cancer (86%), followed by seeing clearly the Squamocolumnar Junction (78%). The lowest competencies were in correctly classifying the images shown as VIA negative/positive (64%), and specifying the required treatment (none, cold coagulation, refer for cancer treatment) with only 50% correct. Regression analysis of the post-test knowledge score derived from a comprehensive final examination regressed on the competency score gave a linear regression coefficient of  $R^2 = 0.8234$ , indicating that the competency assessment was a good predictor of clinical knowledge. On average, a little more than a third (38%) of the participants obtained correct answers in all 4 questions. This indicates that at this early stage of the program it is more useful to analyze the 4 competencies separately and identify and prioritize the next training steps accordingly.

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### SUBSTANTIATING FREEDOM FROM DISEASE BY COMBINING DYNAMIC MODEL PREDICTIONS WITH INFECTION SURVEYS

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Regions endemic for onchocerciasis, a debilitating vector-borne helminth infection, are currently approaching elimination targets after years of mass drug administration and vector control efforts. As large scale control programs transition their focus from active treatment deployment to post-treatment surveillance, the need for evidence-based measures of disease absence grows more pronounced. Freedom from infection tools, originally established in veterinary science, offer a framework for integrating surveillance statistics and mathematical transmission models to quantify the confidence of freedom, thus providing policy makers with a reproducible metric on which to base program decisions. We describe an application of this approach using Ugandan onchocerciasis monitoring data from eight sentinel sites collected by country programs and The Carter Center as a case example. Our dynamic Bayesian transmission model was calibrated to the local baseline conditions of each site, ranging in pre-intervention microfilaria prevalence from 53.8% to 100%. The locally parameterized models were used to estimate site-specific transmission thresholds, which range from 0.03% to 0.68% microfilaria prevalence. The current infection status of each site was determined by hypothesis testing based on probability calculations which consider post-intervention survey sample size, test sensitivity and specificity, and model-generated transmission thresholds. Four Ugandan sites where infection is thought to be interrupted were indeed declared free from infection with greater than 96% confidence while infection was considered to be present in two sites in a region with ongoing transmission. An important finding was that in the four sites found to be infection-free, the method showed that declarations of freedom from infection could have been made at least five years earlier, a result which could have saved unnecessary annual treatments. These results highlight how the synthesis of model predictions and survey data by this new freedom from infection framework can facilitate robust decision making in parasite elimination programs.

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### THE GLOBAL HEALTH NETWORK: IMPLEMENTING RESEARCH CAPACITY DEVELOPMENT, IMPROVING EVIDENCE AND DATA QUALITY ACROSS THE WORLD

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The Global Health Network (TGHN) is a platform that is implementing research capacity development, improving evidence generation and data quality across the world, with a specific focus on low and middle-income countries (LMICs). This is being achieved through two key interdependent mechanisms: a platform for delivering training and research career development for healthcare workers and research staff, and a mechanism for sharing knowledge, experience and tools between regions, organisations and disease areas. TGHN is an online science park that enables research groups to disseminate and share their methods and know-how, thus speeding up research by raising standards and removing duplication. The network achieves this by adapting and applying the latest digital technology and harnessing the online 'sharing' phenomenon to develop, strengthen and encourage clinical research efforts. Since 2010, TGHN has garnered over 1 million visits from a broad spectrum of researchers and health care professionals. Over 100,000 members have registered to the network to take part in online community activities from 196 country locations. TGHN now comprises 37 membersites with world class scientific collaborators that cover specific research areas such as Zika virus and Dengue to broader subject areas such as new-born and maternal health, methodologies and data management. Cross-cutting tools such as Sitefinder aim to match research sites with researchers looking for suitable study locations and capacity to take forward clinical trials. E-Learning

within The Global Health Training Centre has become a trusted and well-regarded training hub offering free online training. Over 214,000 modules have been taken covering a wide range of subjects. TGHN runs free face-to-face workshops with 23 workshops across 15 countries have reached nearly 2,000 research staff to gain practical skills for running successful research projects. TGHN has demonstrated that the research community do want to work together and speed up the process of developing life-saving treatments and new ways to manage disease in the most vulnerable populations across the globe.

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### DOMINANCE OF SIALIC ACID INDEPENDENT INVASION PATHWAYS IN *PLASMODIUM FALCIPARUM* ISOLATES FROM SENEGAMBIA

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Erythrocyte invasion by *Plasmodium falciparum* merozoites is a vital step in establishing parasitaemia during malaria. This process is mediated through several combinations of parasite ligands and erythrocyte receptors depicting the major sialic acid dependent and independent pathways. The influence of declining prevalence of malaria on the epidemiology of these pathways across endemic regions is not known. Here we determined invasion pathways for 60 isolates from uncomplicated malaria, collected from Gambia (Basse) and Senegal (Pikine), where there have been intensive interventions towards elimination. Invasion of each isolate was assayed against erythrocytes treated; Neuraminidase to cleave glycophorin A-C, trypsin removes glycophorin A and C, and chymotrypsin which removes glycophorin B and other surface proteins. The mean inhibition for neuraminidase, trypsin and chymotrypsin was 10%, 49% and 53% respectively across all isolates. There was a positive correlation between neuraminidase and low trypsin inhibition (Spearman  $P=0.0007$ ). Inhibition by low trypsin also positively correlated with chymotrypsin (Spearman  $P=0.0022$ ). Unlike previous reports, this study found higher levels of sialic acid independent invasion. This suggests switching of invasion pathways as malaria prevalence and immunity to non-sialic acid ligands declines.

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### CIRCULATING MICRORNA MIR-1976 PROMOTES MALARIA PATHOGENESIS BY INTERACTING WITH CD40 TRANSCRIPT

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There are multiple theories of how the malaria parasite evades the host immune system. One theory proposes that the parasite acquires host's antigens to make them unrecognizable by the host immune system prior to initiation of the erythrocytic stages. The parasites also modulate host immune cell populations by increase systemic cellular apoptosis. MicroRNAs are small non-coding RNAs that endogenously regulate the gene expression in a post transcriptional manner. MiRNAs have shown to be significantly altered during infections with microbes and are being utilized more in studies relating to biomarkers for diagnostic and drug targeting purposes. However, the role of microRNAs (miRNAs) in mediating malaria pathogenesis is not known. Deep sequencing of circulating miRNAs of five malaria infected patients and three individuals without malaria resulted in twenty-one differentially expressed miRNAs. By using five different gene predictions software (MirBD, DIANA, TargetScan, RNA22, MicroRNA), we identified possible targets of the microRNAs including an upregulated microRNA of interest (has-mir-1976). Using all five-prediction software, the CD40 gene was identified as a predicted target

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for has-mir-1976. CD40 has been shown to enhance immunity via T cells activation and is needed for full development of liver dendritic cells during a *Plasmodium* infection. Inoue et al. showed that *Plasmodium berghei* can be eliminated in mice by stimulating CD40. Additionally, an anti-malarial plant extract (*Artemisia rupestris* L) increases signaling of CD40 which mediates the TLR4 pathway during dendritic cell development. Our data suggests that mir-1976 may interfere with CD40 function to promote severe malaria pathogenesis. Understanding the mechanisms mediating has-mir-1976 induction and its effects on CD40 expression will enable the development of novel treatments against severe malaria.

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### IDENTIFICATION OF PFEMP1 PROTEINS ASSOCIATED WITH THE DEVELOPMENT OF PROTECTIVE IMMUNITY

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*Plasmodium falciparum* is the deadliest of the human malaria parasites. The virulence of *P. falciparum* is related to the ability of infected erythrocytes (IEs) to bind to the endothelium and sequester in vascular beds. The *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) family of clonally variant proteins mediates adhesion of infected RBCs and is responsible for antigenic variation. Studies in malaria-exposed individuals identified naturally acquired protective antibodies to PfEMP1, blocking cytoadhesion in pregnant women, demonstrating the potential of a PfEMP1 based vaccine. PfEMP1 is encoded by the *var* multi-gene family with each parasite expressing a single gene while the remaining ~60 are silenced. PfEMP1 sequences are very diverse; however, they possess organized domain architectures, providing the parasites with a conserved PfEMP1 repertoire that determines the binding phenotype. PfEMP1 proteins are composed of 2-9 Duffy-binding-like (DBL) and cysteine-rich interdomain region (CIDR) domains that are subdivided into domain cassettes (DCs). Previous qPCR-based transcriptome studies have shown that DC8 and DC13 are upregulated in parasites from children with severe malaria. The purpose of this work is to use a global unbiased approach to identify PfEMP1 proteins in circulating parasites of young children using proteomics tools with the goal of identifying targets of immune protection. 289 PfEMP1 proteins identified in 34 clinical isolates were analyzed to determine the most commonly expressed. It is hypothesized that cross-reactivity will be observed between similar PfEMP1s; therefore, identified PfEMP1 proteins were subjected to phylogenetic clustering. 35 representatives were selected from the phylogenetic trees to best represent the entire population. These potentially cross-reactive population representatives will be investigated further through immunosurveillance assays to associate antibody levels to recombinant PfEMP1 domains with protection from disease.

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### ELIMINATION OF MALARIA: A PARASITE RESERVOIR QUESTION

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The hypnozoite theory of malarial relapse does not adequately explain the fact that genetically homologous relapse-like *Plasmodium vivax* recurrences are so commonly encountered. It is feasible, theoretically, that hypnozoites can be responsible for such recurrences, despite there being no *in vivo* parasitological proof hereof in humans. However, considering the ubiquity of genotypic homology in recurrent *P. vivax* malaria, the question arises as to whether there could be, in addition to a presumed hypnozoite origin, another non-circulating source(s) of homologous relapse-like *P. vivax* recurrences. Associated with this question, it should be noted that accumulation of blood stages of *P. vivax* in bone marrow and the spleen has been reported, although the frequency and extent of their occurrence

in these sites are currently unknown. Are such erythrocytic forms a part of the hidden parasite reservoir? If so, they may be responsible for renewed peripheral parasitemia as immunity wanes; or, alternatively, merely contribute to an increase in the number of homologous parasites in the bloodstream (where erythrocytic schizogony might also be taking place). Either way, parasitemic recurrence would then lead to formation of gametocytes, the possibility of ingestion thereof by mosquitoes, and potential ongoing transmission of malaria. A novel (late 2017) analysis of relevant aspects of the biology of recurrent *P. vivax* infection will be presented and research suggestions made. This subject is both topical and practically important in relation to malaria eradication.

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### DEVELOPMENT OF SEE-THROUGH IMAGING METHODS FOR SEXUAL REPRODUCTION OF MALARIA PARASITES

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Similar to animals and plants, malaria parasites perform sexual reproduction based on fusion between male and female gametes. The stage for sexual reproduction is the midgut of mosquito vector, in which the male- and female gametocytes get mature and fuse with each other. The resulting zygote differentiates into a motile ookinete, and migrates outside the midgut to form an oocyst, in which numerous sporozoites are produced. Because only one pair of gamete fusion leads to production of tens of thousands sporozoites, the sexual reproduction of malaria parasites is one of the most formidable stage in their life cycle. As development of transmission blocking vaccine is recently highlighted, analytic methodologies of sexual reproduction of malaria parasites in the mosquito vector may be further required. However, the complicated midgut tissues and the pigment of red blood cells have prevented observation of parasite gamete behaviors in the mosquito body. To overcome such technical difficulties, we tried to develop methods to make the mosquito body transparent and produce gamete marker lines, in which mature male and female gametes are differently labeled with fluorescent proteins. Our methods should enable to observe not only the whole process of sexual reproduction from gamete maturation to fusion moment, but also sporozoite behaviors of malaria parasites in the mosquito stage.

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### TARGETING THE HAP2 FUSION LOOP INHIBITS TRANSMISSION OF *PLASMODIUM BERGHEI* AND *P. FALCIPARUM*

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Fertilization in *Plasmodium* is a complex process that occurs in the gut of the *Anopheles* mosquito upon uptake of blood meal. HAP2 has been postulated to mediate membrane adhesion in *Plasmodium* fertilization between male and female gametes. The presence of HAP2 in a number of Eukaryotic taxa suggests a common gamete fusion mechanism. Recently, *Chlamydomonas reinhardtii* HAP2 was revealed to be a class II fusion protein, additionally suggesting that HAP2 function is largely analogous to viral fusion. This opens novel avenues to block HAP2-mediated transmission of *Plasmodium* through the mosquito host. Here we target a region of HAP2 with homology to the hydrophobic cd loop of *C. reinhardtii* in *P. falciparum* and *P. berghei* using a combination of *in vivo* and *in vitro* assays to assess the potential of targeting the HAP2 fusion loop to inhibit transmission. Immunization with peptides to the cd loop in *P. berghei* reduces infection prevalence within the mosquito host by 58.9% and prevalence of infection by up to 38.44%. Targeting the cd loop in *P. berghei* with peptide antibodies reduces *in vitro* formation of ookinetes by

up to 89.9%. whilst targeting the *P. falciparum* cd loop with purified IgG in field isolated by DMFA reduces oocyst intensity by 75.5% and infection prevalence by 36.46%. These results emphasise conserved mechanisms of fusion in Apicomplexa, suggesting that HAP2 fertilization mirrors that of viral fusion, potentially highlighting a way to design for future anti-malarial transmission-blocking vaccines.

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### A SYSTEMATIC REVIEW AND META-ANALYSIS OF THE RISK OF TRANSFUSION TRANSMITTED MALARIA FROM BLOOD DONORS IN SUB-SAHARAN AFRICA

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Transfusion transmitted malaria (TTM) is a major component of global transfusion transmitted infections, however estimates of its incidence are scarce. It is important as it may increase the morbidity and mortality of blood donation recipients, the majority of whom are young children and pregnant women who are often anaemic or suffering from other co-morbidities. It may also jeopardise global malaria elimination efforts by acting as a reservoir for subclinical parasite transmission. This systematic review aims to assess the current prevalence of *Plasmodium* parasite carriage in blood donors in high-endemic regions in sub-Saharan Africa and to provide estimates of spatial and temporal heterogeneity of TTM risk across Africa. Publication databases and clinical trial registries were searched for articles reporting prevalence studies of malaria parasitemia amongst blood donors in sub-Saharan Africa, written in English and French published between 2000 and 2016. Grey literature sources such as the World Health Organization (WHO) website and individual countries' ministry of health websites were searched for published reports, and reference lists of papers were also screened. Risk of Bias was assessed using the Joanna Briggs Institute Prevalence Critical Appraisal Tool. Fourteen studies were included in the qualitative synthesis and 13 in the meta-analysis (4,035 subjects). This included ten studies from Nigeria, one from the Democratic Republic of Congo, Benin, Cameroon and Senegal. Pooled prevalence of malaria parasitaemia was 34.1% (95%CI: 24.3% - 43.8%), ranging from 6.5% to 74.1%. Considering the location of included countries in this review, a prevalence of one third of blood donors carrying *Plasmodium* provides a robust estimate of the risk of TTM in high transmission areas. As transmission and immunity wane in Africa this prevalence will likely decrease but the severity of TTM will likely increase, thereby posing a potentially serious threat to recipients, and possibly interfering with elimination efforts.

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### SEXUAL DIMORPHISM IN DEVELOPING GAMETOCYTES

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Malaria remains a devastating global health problem. *Plasmodium* parasites, the causative agent of malaria, has a complex life cycle, involving asexual and sexual replication in the human host and sporogony in the *Anopheles* vector. During asexual replication in host red blood cells, a small proportion of asexual parasites develops into gametocytes (sexual stages) of the parasite, the only transmissible stage to mosquitoes. It is known that environmental triggers such as anemia, antimalarial drug exposure, and mixed parasite genotype infections could affect the sexual conversion rates and sex ratios. However, a detailed description of the molecular mechanism controlling male/female differentiation during gametocytogenesis is lacking. As the gametocytes progress through the five stages of maturation, the morphology of male and female gametocytes can be differentiated under a microscope. However, bona fide protein biomarkers for developing male and female gametocytes that can help unravel sexual divergence remain missing. It was reported

that the disruption of PfPuf2 gene, a translational repressor increases the number of male gametocytes generated. In addition, the majority of the male-specific proteins are expressed early from stage II onwards. Therefore we hypothesized that gametocytes are predestined to be male but actively switch to female gametocyte maturation during the stage III-IV transition phase. To establish a list of putative early sex-specific gametocyte markers, we used Click-Chemistry to profile de novo protein synthesis over 5 days; spanning the gametocyte transition period from stage III-IV. We compared the protein list for each 24 period to our predicted male- and female-specific gametocyte proteome to identify proteins and pathways that are putatively involved in gender switching.

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### ANTI-PHOSPHATIDYLSERINE IGM AND IGG ANTIBODIES ARE INCREASED IN FALCIPARUM AND VIVAX MALARIA AND CORRELATE WITH ANAEMIA

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Malarial anaemia results primarily from the loss of uninfected erythrocytes, although its aetiology is incompletely understood. Phosphatidylserine (PS) is a membrane phospholipid normally located on the internal leaflet of the lipid bilayer, which in malaria becomes exposed on the surface of infected and uninfected erythrocytes. In a mouse model anti-PS antibodies have been shown to contribute to anaemia by binding to the surface of uninfected erythrocytes and mediating their phagocytosis. To investigate the role of anti-PS antibodies in malarial anaemia in humans, we measured anti-PS IgG and IgM antibodies in Malaysian adults hospitalised with falciparum and vivax malaria, and correlated with day 0 and nadir haemoglobin. Compared to controls, anti-PS IgM and IgG in patients with falciparum malaria were increased at baseline: 129 (80 - 205) U/ml vs. 27 (19 - 41) U/ml for IgM ( $p < 0.0001$ ), and 81 (65 - 116) U/ml vs 17 (14 - 21) U/ml for IgG ( $p < 0.0001$ ). The corresponding values for *P. vivax* were 117 (75 - 215) U/ml vs. 27 (19 - 41) U/ml ( $p < 0.0001$ ) and 65 (54 - 172) U/ml vs 17 (14 - 21) U/ml ( $p = 0.0001$ ). In falciparum malaria, anti-PS IgG correlated inversely with day 0 haemoglobin ( $r = -0.37$ ,  $p = 0.041$ ) and haemoglobin nadir ( $r = -0.41$ ,  $p = 0.025$ ). In vivax malaria, anti-PS IgM correlated inversely with baseline haemoglobin ( $r = -0.72$ ,  $p = 0.029$ ) and haemoglobin nadir ( $r = -0.81$ ,  $p = 0.008$ ). In vivax malaria, anti-PS IgM antibodies also correlated inversely with the percentage loss of haemoglobin, from baseline to nadir ( $r = 0.70$ ,  $p = 0.036$ ). No correlation was detected between anti-PS antibodies and parasitemia. In adults with vivax and falciparum malaria, anti-PS antibodies are increased and correlate inversely with haemoglobin. This may have implications for development of therapeutic strategies for malarial anaemia.

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### ELEVATED CEREBROSPINAL FLUID TAU PROTEIN LEVELS ARE ASSOCIATED WITH LONG-TERM NEURODEVELOPMENTAL IMPAIRMENT IN UGANDAN CHILDREN WITH CEREBRAL MALARIA

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Microtubule associated protein (MAP) tau is a known marker of brain parenchymal damage. Elevated levels of total tau protein in cerebrospinal fluid (CSF) has been linked with neurodegeneration and cognitive impairment in several forms of dementia and Alzheimer's disease. Studies conducted in Vietnamese adults and in Kenyan children with severe malaria (SM) have shown that levels of tau proteins were increased in patients with SM and associated with poor acute neurologic outcomes. To assess the association of total tau with mortality and long-term neurodevelopmental outcomes, we measured CSF tau levels in a prospective study of 145 Ugandan children 18 months-12 years of age with cerebral malaria (CM). CSF tau levels did not differ significantly between those who died ( $n = 8$ ) and those who survived ( $n = 137$ ,  $P = 0.27$ ). However, CSF tau levels were significantly higher in children with retinopathy positive CM ( $n = 100$ ) compared to retinopathy negative CM ( $n = 45$ ,  $P = 0.03$ ). CSF tau levels also associated with duration of coma ( $\beta = 0.2$ ,  $P < 0.0001$ ) but not with number of seizures, and CSF tau levels were higher at discharge and 6, 12 and 24 months after discharge in children with neurologic deficits than children without deficits (all  $P < 0.05$ ). Finally, higher CSF tau levels were associated with worse scores for overall cognition ( $\beta = -0.8$ ,  $P = 0.03$ ) and working memory ( $\beta = -0.9$ ,  $P = 0.05$ ) 12 months after discharge, but only in children over 5 years of age. Higher CSF tau levels also correlated with higher plasma levels of the endothelial function markers soluble P-selectin (Spearman's  $\rho = 0.22$ ,  $P = 0.007$ ) and angiotensin-2 (Spearman's  $\rho = 0.47$ ,  $P < 0.0001$ ). Thus, in children with CM, CSF tau levels are associated with endothelial damage and predict acute and long-term neurologic and cognitive impairment.

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### RETICULOCYTE DISCONTINUOUS ENRICHMENT USING PERCOLL GRADIENT HAS A NEGATIVE IMPACT ON *IN VITRO* GROWTH OF *PLASMODIUM VIVAX*

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*In vitro* cultures of *Plasmodium* parasites have provided important insights into malaria research. Unfortunately, culturing *P. vivax* (*Pv*) in adult human peripheral blood has presented significant challenges. Recent improvements have made short-term culturing possible with the addition of reticulocyte-enriched human blood. Reticulocyte enrichment is considered an essential process to increase invasion events by providing numerous preferable target cells for the parasites to invade. However, the process of isolating reticulocytes, based on density properties requires further optimization to justify efforts, and results in small quantities. In this study, we tested the invasion efficiency of cryopreserved *Pv* Vietnam/ Palo Alto strain in human adult peripheral blood that was reticulocyte enriched using 40%, 70%, and 80% Percoll gradients, with reticulocytes harvested from the 70% layer. Initial flow cytometry of *Pv* invasion events after 48 hours of *in vitro* culture was higher in reticulocyte enriched blood ( $41.0 \pm 10$ ) compared to unenriched blood ( $13.5 \pm 3.5$ ). However, continued parasite maturation with subsequent invasion cycles is more successful at 72 hours in unenriched blood ( $92 \pm 1$ ) when compared to enriched blood ( $51 \pm 1$ ). After repeated observation of improved parasite propagation in unenriched human blood and the obligate binding of *Pv* Duffy Binding Protein (PvDBP) to host cell Duffy antigens we further investigated, by flow cytometry, the impact of discontinuous gradient Percoll selection of reticulocytes on the red blood cells from three adult donors expressing three different Duffy blood groups  $FY^*A/*A$ ,  $FY^*A/*B$ , and  $FY^*B/*B$ . All three donors showed decreased binding of PvDBP to their RBCs after Percoll treatment  $FY^*A/*A$  (45.3 Unenriched, 36.2 Enriched),  $FY^*A/*B$  (67.1 Unenriched, 41.3 Enriched), and  $FY^*B/*B$  981.9 Unenriched, 62.9 Enriched). Our data illustrates that the use of Percoll to isolate reticulocytes

is potentially detrimental to the *in vitro* culturing of *Pv* in adult peripheral blood and helps to explain early observations made by Lanners (1992) that Percoll enriched blood could not support *Pv* growth.

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### EX-VIVO RING-STAGE SURVIVAL ASSAYS (RSAs), PFKELCH13-PROPELLER MUTATIONS, AND PFMDR1 VARIANTS IN PLASMODIUM FALCIPARUM ISOLATES FROM MALARIA PATIENTS IN COLOMBIA

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Delayed parasite clearance times observed in Southeast Asia provided the first evidence of *Plasmodium falciparum* resistance to artemisinins. The *ex vivo* ring-stage survival assay (RSA) was subsequently developed in the laboratory to better mimic parasite exposure to pharmacologically-relevant artemisinin concentrations achieved during patient treatment. *In vitro*, the RSA0-3h is able to discriminate resistant and sensitive parasites, showing survival rates above and below 1%, respectively. Mutations in the C-terminal propeller domain of the putative kelch protein PF3D7\_1343700 are associated with artemisinin resistance. Additionally, amplification and mutations in the *Pfmdr1* gene are associated with resistance to artemisinin partner drugs like mefloquine and lumefantrine. To clarify the unknown landscape of artemisinin resistance in Colombia, 71 uncomplicated malaria patients from 3 localities were enrolled in an observational study from June 2014 to July 2015. *Ex-vivo* RSAs were performed using parasite samples obtained from each patient, and all isolates were examined for *Pfkelch13*-propeller mutations, *Pfmdr1* copy number variations, and known resistance-associated *Pfmdr1* mutations at codons 86, 184, 1034, 1042, and 1246 using new amplification-sequencing protocols for both genes. *Ex-vivo* RSAs were successfully completed for 56% (40/71) of isolates, 9 of which showed greater than 1% survival. A cycle sequencing of dried blood spots revealed that all isolates were *Pfkelch13* wild-type. Sequencing also revealed that 75.8% of isolates possessed the *Pfmdr1* haplotype NFSDD, and 24.2% had the haplotype NFSDY. Two of 40 isolates showed *Pfmdr1* amplification by qPCR; there were no correlations between increased *ex vivo* RSA survival and *Pfmdr1* haplotype or copy number. The unexpected identification of Colombian parasite isolates with increased *ex vivo* RSA survival rates is of concern, particularly since none of the parasites possessed *Pfkelch13* mutations. Continued monitoring for the emergence of artemisinin resistance is necessary in Colombia and other countries using artemisinin-based therapies.

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### PLASMODIUM FALCIPARUM FALCIPAIN 2A POLYMORPHISMS IN SOUTHEAST ASIA AND ITS CONTRIBUTION TO ARTEMISININ RESISTANCE

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Falcipain-2a (FP2a; PF3D7\_1115700) has been linked to artemisinin resistance recently due to its role in hemoglobin degradation. The inhibition of this protease resulted in decreased artemisinin activity. A mutation at codon 69 of the FP2 gene was detected in Tanzanian isolate exposed to a 5-year *in vitro* artemisinin selection previously by another research group. In order to investigate the association of FP2a with artemisinin resistance and characterize the diversity of the FP2a haplotypes in parasites from the China-Myanmar border, we sequenced the full-length FP2a gene in 141 *Plasmodium falciparum* isolates collected between 2007-

2011. We identified 34 point mutations and 26 distinct haplotypes in 97.1% (137/141) parasite isolates, of which five point mutations were not reported earlier. Nine isolates also showed a deletion at residue 115-116. However, when the samples were stratified by the year of collection, no major change was observed in the frequencies of the different mutations/haplotypes. T343P, D345G, A353T, V393I, A400P, and Q414E mutations lie in the protease domain, while M245I, E248D, E249A, K255R, and N257E mutations are present upstream of the protease domain. Residues A353, A400 and Q414 lie in the binding interface of FP2a and its inhibitor (falstatin) and mutation in these residues may hinder the interaction of FP2a with its inhibitor. It would be of interest to characterize the contribution of these FP2a SNPs to drug resistance, and ART resistance in particular. There has been no previously reported significant correlation between the Falcipain 2a SNPs and artemisinin resistance. The preponderance of Falcipain 2a mutations prompted us to analyze our K13 sequences and RSA Survival rates in the context of Falcipain 2a SNPs, haplotypes, as well as growth and fitness phenotypes. Our preliminary analysis hint of Falcipain 2a mutations/haplotypes those are present in isolates that show artemisinin resistance *in vitro*.

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### HIGH RESOLUTION MELT ANALYSIS REVEALS A POTENTIAL SHIFT IN THE MOLECULAR EPIDEMIOLOGY OF ANTIMALARIAL DRUG RESISTANCE IN NIGERIA

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Artemisinin resistance and decline in the efficacy of first line artemisinin-based combination (ACT) drugs for malaria treatment in some endemic regions threatens the success towards global elimination of malaria. Though resistance has not been confirmed in Africa, molecular survey for drug resistance alleles is vital to detect the emergence, and spread of resistant strains. Here we describe the prevalence of antimalarial drug resistance markers during an ACT efficacy trial in Nigeria. Sixty-five patients were screened pre- and post-treatment with ACT on days 0, 1, 3, 7, 14, 21 and 28. Parasite clearance rates during treatment were determined by microscopy and the highly sensitive VarATS diagnostic PCR which targets multi-copy subtelomeric DNA. 18.5% of participants presented with parasitaemia 3 days post-treatment. 17% of patients presented with day 28 parasitaemia. High resolution melt analysis was carried out to amplify codons Pfcrt-76, Pfmdr1-86, Pfmdr1-184, and PfK-13 C580Y in isolates. 18.5% participants presented with parasitaemia 3 days post-treatment. 17% of patients presented with day 28 parasitaemia. The drug resistance Pfcrt-76T allele was present in 37.2% of isolates in the population. Pfmdr1-86 mutant allele was found in 12.5% of isolates. No mutant allele of the K-13 C580Y was recorded. These results indicate that withdrawal of chloroquine and use of ACTs are selecting for wildtype variants at these chloroquine resistance *loci* in Nigeria. However, persistence of parasitaemia in more than 10% of cases after treatment warrants further investigation of a larger population for any signs of reduced ACT efficacy in Nigeria.

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### THE CONTRIBUTORY ROLE OF SOCIOECONOMIC FACTORS TO THE DEVELOPMENT AND SPREAD OF ANTIMALARIAL DRUG RESISTANCE

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Malaria remains a global health issue with the burden unevenly distributed to the disadvantage of the developing countries of the world. As a socioeconomic issue, the high level of poverty in Nigeria is an important

factor that reinforces the persistent malaria burden in Nigeria. The development of resistance to artemisinin-based combination therapies threatens the sustainability of the present success in malaria control. Antimalarial drug use practices/behaviours remain critical drivers of drug resistance as they can affect some of the other factors. This study adopted a social epidemiological stance in investigating the role of socioeconomic factors in determining drug use behaviours promoting antimalarial drug resistance. Methods: A cross-sectional survey design using questionnaires developed from the findings of a previous qualitative study on the same population. Survey data was analysed for relationships between variables using statistical packages. Findings: Significant differences were found between participants of different levels of socioeconomic measures on antimalarial drug use practices like adherence to malaria treatment guideline (educational level:  $p < 0.001$ , partial eta squared = 0.33; HH income:  $p = 0.025$ , partial eta squared = 0.022; type of settlement:  $p < 0.001$ , partial eta squared = 0.038); getting the complete course of antimalarial drugs (HH income:  $p < 0.001$ , OR = 1.46, 95% CI = 1.20-1.76; Urban settlement  $p < 0.002$ , OR: 0.32, 95% CI: 0.16-0.66); and use of mixed drugs (a form of polypharmacy) for malaria treatment (HH income  $p < 0.001$ , OR: 0.72, 95% CI: 0.61-0.85). Also, the adoption of some of these treatment behaviours was significantly associated with the outcome of suspected treatment failure. Conclusions: The distribution of drug use behaviours that promote the development and spread of antimalarial drug resistance is patterned in line with the stratification of socioeconomic position in the population. Most of the resistance-promoting behaviours reported in this study were more of coping strategies by those at the lower levels of socioeconomic measures.

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### ASSESSMENT OF PFMDR1 AND PF CRT MUTATIONS AFTER SIX YEARS OF IMPLEMENTATION OF ARTEMISININ BASED COMBINATION IN DAKAR, SENEGAL

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ACT have been shown to be effective for uncomplicated *P. falciparum* malaria in Africa including in Senegal. In South East Asia, Pfm<sub>dr1</sub> Single Nucleotide Polymorphism are frequent and tentatively associated with reduced susceptibility to ACT partner drugs mefloquine and lumefantrine. In Africa where amodiaquine is one the most partner drugs of ACT, studies on molecular marker of AQ resistance are urgent. The objective of this study is to monitor molecular markers of AQ the partner drug of ACT in Senegal. Methods: Blood samples were collected from patients with uncomplicated malaria in Deggo health post in 2010 N=124 and 2012 N=160. Pfm<sub>dr1</sub> and Pfcrt SNPs were determined by PCR-RFLP in *Plasmodium falciparum* positive samples. Results: A total of 283 samples positives were analysed for various Pfm<sub>dr1</sub> and Pfcrt SNPs. Pfcrt-76T mutant type haplotype was present at 12.90% and 15.90% in 2010 and in 2012 respectively. Prevalence of 16.94% and 15.62% were found for Pfm<sub>dr1</sub>-86Y in 2010 and in 2012 respectively. Low prevalence of Pfm<sub>dr1</sub>-184F was noted in 2010 7.26% and in 2012 6.88%. Conclusions: Overall a low prevalence of Pfcrt and Pfm<sub>dr1</sub> SNPs associated with CQ and AQ resistance were noted in our study area. Similar results were found in West Africa. Results suggested that partner drug of ACT still be effective in Senegal, however a regular monitor of antimalarial drug is essential in the context of while use of ACT.

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### SUBPATENT PLASMODIUM FALCIPARUM INFECTIONS AFTER TREATMENT OF UNCOMPLICATED FALCIPARUM MALARIA WITH DIHYDROARTEMISININ-PIPERAQUINE AND ARTEMETHER-LUMEFANTRINE IN WESTERN INDONESIA

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Recent evidence of treatment failure following dihydroartemisinin-piperazine (DHA-PQ) treatment for *Plasmodium falciparum* infection in Cambodia and Myanmar is a cause for concern in neighbouring countries. Western Indonesia previously used artesunate-amodiaquine (ASAQ) as the first-line ACT from 2004 before frequent drug failures led to a policy change in 2012 to DHA-PQ. We conducted a randomised controlled trial of DHA-PQ vs artemether-lumefantrine (AL) for the treatment of uncomplicated falciparum malaria. Between January and June 2015, we enrolled 302 adults and children in Batubara, Langkat and South Nias regencies in North Sumatera, Indonesia. Patients were given a standard 3-dose of DHA-PQ or 6-dose of AL, and followed for 6 weeks. Drug resistance markers for *P. falciparum* were determined. Primary outcome was cumulative risk for parasitological failure at day-42 determined by microscopy following a standard WHO protocol. Subpatent parasitaemia during follow-up detected by PCR targeting the *pfmdr1* gene was a secondary outcome. Of the 302 enrolled participants, 12.9% (39/302) had mixed infections with *Plasmodium vivax*. Most patients harboured genotypes Pfcrt-SVMNT (88.8%, 63/71), Pfm<sub>dr1</sub>-86Y/184Y (70.1%, 68/97) and Pfk13-wild type alleles (88%, 66/75) prior to treatment. The known-mutants of Pfk13 T474A and M476I were identified in 2 and 1 patients, with the remainder being previously undescribed *pfk13* mutations. Per-protocol analysis showed day-42 uncorrected treatment failures at 6.0% (8/133) and 2.21% (3/136) in DHP and AL groups ( $P > 0.05$ ). By PCR, the parasitological failures increased to 27.1% (36/133) and 27.8% (37/133) ( $P > 0.05$ ), with a reduction in the Pfm<sub>dr1</sub>-YY haplotype prevalence to 0% and 13.8% ( $P < 0.05$ ). Subpatent recurrence was most common among patients from Langkat regency (OR 3.3, 95% CI 1.6-6.7;  $P < 0.001$ ). The predominant SVMNT and Pfm<sub>dr1</sub>-YY alleles in this population may have been selected by ASAQ in the past. DHP and AL appear to remain effective for *P. falciparum* infection in western Indonesia, however submicroscopic recurrence may contribute to further transmission from treated individuals.

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### MOLECULAR EPIDEMIOLOGY AND GENETIC DIVERSITY'S ANALYSIS OF THE CHLOROQUINE RESISTANT GENE PF CRT IN CAMEROONIAN FIELD ISOLATES REVEAL NOVEL INSIGHTS WHICH COULD IMPACT THE MALARIA CONTROL STRATEGIES IN CAMEROON

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Understanding the epidemiology and population genetics of drug-resistant genes can help in devising novel control strategies. The high spread of the resistant strains of the malaria parasite *Plasmodium falciparum* pose a greater challenge than before to the control programs across the world. Specific mutations in the *P. falciparum* chloroquine resistant transporter gene "Pfcrt" have been associated with resistance to not only chloroquine, but also to amodiaquine, one of the artemisinin partners used in Cameroon for the treatment of uncomplicated malaria. We here present not only data of the first attempt to assess genetic variation at the Single Nucleotide Polymorphisms (SNPs) level in the Pfcrt gene in five distinct geographical settings of the Southern-Cameroon (the

most malaria endemic part), but also the distribution of different Pfcrt genotypes in these areas. Mutations in the Pfcrt gene and chloroquine-resistant Pfcrt haplotypes hitherto unreported in Cameroon were detected, with the surprise appearance of the S(agt)VMNT haplotype. Variable genetic diversity was observed across the populations. High linkage disequilibrium was found between few SNPs suggesting a synergistic work for conferring/maintaining a higher level of resistance. Inference of evolutionary pattern of this gene in Cameroon based on the genetic diversity data depicts a signature of Darwinian positive natural selection on these *loci*. Observation of novel mutations might traduce new variants in chloroquine/or amodiaquine resistance (proposal awaiting an experimental verification) and signal of positive selection can be the result of drug pressure exerted by misuse of chloroquine (though officially banned from the country) and/or amodiaquine. Our findings thus, provide a baseline understanding of the evolution of a malaria drug resistant gene in Cameroon and suggest a successful establishment of chloroquine-resistant strains which requires urgent attention of the malaria control program in Cameroon.

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### SUSTAINED HIGH CURE RATE OF ARTEMETHER LUMEFANTRINE IN TANZANIA

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We assessed the temporal trend of artemether-lumefantrine (AL) cure rate after 8 years of its wide-scale use for treatment of uncomplicated *Plasmodium falciparum* malaria from 2006 to 2014 in Bagamoyo district, Tanzania. A trend analysis was performed for 4 studies conducted in 2006, 2007-8, 2012-13 and 2014. Patients with acute uncomplicated *P. falciparum* malaria were enrolled, treated with a standard AL regimen and followed-up for 3 (2006), 28 (2014), 42 (2012-13) or 56 (2007-8) days for clinical and laboratory evaluation. The primary outcome was day 28 polymerase chain reaction (PCR)-adjusted cure rate across years from 2007 to 2014. Parasite clearance was slower for the 2006 and 2007-8 cohorts with less than 50% of patients cleared of parasitemia on day 1, but was rapid for the 2012-13 and 2014 cohorts. The day 28 PCR-adjusted cure rate was 168/170 (98.8%) (95% confidence interval [CI], 97.2-100), 122/127 (96.1%) (95% CI, 92.6-99.5) and 206/207 (99.5%) (95% CI, 98.6-100) in 2007-8, 2012-13 and 2014, respectively. There was no significant change in the trend of cure rate between 2007 and 2014 ( $\chi^2$ -trend test=0.06,  $p=0.90$ ). Pre-treatment *Pfmdr1* N86 prevalence increased significantly across years from 13/48 (27.1%) in 2006 to 183/213 (85.9%) in 2014 ( $p<0.001$ ), and *Pfcr* K76 prevalence increased significantly from 24/47 (51.1%) in 2006 to 198/205 (96.6%) in 2014 ( $p<0.001$ ). The AL cure rate remained high after 8 years of its wide-scale use in Bagamoyo district for the treatment of uncomplicated *P. falciparum* malaria despite an increase in the prevalence of pre-treatment *Pfmdr1* N86 and *Pfcr* K76 between 2006 and 2014.

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### FORMULATION AND EVALUATION OF ETHOSOMES CONTAINING ARTESUNATE

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Ethosomes are nano-sized drug delivery systems that can permeate biological barriers. Nasal delivery is becoming a promising technique considered as an alternative route to achieve non-invasive delivery of drugs directly and rapidly to the brain (by-passing the blood-brain-barrier) and the systemic circulation. Artesunate administered parenterally and orally is used for treating cerebral and server malaria and uncomplicated

malaria respectively. Parenteral drug administration is difficult to achieve in some rural areas in Africa where access to healthcare professionals may be challenging. Moreso, the oral route may not be convenient for some groups of patients. The aim of this research work was to formulate and investigate the intranasal delivery of artesunate-loaded ethosomes as an alternative, non-invasive route of administration for the treatment of cerebral, server and uncomplicated malaria. Ethosomes containing artesunate were formulated using 30% and 50% ethanol, Phospholipon 90H® (2%W/V) and Tween 80 (5.2%). pH stability test, encapsulation efficiency, *in vitro* release and *in vivo* studies were carried out on all batches of ethosomes. The results obtained from *in vitro* release analysis were fitted into mathematical models. All batches of artesunate-loaded ethosomes showed high encapsulation efficiencies (77.46-84.63%). However, the pH stability study revealed reduction in pH after two months. *In vitro* release analysis of formulations showed sustained release of drugs with an initial burst release after 30 mins. All batches of ethosomes followed zero order drug release. *In vivo* evaluation of artesunate in ethosomes and a commercial sample administered intramuscularly showed a significant decrease in parasitaemia ( $p<0.05$ ). However, ethosomes and commercial formulations of artesunate could not reduce parasitaemia level when administered through the nasal route. Artesunate-loaded ethosomes were successfully formulated and achieved sustained release of drugs with significant clearance of parasitaemia when administered intramuscularly but not intranasally.

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### SURFACE CHARACTERIZATION OF HEMOZOIN: IMPLICATIONS IN UNDERSTANDING MALARIA PATHOGENESIS IN THE DEVELOPMENT OF NOVEL ANTI-MALARIAL DRUGS

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During intra-erythrocytic multiplication, *Plasmodium* parasites convert heme into the inert crystal, hemozoin (Hz), to detoxify hemoglobin. Because Hz is associated with the pathogenesis of malaria, including severe malarial anemia, and affects the function of phagocytic cells that play an important role in immunity to malaria, Hz remains an attractive target for drug development. An important strategy is to develop novel anti-malarials that bind to the surface of Hz and inhibit further accumulation of this crystal and, thereby, reduce parasite multiplication within the host red blood cells. Previous studies identified several biochemical properties of Hz as well as its synthetic analog,  $\beta$ -hematin, but little is known about the surface properties of either. Such information is crucial to understand the biochemical nature of Hz more precisely and identify target sites for drug development. Here, we used X-ray photoelectron spectroscopy (XPS) combined with matrix-assisted laser desorption/ionization time of flight (MALDI-ToF) to study the surface of Hz obtained from *P. falciparum* and *P. chabaudi* AS. These analyses revealed for the first time the presence of inorganic species on the Hz surface. Based on our findings, we hypothesized that naturally-occurring molecules, such as riboflavin or epigallocatechin gallate, may exert anti-plasmodial activity by interacting with the surface of Hz. To address this, we used a screening assay to assess the potential of these compounds as inhibitors of  $\beta$ -hematin growth and analyzed the resulting crystals using MALDI-ToF and Fourier Transform-Infrared (FT-IR) spectroscopy. Our preliminary studies show that both riboflavin and epigallocatechin gallate dramatically modified the morphology of  $\beta$ -hematin. Together, these data will help to understand how immunogenic molecules may bind to Hz as well as provide new insight on Hz biomineralization. Importantly, our findings support the usefulness of this approach to develop new anti-malarial drugs.

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## CHANGING ANTIMALARIAL DRUG EFFICACIES IN UGANDA

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Dihydroartemisinin-piperazine (DHA/PQ) has demonstrated excellent efficacy for the treatment and prevention of malaria in Uganda. However, resistance to both components of this regimen has emerged in SE Asia. The antimalarial efficacy of artemether-lumefantrine (AM/LM), the first-line regimen to treat malaria in Uganda, has also been excellent, but continued AM/LM pressure may select for parasites with decreased sensitivity to LM. We characterized the *ex vivo* drug sensitivity and molecular features of 58 *P. falciparum* isolates collected in the Tororo district of Uganda from May-July, 2016 and compared results with those for 442 isolates collected in 2010-13. Drug sensitivity was assessed by *ex vivo* 72 h growth inhibition (IC50) assays; molecular results were assessed by standard methods. The sensitivities of the aminoquinolines chloroquine, amodiaquine, and PQ all improved significantly from 2010-13 to 2016 (geometric mean IC50s 248, 76.9, and 19.1 nM in 2010-13 and 36.1, 15.6, and 7.3 nM in 2016, respectively), sensitivity to LM decreased marginally (IC50 3.0 nM in 2010-13 and 4.3 nM in 2016), and sensitivity to DHA was unchanged (IC50 1.4 nM in 2010-13 and 2016). Molecular analysis showed decreased prevalence over time of the transporter mutations *pfcr*t 76T, *pfmdr*1 86Y, and *pfmdr*1 1246Y, consistent with the observed sensitivity changes. Sequencing of the *k13* propeller-encoding domain showed 2 of 58 samples from 2016 with an A578S mutation, which has been previously reported in Africa. All samples from 2016 had one copy of *pfmdr*1. Increased *plasmepsin-2* copy number (> 1.6 copies by qPCR) was seen in 7 of 58 samples from 2010-13 and 7 of 58 samples from 2016, but was not associated with increased PQ IC50. The E415G mutation in a putative *exonuclease* gene, linked with PQ resistance in SE Asia, was not present in any samples from 2010-13 or 2016. In summary, after heavy use of AM/LM, parasites demonstrated improved sensitivity to aminoquinolines and decreased sensitivity to LM over time. These results may portend decreasing antimalarial efficacy of AM/LM. Continued studies of the relative antimalarial efficacies of ACTs are needed.

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FITNESS LOSS UNDER AMINO ACID STARVATION IN ARTEMISININ-RESISTANT *PLASMODIUM FALCIPARUM* ISOLATES FROM CAMBODIA

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Artemisinin is the most effective drug for *Plasmodium falciparum* malaria treatment currently in clinical use without any parallel in its fast parasite clearance. Emerging artemisinin-resistant parasites poses a great global health risk. At present, the level of artemisinin resistance is still relatively low with evidence pointing towards a trade-off between artemisinin resistance and fitness loss. Here we show that artemisinin-resistant isolates from Cambodia manifested fitness loss, showing fewer progenies during the intra-erythrocytic developmental cycle. The loss in fitness was exacerbated under the condition of low exogenous amino acid supply. The resistant parasites failed to undergo maturation, whereas their drug-sensitive counterparts were able to complete the erythrocytic cycle under amino acid deprivation condition. The artemisinin-resistant phenotype

was not stable, and loss of the phenotype was associated with change in the expression of a putative target of artemisinin, Exp1, a membrane glutathione transferase. Analysis of SNPs in haemoglobin processing genes revealed association with shift in clearance time, suggesting relevancy of changes in haemoglobin catabolism and artemisinin resistance. Analysis of SNPs associated with fast clearance time revealed Plasmeprin III to be linked to artemisinin sensitivity. These findings on fitness and protein homeostasis could provide clues on how to contain emerging artemisinin-resistant parasites.

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HIGH FREQUENCY OF MULTI-CLONALITY INFECTION AND DRUG-RESISTANT ALLELES OF *PLASMODIUM FALCIPARUM* ISOLATES FROM GABON FOUR YEARS AFTER THE IMPLEMENTATION OF ACT

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The main objective of this investigation was to compare the assessment of the frequencies of *Pfcr*t-76T, *Pfmdr*1-86Y, *Pfdhfr* and *Pfdhps* before (2005) and after the implementation of Artemisinin based Combination Therapy (ACT) (2008) and multi-clonal infections based on analysis of merozoite surface protein 1 and 2 genes (*msp*1 and *msp*2) during rebound malaria of country in 2011. The *Pfcr*t-76T, *Pfcr*t-86Y alleles and polymorphic codons 108, 59 and 51 of *Pfdhfr* and 437, 580 and 540 of *Pfdhps* genes were analyzed by PCR-RFLP. The *msp*1 and *msp*2 genes were analyzed using nested PCR-method. A total, 107 *Plasmodium falciparum* isolates of 2005 (n=29) and 2008 (n=78) at the regional hospital were analyzed. The frequencies of *Pfcr*t-76T mutant allele in 2005 (82.5%) and 2008 (70.0%) were comparable (p=0.3). The prevalence of *Pfmdr*1-86Y mutant allele was comparable between 2005 (82.8%) and 2008 (70.5%) (p=0.1). The proportion of the triple *Pfdhfr* was increased: 72.4% in 2005 vs 91.9% in 2008 (P= 0.03); as with quadruple mutation including A437G: 34.4 (35/55) vs 64.8 (p= 0.01). The Multiple Infections (MI) was found in 61.7% (29/46) of isolates from Oyem. For *Pfmsp*2 gene, MI were more frequent at Oyem (55.3%; 26/47). ≥5 genotypes were found with analysis of each gene. Using *Pfmsp*1 gene, complexity of infection (COI) was Oyem (1.85±0.62). The COI was at 2.1±0.48, Oyem with *Pfmsp*2 gene analyzed. In conclusion, an increase of the frequency of *P. falciparum* wild type allele *Pfcr*t-76T, *Pfmdr*1-86Y and quadruple mutation (triple mutation *Pfdhfr*+*Pfdhps*-437G) were observed within a short period after chloroquine withdrawal and higher of MI should increased the morbidity in Gabon.

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SURVEILLANCE *IN VIVO* OF THE EFFICACY OF ARTESUNATE-AMODIAQUINE FOR UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN MADAGASCAR

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The malaria situation has been worsening in Madagascar. In 2005, the Ministry of Public Health adopted a new antimalarial treatment policy, with Artesunate Amodiaquine (ASAQ) and Artemether-Lumefantrine (AL) as the first and second line antimalarial drugs, respectively. As part of routine surveillance on the therapeutic efficacy of ACT in Madagascar, the efficacy of ASAQ was studied. The villages of Anjoma Ramartina situated in highland fringe with seasonal transmission were selected for the study. In the site, the study was a one arm prospective evaluation of the clinical parasitological and hematological responses to directly observed therapy for uncomplicated malaria with ASAQ among all patients recruited. The study was powered to estimate site-specific efficacy for ASAQ. Blood was collected for malaria microscopy and molecular testing on days 0-3,

7, 14, 21, and 28. Recrudescence and reinfection were differentiated using polymerase chain reaction (PCR) genotyping of merozoite surface protein and Glurp. The primary outcome was the PCR-corrected day 28 Kaplan-Meier cumulative success rate. A total of 60 patients with uncomplicated malaria were recruited. Per protocol analyses on day 28 showed an overall PCR uncorrected cure rate of 100% for ASAQ (95% CI 93.3 - 100), Kaplan-Meier survival analysis showed similar outcomes. Prevalence of fever decreased by 90% after the first day of treatment with ASAQ in the site. By microscopy, no patient studied was parasitaemic on day 3. With PCR, four patients were detected to be infected on day 28. The polymorphism (msp1, msp2 and glurp) study outcomes showed that these infections were new infections and not recrudescences due to treatment failure. In conclusion, as evidenced by the day 28 PCR-corrected cumulative success rates observed in the study site, ASAQ remains an efficacious first-line treatment for uncomplicated malaria in Madagascar. Evidence of rapid reinfection suggests that there is a continued need to scale-up effective malaria prevention interventions and surveillance to reduce transmission.

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### THE WWARN VIVAX SURVEYOR: OPEN ACCESS ONLINE MAPPING DATABASE FOR CLINICAL TRIALS OF *PLASMODIUM VIVAX*

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Recurrent infection with *Plasmodium vivax* is associated with significant morbidity and mortality. Although radical cure can reduce recurrent infection, it is confounded by antimalarial resistance and the lack of safe and effective hypnozoitocidal treatment. The WWARN Vivax Surveyor documents the available literature of published clinical trials of *Plasmodium vivax*, providing an up-to-date, online, open access tool to view and download available information. A systematic review was conducted to identify prospective *P. vivax* therapeutic clinical trials with at least 28 days follow-up published between January 1960 and October 2016. Treatment arms and evidence of chloroquine resistance based, on the risk of recurrent *P. vivax* by day 28 and whole blood chloroquine concentrations, were mapped to trial sites. Since 1960, a total of 1,152 antimalarial clinical trials with a minimum 28 days follow-up have been published, of which 230 (20.0%) enrolled patients with *P. vivax* and were included. Trials were conducted in 38 countries: 168 (73.0%) in the Asia-Pacific, 13 (5.7%) in Africa and 43 (18.7%) in the Americas. The proportion of antimalarial trials assessing *P. vivax* rose from 10.7% (12/112) in 1991-1995, to 24.9% (56/225) in 2011-2015. Overall, 188 (81.7%) *P. vivax* trials included a chloroquine treatment arm, either alone or in combination with primaquine, and 107 (46.5%) trials included a chloroquine treatment arm with early primaquine to assess radical cure. There has been a recent increase in treatment arms with artemisinin derivatives. Of the 131 sites in which chloroquine resistance could be quantified resistance was present in 59 (45.0%) sites in 15 endemic countries. Over the last 20 years there has been a substantial increase in clinical research on the treatment of *P. vivax*, which has generated a greater awareness of the global extent of chloroquine resistance. The review also highlights the lack of safe and effective hypnozoitocidal regimens and information gaps in some regions. The open access, interactive WWARN Vivax Surveyor provides up to date information of areas where drug resistant *P. vivax* is emerging.

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### THE EFFECT OF AGE, WEIGHT AND PHARMACOGENETICS ON THE PHARMACOKINETICS OF PRIMAQUINE IN CHILDREN - IMPLICATIONS FOR DOSING AND THE RELATIONSHIP WITH DRUG-INDUCED HAEMOLYSIS

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Low dose primaquine is recommended to prevent *Plasmodium falciparum* malaria transmission in areas threatened by artemisinin resistance and areas aiming for malaria elimination. Community treatment campaigns with artemisinin-based combination therapy in combination with the gametocytocidal primaquine dose target all age groups but no studies thus far have assessed the pharmacokinetics of this gametocytocidal drug in African children. We recruited forty children with normal G6PD function participating in a primaquine efficacy trial in Burkina Faso to study primaquine pharmacokinetics. These children received artemether-lumefantrine and either a 0.25 or a 0.40 mg/kg primaquine dose. Physiological population pharmacokinetic modelling was used to assess the impact of weight, age and CYP2D6 genotype on primaquine and carboxy-primaquine pharmacokinetics. Furthermore, as primaquine may induce haemolysis, the relationship between pharmacokinetics and haemolysis was investigated. Despite weight-normalized dosing, the areas under the plasma concentration-time curves and the peak concentrations for both primaquine and carboxy-primaquine increased with age and body weight. Children who were genetically determined CYP2D6 poor metabolizers had higher primaquine levels, indicating lower PQ CYP2D6-mediated metabolism. Furthermore, higher primaquine exposure was correlated with a greater decline in haemoglobin. In conclusion, our data indicate that primaquine and carboxy-primaquine pharmacokinetics are influenced by age, weight and CYP2D6 genotype and suggest that dosing strategies may have to be re-considered to maximize the transmission-blocking properties of primaquine. Furthermore, besides G6PD phenotype, drug exposure may be an important covariate explaining development of primaquine induced haemolysis. We are currently expanding our PK/PD-model to the adult population.

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### HIGH PREVALENCE OF MALARIA SUBMICROSCOPIC INFECTION IN WOMEN UNDER SULFADOXINE-PYRIMETHAMINE PREVENTIVE TREATMENT AT DELIVERY IN THE REPUBLIC OF CONGO

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Malaria infections undetectable by microscopy but detectable by Polymerase Chain Reaction (PCR) (defined as submicroscopic malaria) are common in endemic areas. A cross-sectional study was conducted in Madibou, a southern district of Brazzaville in Republic of Congo (country with high malaria resistance to SP), between March 2014 and April 2015. The objective of the present cross-sectional study was to assess the frequency of submicroscopic *Plasmodium falciparum* infection among Congolese women with asymptomatic *P. falciparum* infection at delivery and its relationship with IPTp administration. We recruited 366 women at delivery. Placental blood samples were obtained and tested from all asymptomatic women for *P. falciparum* infection by microscopy

and nested polymerase chain reaction, using the *P. falciparum* merozoite surface protein-2 (*mssp2*) gene. Of the 366 pregnant women recruited, 83.6% had received IPTp-SP. With regard to peripheral and placental blood, 25.8% (79/306) and 17.3% (53/306) of those who received IPTp-SP were submicroscopic malaria positive while 74.2% (227/306) and 82.7% (253/306) were negative, respectively. In multivariable logistic regression analysis, pregnant women who received  $\geq 2$  doses of SP in their third trimester showed decreased risk of peripheral (Adjusted OR (AOR) = 0.5; 95% CI [0.3-0.9];  $P = 0.01$ ) and placental (AOR = 0.4; 95% CI [0.3-0.8];  $P = 0.003$ ) *P. falciparum* infection. We observed a high frequency of submicroscopic peripheral and placental *P. falciparum* infection, probably due to residual uncleared gestational infections. The study shows that 2 or 3 SP doses during pregnancy were associated with a lower rate of peripheral and placental *P. falciparum* infection. These findings confirm that IPTp-SP in Congolese pregnant women reduces the risk of *P. falciparum* infection and remains a necessary and efficient preventive tool. This might suggest that IPTp-SP remains a valid recommendation for malaria prevention in the Republic of Congo.

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### NEW INSIGHTS ON EPIGENETICS TARGETS TO TREAT PARASITIC DISEASES

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There is a growing appreciation that epigenetic misregulation makes a significant contribution to human diseases. Many of the recently identified protein families that control the expression of genes through epigenetic mechanisms are finally proving tractable for the discovery and development of small molecules that modulate their function; hence representing new target classes for drug discovery<sup>1</sup>. Particularly outstanding is the recent development of potent and selective small molecule inhibitors of bromodomains (BRD). BRDs are conserved structural modules in chromatin-associated proteins that recognize acetyl-lysine residues on histone and non-histone proteins. Despite these advances, the role of BRDs in the regulation of cellular processes in parasites that cause human disease remains poorly understood. Therefore, a systematic evaluation of BRD protein essentiality in parasitic diseases is needed to identify the best targets for intervention. In contrast to current drug intervention strategies under investigation for parasitic diseases, which primarily affect parasite cell physiology, metabolism and homeostasis; epigenetic target approaches offer an opportunity to explore new mode of actions including the disruption of interactions between the host immune system, host cell receptors and parasite ligands at specific stages of the infection cycle.

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### MALARIA PREVENTION WITH NUTRIENT SUPPLEMENTATION IN ADDITION TO SEASONAL CHEMOPREVENTION IN CHILDREN AGED 6-59 MONTHS IN RURAL MALI

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Susceptibility to malaria and malnutrition are inter-related in young children. We hypothesized that the response to seasonal malaria chemoprevention (SMC) with sulfadoxine-pyrimethamine and amodiaquine may be boosted by concurrent distribution of a lipid-based nutrient supplement (LNS). The objective of this study was to determine

whether the association of SMC+LNS reduces the occurrence of malaria episodes when compared to SMC alone among children aged 6-59 months. A paired cluster cohort was carried out between August and November 2016 in 18 rural health areas of Koulikoro region, Mali. All children aged 6-59 months participating in community-based SMC distribution sessions were eligible: they received 4 monthly rounds of SMC+LNS or SMC alone in the intervention and control groups, respectively. At each round, malaria cases were identified by community health workers through rapid diagnostic tests among children presenting with fever or a history of fever in the past 48 hours. The primary outcome was the occurrence of malaria episodes detected from the 2nd through the 4th round. The secondary outcome was the occurrence of a repeat malaria episode in the 3rd or 4th round. Generalized linear mixed model were used for both outcomes in intention-to-treat (ITT) and per-protocol (PP) analyses; PP analysis included children who actually attended all 4 rounds of SMC distribution. ITT and PP analyses included 11,666 and 8,497 children: 30.1% and 31.5% in the intervention group, 69.9% and 68.5% in the control group, respectively. No significant association was found between the intervention and the occurrence of malaria episodes (aOR: 0.93; 95% CI: 0.61-1.41 and 0.93; 95%CI: 0.62-1.38 for ITT and PP, respectively). However, a statistically significant positive association was found between the intervention and the occurrence of repeated malaria episodes in PP analysis (aOR: 0.61; 95% CI: 0.37-1.01 and 0.49; 95% CI: 0.29-0.82 for ITT and PP, respectively). While the association of LNS and SMC did not impact on the occurrence of malaria episodes in children when compared to SMC alone, it seemed to reduce the risk of repeated malaria episodes.

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### MOLECULAR EVIDENCE FOR PLASMODIUM FALCIPARUM RESISTANCE TO SULFADOXINE-PYRIMETHAMINE BUT ABSENCE OF K13 MUTATIONS IN MANGALORE, SOUTHERN INDIA

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The first-line treatment of *Plasmodium falciparum* malaria in most of India is sulfadoxine-pyrimethamine-artesunate (*plus primaquine*). Among 107 *P. falciparum* isolates obtained from patients attending Wenlock Hospital in Mangalore, Karnataka, southern India, we determined by sequencing mutations associated with sulfadoxine-pyrimethamine in the dihydrofolate hydrogenase gene (*pfdhfr*; codons 51, 59, 108, 164) and dihydropteroate synthase gene (*pfdhps*; codons 436, 437, 540, 581, 613). In addition, the *P. falciparum* Kelch 13 (*K13*) propeller domain was sequenced to identify variants associated with artemisinin resistance. For *pfdhfr*, all isolates revealed wildtype alleles at codons 51 and 164 but the mutations 59R and 108N co-occurred in 71%. *Pfdhps* codons 436, 581, and 613 were wildtype. The *pfdhps* variants 437G and 540E were present in 72% and 24%, respectively. All isolates exhibiting *pfdhps* 540E also had 437G. Combined, *pfdhfr/pfdhps* triple (59R-108N-437G) and quadruple (59R-108N-437G-540E) mutants were seen in 45% and 25%, respectively. No *K13* variants were observed. These molecular data show a substantial degree of sulfadoxine-pyrimethamine resistance in the region of Mangalore, southern India, but the absence of antifolate super-resistance (*pfdhfr* 164L, *pfdhps* 581G). Reassuringly, *K13* mutants affecting artesunate efficacy were absent. Nevertheless, sulfadoxine-pyrimethamine resistance may affect the protection against emerging artemisinin resistance afforded by combinatory treatment. Anticipating the intensification of sulfadoxine-pyrimethamine resistance in this region, monitoring clinical efficacy and drug resistance markers including non-antifolate partner drugs and *pfmdr1* alleles is needed.

### SPREAD OF ARTEMISININ RESISTANT *PLASMODIUM FALCIPARUM* IN FIVE SOUTHERN PROVINCES OF LAO PDR IN 2015-2016

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Government of the Lao PDR and WHO have set an ambitious goal to eliminate malaria by 2030, and malaria burden in the country has been gradually decreasing. However, malaria is still prevalent especially in remote rural areas in the country, and in 2013, a trial conducted in a southern part of the Lao PDR showed that 5%-22% of the *Plasmodium falciparum* malaria patients treated with artemether-lumefantrine were still parasitemic on day 3 after treatment. Moreover, about 20% of isolates of *Plasmodium falciparum* collected from the southern part of the country in 2013 possessed mutations in *kelch 13 propeller (k13)* gene that was associated with artemisinin resistance. In the present study, we examined 1,156 *P. falciparum* isolates collected from malaria patients who visited health facilities in 5 southern provinces in Lao PDR from 2015-2016. DNA analysis revealed that 55.5% (642/1,156) of the isolates showed artemisinin resistant mutations (mainly C580Y) in the *k13* gene. Moreover, frequencies of the mutations in each province were heterogeneous: 73% in Champasak, 69% in Attapeu, 59% in Salavan, 39% in Sekong and 28% in Savannakhet. The frequency was the highest in the southern-most provinces of the country (Champasak and Attapeu) and decreasing toward the north (Savannakhet). These distribution patterns of the *k13* mutations suggest that the resistant isolates may have been introduced by human movement from Thailand/Cambodia and subsequently migrated northward in Lao PDR. We also found that *P. falciparum* infected adult male populations were predominant (65%: 754/1,156) and their occupations were stated as mainly agriculturally related. These results suggest that targeted prevention plans for adult male populations should be designed and implemented to ensure the containment of artemisinin resistance and towards the elimination of malaria in Lao PDR.

### IMPROVING METHODS FOR SURVEILLANCE OF ANTIMALARIAL DRUG RESISTANCE: AN ASSESSMENT OF THE CURRENT LANDSCAPE

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Surveillance of antimalarial drug resistance is undertaken using three approaches: therapeutic efficacy studies to assess the efficacy of drugs in patients; *in vitro/ex vivo* studies to evaluate parasite susceptibility to the drugs; or molecular studies to identify known gene mutations and/or copy number changes associated with drug resistance. These methods are complementary, as they evaluate different aspects of resistance. One key issue when comparing these techniques is the variability of methods used by investigators when conducting such approaches. We have conducted a review of the different methods used to monitor antimalarial resistance, and assessed their strengths and weaknesses. The World Health Organization (WHO) has developed a standard protocol for evaluating the efficacy of antimalarial drugs, used by Control Programs and research groups to conduct antimalarial efficacy studies. Parts of the WHO protocol, however, are not prescriptive and are subject to investigators' choices,

such as aspects concerning microscopy quality control, pharmacology assessment and the genotyping methods used to differentiate recrudescence from new infections. For *in vitro/ex vivo* techniques, a number of different methods are being used, and it has proven difficult to compare results across the different methods. Standardisation of common steps, such as parasite culture, and consistent approaches to data analysis could decrease the high inter-laboratory variability of results. For molecular assessments, different techniques exist as well. The technology landscape for molecular analysis of drug resistance genes is changing quite rapidly, and techniques such as sequencing and real time polymerase chain reaction (PCR) are becoming available at low cost. Strengthening the work of national or regional reference laboratories and research institutions with precise procedures, quality control and assurance schemes and training could improve the quality of antimalarial resistance assessment. Moreover, new technologies in development may be simpler and more user friendly, and could potentially be used directly at peripheral level.

### RETURN OF CHLOROQUINE SENSITIVE *PLASMODIUM FALCIPARUM* MALARIA OF MUTASA DISTRICT, ZIMBABWE

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The emergence of *Plasmodium falciparum* drug resistance erodes the arsenal of effective antimalarials and poses one of the major threats for malaria control and elimination programmes. It is imperative to monitor parasite resistance profiles to available programme antimalarials, both old and new. It has been shown that *P. falciparum* resistance to chloroquine, the former safest and most effective antimalarial, may recede after years of drug withdrawal from use. The current study determined the levels of the chloroquine resistance-conferring PfCRT K76T mutation among *falciparum* malaria infections of Mutasa District, Zimbabwe in 2013, compared to 2003, when chloroquine monotherapy was suspended. Cross-sectional surveys were conducted in 2003 and 2013, on 408 and 373 study participants, respectively, from representative households of Mutasa District. Parasite DNA samples were collected using dry blood spots. DNA was extracted by the Chelex method and *P. falciparum* infections were genotyped at PfCRT amino acid codon 76 using nested PCR and restriction enzyme digestion. Of the participants screened in 2003 and 2013, 66 (17.7%) and 49 (12%), respectively were PCR-positive for *P. falciparum* and all were successfully genotyped at the key chloroquine resistance-conferring PfCRT codon 76. In 2003, there was a high prevalence (63.6%) of the K76T chloroquine resistance mutant, including 3% mixed mutated and wild type infections, leaving only 33% wild type infection. By contrast, 2013 malaria infections had only 3% K76T mutants, the rest (97%) being wild type. No K76T mutant was found among *P. falciparum* infections in the mosquito mid-gut phase. There has been a considerable return of chloroquine-sensitive *P. falciparum* malaria in Mutasa, less than ten years after suspension of chloroquine use. Resumption of indoor residual spraying, sporogonic selection in the local vector *An. funestus* and adoption ACTs since 2008 may be contributing to the rapid restoration of the wild type parasitaemia. Further monitoring is recommended as it may later be viable to consider incorporating chloroquine as an ACT partner drug.

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## PERFORMANCE OF PFHRP-2 BASED RDT MALARIA DIAGNOSTIC TESTS IN RURAL ZIMBABWE

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Accurate identification of *Plasmodium* infections in community surveys is essential to successful malaria control. Microscopy and rapid diagnostic tests (RDTs) are the main techniques used to diagnose malaria in field-based surveys. RDTs are growing in popularity as they allow for rapid and inexpensive diagnosis. Using data from a malaria survey conducted in Mutasa District, Zimbabwe, Paracheck P.f® and Immunochromatographic Test (ICT) RDTs performance using cytochrome b gene polymerase chain reaction (Cyt b PCR) as a gold standard were compared. This was a cross-sectional survey in which households were identified using the Geographic Position System (GPS). Paracheck P.f® and ICT RDT kits were used in parallel to screen for malaria in all age groups in the selected households from October 2012 up to June 2014. The smears were stained with Giemsa stain and examined for parasites using a compound microscope. Parasite DNA was extracted from the dried blood spot (DBS) samples using the Chelex method and a nested PCR for mitochondrial cytochrome b gene. The proportion of *P. falciparum* infections confirmed by PCR, microscopy, Paracheck- Pf® and ICT were 7.17%, 1.90%, and 6.78%, respectively. The sensitivity of microscopy was 60.7% with a 95% confidence interval (CI) of (40.6-78.5) and a specificity of 93.8%, CI (92.5-94.9). Microscopy had a positive predictive value of 14.5%, CI (8.7-22.2). The sensitivity of the two RDTs was 47.8% with a 95% confidence interval (CI) of (37.1-58.6) and a specificity of 95.3%, CI (94.1-96.3). The RDTs had a positive predictive value of 37.4%, CI (28.5-46.9). There was no significant difference between the two RDT brands, Paracheck- Pf and ICT, so they can be used interchangeably. Both microscopy and RDTs showed limited sensitivity of 60.7% and 37.4%, respectively. However, microscopy performed better than RDT possibly owing to low parasitemia.

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## CASE REPORT: RECURRENT FALCIPARUM MALARIA IN A COMMUNITY HOSPITAL IN NEW YORK

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Case: A 38-year-old man presented with acute-onset fever and chills with nausea and vomiting for three days. The symptoms relapsed on the third day. Fever could be resolved by ibuprofen. He denied diarrhea, dizziness, confusion, neck pain or stiffness, visual or hearing changes, or rashes. He recently visited Senegal in Africa for two weeks and came back six days ago. He didn't take malaria prophylaxis. He denied any tick or rodent bites but reported exposure to mosquitos. Patient was treated for malaria 5 years ago in Africa. He came to the US 4 years ago. Blood parasites showed *Plasmodium falciparum* >5%. Patient was started on quinine sulfate doxycycline. Blood glucose dropped below 60mg/dl after treatment. Patient was on POCT glucose monitoring as both malaria and quinine may induce hypoglycemia. After treatment for 5 days, patient had three consecutive negative blood parasites smear. Patient was discharged with quinine and doxycycline to finish 7-day course. Discussion: The attack occurred every other day, which is a characteristic of the "tertian" parasites (*Plasmodium falciparum*, *P. vivax*, and *P. ovale*). Patient had hyperparasitemia according to the criteria (*Plasmodium falciparum* >5%). However, he didn't develop severe complications except transient hypoglycemia. It is noted that patient had previous malaria infection five years ago. Even though it might not be *P. falciparum* infection at that time. Patient could have developed partial immunity that prevented him from severe complications this time. The majority of malaria cases in the US occur in travelers or immigrants returning from malaria-endemic areas. It is recommended to take prophylactic medications considering the drug-resistance in different countries. Conclusion: Periodic fever and chills should arise the suspicion for malaria. Blood smear parasite is the test of choice.

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Faster diagnosis leads to better outcomes even in hyperparasitemia. Travelers to malaria-endemic areas are recommended to take prophylactic medications and use personal protective measures.

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## ESTABLISHMENT OF THE MAJOR SUBCLINICAL SYMPTOMS ASSOCIATED WITH MALARIA IN OWERRI SOUTHEASTERN NIGERIA

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Malaria still remains a life threatening disease of great public health importance especially in sub-Saharan Africa. Detection of malaria at the early stage of the disease could prompt early treatment and subsequently reduction in the morbidity and burden of the disease. We aimed at establishing the major symptoms associated with subclinical malaria in Owerri, South Eastern Nigeria. We utilized a community based and interventional (therapeutic) approach to conduct the study between September and December in two communities (Naze and Ikenegbu) in Owerri, South Eastern Nigeria. The study population consisted of males and females aged 18 years and above, who fulfilled the inclusion criteria and were living in the study areas as at the period of study. We employed a systematic house to house sampling technique and a total of 117 participants from the study sites who tested positive to malaria parasite by microscopy but with no classical clinical symptoms were enlisted. The subjects were later treated with artemeter-lumefantrine. The major symptoms of subclinical malaria we established were; body itching, 111(12.9%), bone pains, 110(12.8%), sleepiness, 110 (12.8%), bad dreams, 107(12.4%), dizziness, 92(10.7%), bitter/soar taste, 90(10.5%), joints/muscular pains, 77(9.0%), and weakness of the body, 75(8.7%). Others, 88 (10.2%) participants reported cough/catarrh 23(2.7%), abdominal discomfort 23(2.7%), loss of appetite 23(2.7%), yellow urine 7(0.8%), constipation 7(0.8%), and sleeplessness 5(0.6%). These subclinical symptoms create suspicion of impending malaria attack and could cause patients to take immediate actions towards proper diagnosis and treatment at the early stage. A recent study done in the same study area showed a better treatment outcome with artemeter-lumefantrine initiated at the subclinical stage than during the clinical stage. Early detection and treatment of malaria could lead to reduction of morbidity, mortality and burden associated with malaria.

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## HIGH-THROUGHPUT SCREENING OF *PLASMODIUM FALCIPARUM* HRP2 DELETION FOR MONITORING OF RDT EFFICACY FOR MALARIA DIAGNOSIS

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Due to ease of use and low costs, rapid diagnostic tests (RDTs) have become the main alternative to microscopy for malaria diagnosis in the field, with over 300 million RDTs sold in 2014. The majority of RDTs detects the *Plasmodium falciparum* HRP2 protein, yet recent reports of *hrp2* deletion question the efficacy of these tests. Due to a lack of appropriate laboratory methods for high-throughput screening, the extent of *hrp2* deletion is not well understood. We present a novel assay to detect *hrp2* and *hrp3* deletions based on droplet digital PCR (ddPCR). The assay is highly sensitive and thus suited to screen asymptomatic infections, can detect mixed infections with *hrp2*- and wild-type parasites, and greatly reduces the number of reactions to be run and the amount of template DNA required. Each sample is partitioned into approx. 15000 droplets that are amplified and screened individually. Depending on parasite density, dozens to thousands of repeats are run in a single well, thus minimizing the risk of false-negative results. Among samples from Eastern Africa, where previously no reports on *hrp2* deletion were available, *hrp2* deletion

was found in 1/23 isolates from southwestern Ethiopia, while all 71 *P. falciparum* positive samples from Western Kenya carried the *hrp2* gene. Screening of samples from other African countries is ongoing. In summary, ddPCR allows for simplified screening for *hrp2* deletion and thus can inform control programs on the efficacy of RDTs for malaria diagnosis.

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### FIELD EVALUATION OF SELEXON MALARIA ANTIGEN *PLASMODIUM FALCIPARUM* SYSTEM FOR MALARIA DIAGNOSIS IN WESTERN KENYA

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Use of rapid diagnostic tests (RDTs) for malaria is increasing as the tests are relatively inexpensive and do not require highly specialized personnel or infrastructure. However, RDT results are dependent on accurate interpretation of test bands, and bands may be faint, leading to inter-operator variability and misclassification. The SelexOn™ Malaria Antigen *Plasmodium falciparum* point of care automated reader system (Dong-A ST, Seoul, South Korea) consists of a RDT and an automated reader that uses a lateral flow immunoassay to qualitatively detect *Plasmodium falciparum* histidine-rich protein II (HRP-II) antigen in blood. This system may improve diagnostic accuracy compared to light microscopy and visually-read RDTs. However, it has not been evaluated in the field. We enrolled individuals presenting to four health facilities in western Kenya with presumed malaria infection and tested their blood samples by the SelexOn™ automated reader, and Carestart™ Malaria HRP-2/pLDH (Pf/PAN) Combo Test (Access Bio, Inc., Somerset, NJ, USA) RDTs to evaluate the performance of these tests to diagnose malaria infection. We calculated the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of each test, in Graph Pad Prism version 5 (Graph Pad Software, La Jolla, CA, USA), using Fisher's exact test to determine 95% confidence intervals (CI), using expert microscopy as the gold standard. We enrolled 465 patients (age range: 6-1085 months; median= 192 months). Malaria rates were: 29% (n=136) based on expert microscopy; 39% (n=181) by the SelexOn™ automated reader; and 36.9% (n=170) by Carestart™ RDT. The sensitivity, specificity, PPV, and NPV of SelexOn™ automated reader were 93.4% (CI: 87.8-96.9), 83.6% (CI: 79.1-87.6), PPV=70.2%, and NPV=96.8%; for CareStart™ RDTs sensitivity was 92.6% (CI: 86.8-96.4), specificity was 86.2% (CI: 81.2-89.8), PPV was 73.5% and NPV was 96.6%. In our study, the performance characteristics of the SelexOn™ system were similar to that of CareStart™ in our study.

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### DETECTION OF MALARIA GAMETOCYTES CARRIAGE IN KISUMU COUNTY, WESTERN KENYA FOR DETERMINATION OF TRANSMISSION DYNAMICS

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Owing to the declining malaria burden globally, malaria endemic countries are endeavouring to enter the pre-elimination phase. *Plasmodium falciparum* gametocytes are vital to sustained malaria transmission. Microscopy is one of the most widely used tools for the diagnosis of malaria; however, molecular methods are more sensitive in detection of both sexual and asexual parasites, especially in asymptomatic carriers.

**Aim:** To determine gametocyte carriage among the asymptomatic malaria cases in Kisumu, Kenya. A cluster designed cross-sectional study was carried out in Kisumu, Kenya between July 2015 and June 2016 to establish the role of asymptomatic individuals in transmission of malaria. Two ml of whole blood was collected from each individual living in the enrolled households. RNA extracts from the blood were used for diagnosis and quantification of the parasite's asexual stage using RT-PCR, and presence of sexual stage parasites was determined as either early (Pf16) or late (Pf25) stage gametocytes. Microscopic analyses of the sexual and asexual parasites were performed by blinded expert microscopists. 2384 (55%) out of 4333 asymptomatic individuals tested positive for malaria by RT-PCR as compared to 1090 (25%) by microscopy. Of the 2384 *P. falciparum* positive specimens, 1292 (54%) were positive for gametocytes with 1055 (82%) having both Pf16 and Pf25, 200 (15%) with Pf16 only and 37 (3%) having Pf25 only. Gametocyte carriage by microscopy was 93 (8%) of the 1090 samples. In conclusion, Kisumu County is in a high malaria transmission setting. This study showed more than half of the asymptomatic residents serve as a reservoir for malaria transmission regardless of the season. This study underscores the importance of understanding the contribution of submicroscopic infections to malaria disease transmission and the value of using molecular diagnostics in malaria control and elimination efforts.

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### LUMINEX-BASED QUANTIFICATION OF *PLASMODIUM SP* HRP2 AND PLDH IN *PLASMODIUM*-INFECTED PREGNANT WOMEN

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Malaria diagnostics and surveillance relies mainly upon the detection of *Plasmodium falciparum* histidine-rich protein 2 (HRP2) and *Plasmodium spp* lactate dehydrogenase (pLDH). The available reference immunoassays for HRP2 and pLDH are based on ELISAs with limited sensitivity. We are currently developing a highly-sensitive Luminex-based assay for the multiplex detection and quantification of HRP2 and pLDH. This assay will be applied for HRP2 and pLDH quantification in plasma from 51 *P. falciparum*-infected pregnant women from Manhiça, Southern Mozambique, and 100 *P. falciparum* or *P. vivax*-infected pregnant women from Urabá-Antioquia region in Colombia. Blood samples from Mozambique were collected at three time points: 2 before receiving

intermittent preventive treatment (IPTp) doses, and 1 at delivery. Quantification of HRP2 in this set of samples will allow us to estimate the persistence of HRP2 in plasma, as currently its dynamics in blood is not well understood. Detection of both antigens is expected to be in the sub-picomogram level. Subsequently, HRP2 half-life and time to negativization will be accurately determined within our sample set. This highly-sensitive multiplex immunoassay will serve as reference method for the validation of malaria rapid diagnostics tests, as a tool for estimating the time of persistence of HRP2 in blood after clearance of infection, and to determine the value of HRP2 detection as an indicator of recent exposure to *P. falciparum*.

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### MULTIPLE ANTIGEN RAPID DIAGNOSTIC TESTS FOR THE DIAGNOSIS OF SEVERE MALARIA IN HIGH-TRANSMISSION, RESOURCE-LIMITED SETTINGS

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Many malaria-endemic countries have adopted community case management programs for the diagnosis and treatment of malaria using lay health workers. While effective for the management of uncomplicated malaria, the ability of these programs to identify and refer cases of severe malaria is limited. New tools are needed to improve diagnostic and management algorithms for severe malaria. This prospective, observational cohort study assessed the accuracy of a multiple antigen RDT compared to clinical and laboratory criteria to identify patients with severe malaria. All individuals presenting to a rural clinic in Western Uganda underwent testing with a HRP-2/pLDH RDT. Those with a positive RDT result had thin/thick blood smears prepared and blood and urine collected for measurement of hemoglobin, serum chemistries, and urinalysis. We estimated the sensitivity, specificity and negative and positive predictive values for a HRP-2+/pLDH+ result compared to WHO defined clinical and laboratory reference standards for severe malaria. A total of 2,678 children <12 years of age underwent testing for malaria with a RDT. Nearly half (1,248, 46.6%) had a positive RDT result, of whom 76 (8.3%) satisfied criteria for severe malaria. The sensitivity and specificity of a HRP-2+/pLDH+ result for severe malaria was 97.4% (95% CI 90.0 - 99.5), and 75.4% (95% CI 73.6 - 77.2%), respectively. A HRP-2+/pLDH+ result was significantly more sensitive (97.4% vs. 67.1%,  $p < 0.001$ ) and had a higher negative predictive value (99.9% vs. 98.8%,  $p < 0.001$ ) for the detection of severe malaria than clinical findings. In conclusion, a multiple antigen RDT has high sensitivity for the detection of severe malaria, making it a promising tool in the management and triage of children with malaria in low-resource settings. Further work is needed to validate and operationalize diagnostic and treatment algorithms so as not to overwhelm tenuous referral networks.

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### IMPROVED POINT-OF-CARE TESTING FOR THE DETECTION OF INFECTION WITH MALARIA PARASITES DURING PREGNANCY IN BUSIA, UGANDA

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Prompt and accurate diagnosis of malaria remains a challenge to malaria elimination efforts especially during pregnancy. Currently available rapid diagnostic tests (RDTs) lack the sensitivity to detect low level parasitemia common during pregnancy among women living in highly endemic areas. In the bid to bridge this gap, we are evaluating two technologies, each of which may independently increase sensitivity by an order of magnitude compared to visual reading of commercial RDTs; sensitive, portable RDT reader based on photo-thermal spectroscopy, and an optimized high sensitivity prototype plasma-specific HRP2 RDT. The study is nested within an ongoing cohort of pregnant women in Uganda. Seven hundred eight two (782) HIV uninfected women were enrolled at 12-20 weeks of gestation and followed through delivery. A Carestart RDT, prototype plasma-specific HRP2 RDT and malaria microscopy were performed on routine visits. Both RDTs were evaluated visually and by the reader, and a whole blood sample is being stored for quantitative PCR (qPCR), which will soon be performed and used as a gold standard for detection of malaria parasites. Of the 366 tested samples, 27% were positive by standard Carestart RDT, while, 55% by the prototype RDT ( $P < 0.001$ ). Of the microscopy negative samples ( $n=274$ ), 6.2 and 41% were positive by Carestart and the prototype RDTs respectively ( $P < 0.001$ ). Of the 28 samples with parasite densities greater than 0 but less than 100 parasites/ $\mu$ l, more samples were identified as positive by the prototype RDT (83% vs. 75%), though this was not statistically significant. The 3 low density infections not detected by either RDTs remain to have the presence and species of parasites to be confirmed by qPCR. All infections with greater than 100 parasites/ $\mu$ l were equally detected by microscopy and both RDTs. Both of these new technologies, used alone or in combination, offer promise for the easy, prompt and accurate diagnosis of submicroscopic malaria infections in pregnancy. Availability of both qPCR and photo-thermal reader data prior to the ASTM meeting will enable us to ascertain this conclusion.

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### AN ULTRASENSITIVE LOOP MEDIATED ISOTHERMAL AMPLIFICATION (US-LAMP) METHOD FOR DIAGNOSIS AND SURVEILLANCE OF MALARIA

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Loop mediated Isothermal Amplification (LAMP)-based methods have proven their potential as field-adaptable molecular test for Point of Care (POCT) diagnosis of malaria. LAMP methods were found to be efficient for diagnosis of symptomatic malaria in multiple settings. At the same time, the efficiencies of LAMP-based methods were inconsistent to detect asymptomatic infections. So, far LAMP-based methods have achieved a lower limit of detection of 1-5 parasites/microliter of whole blood using 18S rRNA gene and mitochondrial cytochrome b gene as the target. We have developed an ultrasensitive (US)-LAMP for diagnosis of falciparum malaria targeting 18S rRNA transcripts. Since a single parasite at the ring stage contains approximately 10,000 copies of 18S rRNA, the limit of detection has improved to 0.05-0.1 parasites/microliter of whole blood. Optimization of US-LAMP from filter paper has also been performed. US-LAMP will be an excellent alternative for both molecular surveillance and POCT in resource-limited settings. This study will be extended on-field specimens.

### HIGH PREVALENCE OF *PLASMODIUM FALCIPARUM* HISTIDINE RICH PROTEIN 2 AND 3 GENE DELETIONS AND THEIR IMPLICATIONS FOR MALARIA DIAGNOSIS IN ETHIOPIA

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PfHRP2-based rapid diagnostic tests have become a mainstay of malaria diagnosis in resource-limited environments throughout the malaria-endemic world. In Ethiopia rapid diagnostic tests are mainly used at village level, whereas microscopy remains the gold standard for malaria diagnosis at health center and hospital level. Deletions of the *pfhrp2*, *pfhrp3* and flanking genes have previously been shown to be associated with negative results from rapid diagnostic devices mainly in Latin America. Conflicting results from rapid diagnostic devices as compared to microscopy and molecular techniques in Ethiopia suggest that *pfhrp2* and *pfhrp3* gene deletions may be common in Ethiopia. Out of a total number of 303 blood samples collected from children under 5 years at three health centers (Aykel, Negade Bahir, and Sanja) in northwestern Ethiopia 33 (10.9%) tested positive in nested PCR for *P. falciparum* malaria, 19 (6.3%) for *P. vivax*, and 3 (1%) for *P. ovale*, respectively. The *pfhrp2*, *pfhrp3* (exon 1, the intron, and exon 2) and flanking genes (MAL7P1\_228 and MAL7P1\_230 for *pfhrp2*, and MAL13P1\_475 and MAL13P1\_485 for *pfhrp3*) were amplified using standard nested PCR. *Pfhrp2* and both of its flanking genes, MAL7P1.230 and MAL7P1.228, were found to be present in only 12 (36.4%) out of the 33 samples testing positive for *P. falciparum*. Twenty one (63.6%) samples tested negative for the *pfhrp2* gene, out of which 19 samples (57.6%) tested positive for at least one of the flanking genes. Only 5 (15.2%) samples gave positive results for the *pfhrp3* gene and both of its flanking genes, whereas 16 (48.5%) tested negative for all three and 12 (36.4%) for *pfhrp3* and at least one of the flanking genes. Our study provides clear evidence of widespread deletions in the *pfhrp2* and *pfhrp3* genes in Ethiopia thereby confirming anecdotal reports of diagnostic failure with HRP2-based rapid diagnostic tests in the region. The potential implications of our findings for the current diagnostic paradigm, which relies on the detection of *P. falciparum* by HRP2-based rapid diagnostic tests in remote areas, may need rethinking.

### POTENTIAL BIOMARKERS OF *PLASMODIUM FALCIPARUM* INFECTIONS IN PREGNANT WOMEN: A CASE-CONTROL STUDY FROM NANORO, BURKINA FASO

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Diagnosis of malaria in pregnancy is problematic due to reduced sensitivity of conventional diagnostic tests (rapid diagnostic tests and microscopy). In this study the potential of six biomarkers for diagnosing malaria in pregnancy was evaluated. At enrollment at the antenatal care clinic, at a follow-up antenatal care visit and at delivery blood was collected from pregnant women for diagnosis of malaria by microscopy and real-time PCR, and for biomarker analyses by ELISA (interleukin 10, IL-10; tumor necrosis factor- $\alpha$ , TNF- $\alpha$ ; soluble tumor necrosis factor receptor II, sTNF-RII; soluble fms-like tyrosine kinase 1, sFlt-1; leptin and apolipoprotein B, Apo-B). At delivery a placental biopsy was collected for histological analysis. Both IL-10 and sTNF-RII were significantly increased at all time points in malaria infected women compared with uninfected women ( $p < 0.001$ ) and the levels were positively associated with parasite density ( $p$

$< 0.001$  and  $p = 0.003$  for IL-10 and sTNF-RII respectively). IL-10 was also negatively associated with birth weight and in malaria infected women with low birth weight ( $< 2500$  g). A scoring model was created using IL-10 and sTNF-RII cut-off points. For primigravida the model had a sensitivity of 92.3% (95%CI 62.3 - 98.9) and specificity of 95.2% (95%CI 71.1 - 99.4) for diagnosing malaria during pregnancy. IL-10 and sTNF-RII are potential biomarkers for diagnosing malaria in pregnancy and can be combined in a model, although this should be confirmed in future surveys. Furthermore, additional markers might be necessary to ensure sufficient specificity when other inflammatory diseases are prevalent.

### GENETIC ORIGINS OF *PLASMODIUM FALCIPARUM* PARASITES WITH *HRP2* GENE DELETIONS IN PERU AND ERITREA

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The worldwide deployment of malaria rapid diagnostic tests (RDTs) has greatly improved malaria diagnosis and case management, contributing to the marked decline of malaria incidence and deaths in recent years. Most of the currently available RDTs detect a specific protein in the patient blood called histidine-rich protein 2 (HRP2) which is expressed only by *Plasmodium falciparum*. HRP2 is not essential for parasite survival, with some parasites deleting the *hrp2* gene encoding this protein. Parasites lacking HRP2 are undetectable by HRP2-detecting RDTs causing false negative RDT results, delaying lifesaving treatment. Parasites lacking HRP2 were first reported in Peru and subsequently reported in several South American, Asian-Pacific and African countries, posing a serious threat to malaria case management and the success of global malaria control/elimination programs. It is important to understand whether the emergence of parasites lacking HRP2 in these regions was due to introduction or simultaneous de novo development. In this study, we investigated genetic origins of parasites with *hrp2* gene deletions in Peru and Eritrea using a 7-loci microsatellite genotyping. The results reveal multiple haplotypes in *hrp2*-positive and *hrp2*-negative parasite populations in each location. This indicates *hrp2*-negative parasites have emerged from different genetic backgrounds. Importantly, in Peru and Eritrea, *hrp2*-negative parasite populations showed distinct haplotypes, strongly suggesting de novo development of these parasite populations in both locations. The outcome of this study will inform malaria control and elimination policy and strategy, and highlight the need for improved non-HRP2 based RDTs.

### ESTABLISHMENT AND APPLICATION OF A NOVEL FIELD BASED LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) ASSAY FOR MONITORING ANTI-MALARIAL DRUG RESISTANCE IN *PLASMODIUM FALCIPARUM*

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*Plasmodium falciparum* malaria is still one of the most threatening diseases and resistance to anti-malarial drugs is one of the main serious evil for the control and elimination of malaria. Relatively high incidence of chloroquine (CQ) and sulphadoxine-pyrimethamine (SP) resistant *P. falciparum* cases have been reported in India. Detection of anti-malarial drug resistance is mainly based on highly sophisticated, costly and time-

consuming molecular techniques including DNA sequencing; these techniques could not cope with the current demand of field based methods for point of care need. In this study we developed a novel field based easy to use loop mediated isothermal amplification (LAMP) assay for rapid detection of chloroquine resistance (*Pfcr* K76T) in *P. falciparum*. Our data provide strong evidence that this new LAMP method could be used for early warning of CQ resistant *P. falciparum* in field conditions. We believe that pre-establishment of a novel isothermal amplification molecular assay for K76T detection could also be useful to develop similar technique valuable for the other important molecular markers including *Plasmodium falciparum* dihydrofolate reductase (*Pfdhfr*), *P. falciparum* dihydropteroate synthase (*Pfdhps*) associated with sulfadoxine-pyrimethamine (SP) resistance and most importantly Kelch13-propeller region constitutes a useful molecular marker for large-scale surveillance of artemisinin resistance in *P. falciparum*. Thus in the view of above targeted marker information we believe that the standardized protocol to detect CQ resistance may be helpful for rapid LAMP optimization to detect the sulfadoxine-pyrimethamine (SP) and artemisinin resistance in malaria-endemic settings.

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#### SCREENING FOR MALARIA IN PREGNANCY WITH RDTs BY COMMUNITY HEALTH WORKERS IN NANORO, BURKINA FASO

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One of the current strategies to prevent malaria in pregnancy is intermittent preventive treatment with sulfadoxine-pyrimethamine (IPTp-SP). However, in order for pregnant women to receive an adequate number of SP doses, they should attend a health facility on a regular basis. In addition, SP resistance may decrease IPTp-SP efficacy. New or additional interventions for preventing malaria during pregnancy are therefore warranted. Our study aimed to evaluate the effect of home-based screening for malaria with rapid diagnostic tests (RDT) by community health workers (CHWs) in a cluster-randomized controlled trial in a malaria endemic region in Burkina Faso. The proposed intervention was added to the standard IPTp-SP strategy. Artemether-lumefantrine was given in case of a positive RDT. RDT results were compared with microscopy or real-time PCR, and the sensitivity and specificity of RDTs performed by CHWs was estimated. The adherence to test results and treatment was assessed for both CHWs and pregnant women. Overall, CHWs were able to conduct

RDTs with a sensitivity of 81.5% (95%CI 67.9 - 90.2) and high specificity of 92.1% (95%CI 89.9 - 93.9). After a positive RDT, 79.1% of women received artemether-lumefantrine. When treatment was not given, this was largely due to the woman being already under treatment. Almost all treated women finished the full course of artemether-lumefantrine (96.4%). CHWs are capable of performing RDTs with high specificity and acceptable sensitivity. Furthermore, CHWs showed excellent adherence to test results and treatment guidelines, suggesting this is a feasible approach. However, the effect of this intervention on malaria-related morbidity during pregnancy needs to be elucidated before firm conclusions can be drawn.

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#### DIAGNOSTIC CHALLENGE OF NON-FALCIPARUM SPECIES IN SENEGAL

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As *falciparum* malaria is decreasing in Senegal, clinicians are challenging with undetermined causes of fever. We investigated the occurrence of non-*falciparum* species and other endemic pathogens that could lead to similar symptoms. From October 2015 to December 2016, we included 4 sites in Senegal: Dakar, Thies (malaria hypoendemic regions), Richard Toll (pre-elimination stage) and Kedougou (high prevalence of malaria). We conducted a prospective study where feverish patients were selected. For each sample, microscopic examination of Giemsa stained slides and RDTs were performed and filter paper was spotted with fingertip blood for PCR. In the site, slides were read by health center (HC) microscopists and all slides were checked once in Dakar by an expert microscopist. PCR was used to resolve discrepancies. In total, 927 samples were collected. Overall *Plasmodium falciparum* was detected in 213 samples by expert microscopist. HC missed 11 *falciparum* positive samples: 7 samples were under 100 parasites/ul and 4 samples were under 500 parasites/ul. All missed samples were located in Richard Toll. Non-*falciparum* species occurred in 6 samples detected by expert microscopy: 4 *P. ovale* and 2 *P. malariae*. Among those samples, 1 *P. ovale* was missed by HC microscopist, 2 *P. ovale* were not detected by RDT LDH bands. One HC microscopist misdiagnosed *P. ovale* for *P. vivax*. *P. malariae* samples were correctly diagnosed by HC microscopist. In 15 samples, spirochetes of *Borrelia* were observed. Only one HC microscopist was able to detect spirochetes in thick smear. As *P. falciparum* is decreasing it becomes an urgent need to train technicians to recognize unfamiliar malarial parasites and other pathogens responsible for fever such as *Borrelia*.

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#### LAMPREY MONOCLONAL VLR ANTIBODIES AGAINST PLASMODIUM FALCIPARUM HISTIDINE RICH PROTEIN-2

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Lampreys use variable lymphocyte receptors (VLR) composed by leucine-rich-repeat (LRR) segments for antigen recognition instead of the immunoglobulin (Ig)-based receptors used by jawed vertebrates. VLRs in lampreys are as diverse and antigen-specific as Ig-based antibodies made by mammals and other jawed vertebrates. Furthermore, they have unique properties that may make them advantageous over the traditional mouse and human antibodies for diagnostic tests, including better thermostability, high avidity, long shelf-life and recognition of novel antigen epitopes when compared to mammalian antibodies. Here we describe the generation of VLR monoclonal antibodies directed against the histidine rich protein-2 (HRP-2) of *Plasmodium falciparum*. HRP2 based rapid diagnostic tests (RDTs) are the most commonly used tests for malaria diagnosis and this test is highly sensitive and specific to *P. falciparum* as HRP2 protein is not produced by other human malaria parasites. As a first step to test the hypothesis that lamprey-based antibodies may enhance the stability and durability of RDTs, especially for use in tropical countries, we immunized lamprey larvae with purified HRP-2 protein to elicit production of high titer VLR antibodies specific for HRP-2. Lymphocytes from immunized lampreys were then used to construct VLR cDNA libraries, which were expressed on the yeast surface by fusion to the Aga2p yeast protein and tagged with a myc epitope to enable detection of surface expression. HRP-2 binding clones were selected by incubating the yeast cells with biotinylated HRP-2 antigen and staining with streptavidin-PE. Using this methodology, we

selected two recombinant VLRB antibodies with high affinity and specificity for recombinant HRP-2 and that also recognize the native HRP-2 protein in *P. falciparum* culture supernatants. The immunological characterization of these antibodies will be presented.

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#### ANALYTICAL SENSITIVITY OF PCR TESTS FOR OPTIMUM VIVAX MALARIA DIAGNOSIS

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The prompt diagnosis of plasmodial species for correct and effective patient treatment prevents the transmission and reintroduction of malaria, as well as the worsening of health condition of the patient. The PCR method allows the diagnosis below the detection threshold of microscopic examination. The precision of real time PCR for diagnosis purposes, comprising the repeatability and reproducibility parameters, is scarcely reported. Thus, we developed a real-time PCR assay with SYBR® Green and TaqMan® systems for the diagnosis of *Plasmodium vivax* malarial infection. Our experimental design included the construction of a standard curve with *P. vivax* DNA, cloned or not, to determine linearity; the setting of the lower detection limit and analytical sensitivity to measure sensitivity and; intra assay variations (repeatability) as well as oscillations between assays, operators and equipment (reproducibility) to set precision. The performance of these parameters in the standardization of real time PCR showed linearity in SYBR® Green and TaqMan® systems with 1 and 5 copies / microliter with cloned DNA and 1 parasite /microliter with uncloned *P. vivax* DNA; quantification threshold of 1.77 and 0.94 and; analytical sensitivity of 1.13 and 1.17 copies / microliter, respectively. When compared conventional PCR with real time one, the detection limit remained 0.00001 parasite / microliter and the precision with 0.1 parasite / microliter for SYBR® Green and 1 parasite / microliter for TaqMan® and conventional PCR was also 100%. We concluded that real-time PCR is the eligible methodology for the detection of *P. vivax* parasites; the TaqMan® system is the most indicated for quantitative assays and that real time PCR should be adopted as a gold standard for vivax malaria diagnosis in reference laboratories.

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#### RANDOMIZED TRIAL TO ASSESS THE EFFECT ON QTc INTERVAL OF REPEATED TREATMENT OF UNCOMPLICATED MALARIA WITH ACTs IN BOBO-DIOULASSO, BURKINA FASO: RELATION BETWEEN PARASITEMIA AND PROLONGED QTc

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ACTs are widely used for the management of malaria and even tested for chemoprevention. In single episode efficacy studies, these drugs were well clinically tolerated but cardiac effects over repeated treatment are less investigated. We conducted a prospective randomized controlled trial in Bobo-Dioulasso from August 2012 to October 2013 where patients aged 6 months and over were randomly allocated to receive either Pyronaridine-artesunate or Dihydroartemisinin-piperaquine or Artemether-lumefantrine on first and on subsequent episodes. Each participant was screened against inclusion criteria including the ECG which was repeated again 2 hours after the last dose and blood smear each 12 hours until the third day. We were considered that QTc interval more than 450 ms is abnormal, but prolonged QTc interval over 500 ms was reported as adverse event. Drugs tolerance was compared using chi square test and p value of less than 0.5 is significant. Patients were randomized to receive PA (n=224) DHAPQ (n=224) or AL (n=296). During the 2 years follow up we observed

a total of 149 (in 1834 electrocardiogram performed on day 2 monitoring) prolonged QTc more than 450 ms (19/521 for PA, 92/548 for DHAPQ and 38/765 for AL, p<0.001). These proportions of QTc prolonged decreased over the subsequent episodes irrespective of the drug (57 QTc>450 in episode 1 to 0 in episode 8 until episode 10). Therefore, there are no relation between prolonged QTc and parasites elimination at day 2. A higher number of QTc prolonged was observed in DHA-PQ group than other groups. Otherwise, three drugs were well tolerated despite repeated treatment of malaria which seems to improve over consecutive episodes.

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#### EFFICACY OF PRIMAQUINE AND METHYLENE BLUE FOR PREVENTING PLASMODIUM FALCIPARUM TRANSMISSION AMONG GAMETOCYTEMIC MALES IN MALI

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Primaquine (PQ) and methylene blue (MB) are two gametocytocidal compounds that have the potential to prevent transmission of *P. falciparum* malaria to mosquitoes. We aimed to establish the efficacy and safety of single low-dose PQ and MB combined with blood schizonticides in male patients in Mali. In this phase 2, four arm, randomized control trial, we enrolled males 5-50 years of age with uncomplicated *P. falciparum* malaria at the Malaria Research and Training Centre (MRTC) field site in Ouelessebouyou, Mali. All participants were positive carriers of gametocytes assessed through microscopy and glucose-6-phosphate dehydrogenase (G6PD) normal defined by rapid diagnostic test (CareStart™ G6PD RDT). Participants were randomized 1:1:1:1 in blocks of 8 to receive either sulfadoxine-pyrimethamine plus amodiaquine (SP-AQ) alone, SP-AQ plus 0.25 mg/kg PQ, dihydroartemisinin piperaquine (DP) alone, or DP plus 15 mg/kg/day for 3 days of MB. The primary endpoint was mean within-person percent change in mosquito infectivity 7 days after treatment initiation among participants who were infectious at baseline. Mosquito infectivity was measured using membrane feeding assays. Between June to December 2016, we enrolled 80 participants. In the primary analysis sample (n=52), all participants who received either PQ or MB exhibited a 100% within-person reduction in infectivity by day 7 and this reduction was significantly higher compared to either SP-AQ (p<0.001) and DP alone (p=0.002), respectively. No clinically meaningful or statistically significant drops in hemoglobin were observed during follow-up. No significant differences in AEs were observed between the SP-AQ (85%) and SP-AQ plus PQ arm (65%) (p=0.10), or between the DP (55%) and DP plus MB arm (60%) (p=0.20), excluding blue urine. One participant in SP-AQ arm reported a severe headache on day 7, but this AE was considered unlikely due to the study drug. A single dose of 0.25 mg/kg of PQ and 15 mg/kg/day x 3 days of MB, given alongside SP-AQ or DP, respectively, were safe and highly efficacious for preventing *P. falciparum* transmission in G6PD normal males. (ClinicalTrials.gov, number NCT02831023)

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### IMPROVING PROSPECTIVE STANDARDIZATION OF MALARIA CLINICAL TRIALS DATA USED TO MONITOR TRENDS IN ANTIMALARIAL EFFICACY AND RESISTANCE: THE WORLDWIDE ANTIMALARIAL RESISTANCE NETWORK RESOURCES PLATFORM

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Standards for conducting clinical trials are fairly uniform irrespective of disease, but have with time been improved by the introduction of guidelines and resources aimed at improving the quality of design and conduct of clinical trials. It is these tools that have continuously evolved according to different diseases and are ideally aimed at simplifying this process. Malaria scientists in endemic settings have been faced with a growing availability of tools and resources. Such a wide unstandardized platform has led to heterogeneity in the quality and completeness of data from clinical trials. The lack of a simplified accessible toolkit to help set up clinical trials, in the presence of a vast source of information, has often resulted in difficulties for malaria investigators to fully utilise the available resources with ease. The WorldWide Antimalarial Resistance Network (WWARN), a collaborative initiative that generates and collates innovative resources to inform the malaria community on factors affecting antimalarial efficacy, has over the past decade developed specific resources for use in malaria studies. We will present findings from WWARN's recent scoping exercise aimed at understanding the variation in tools and resources commonly used to design and conduct malaria clinical trials in low and middle income countries (LMICs), and provide an update of an ongoing project that is aimed at establishing an open access platform of resources to malaria trialists in LMICs. This toolkit will support the standardisation of prospective data collection, aiming at optimising the usability and comparability of data.

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### LOW DOSE PRIMAQUINE EFFICACY AND SAFETY: A REVIEW AND INDIVIDUAL PATIENT DATA META-ANALYSIS

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Primaquine is the only commercially available drug that kills mature *P. falciparum* gametocytes, the lifecycle stage responsible for the transmission of malaria from the human to the mosquito. Primaquine can cause haemolysis in individuals with glucose-6-phosphate dehydrogenase deficiency (G6PD). The severity of haemolysis is dependent on the dose of primaquine used and the level of G6PD enzyme activity. The WHO recommends the use of a single low (0.25 mg/kg) dose of primaquine (SLD PQ) with an artemisinin-based combination treatment, without prior G6PD testing, to individuals with uncomplicated malaria in countries targeting elimination and/or facing drug resistance against artemisinin-based therapies. Adoption of the WHO recommendation to use SLD PQ has been slow, particularly in African countries where there is a perceived lack of evidence for its safety and efficacy. Numerous clinical trials have been conducted, presenting a valuable dataset for further scrutiny. WWARN is undertaking an individual patient data meta-analysis of studies conducted in Africa testing SLD-PQ. Data for inclusion were identified from a review of trial registries. The objectives of the safety analysis are to quantify the reduction in haemoglobin between days 0 and 7 associated with the administration of SLD PQ and to investigate potential factors predicting these reductions. In addition, the analysis characterizes the incidence, type, and study drug relationship of reported serious adverse events and adverse events of special interest with particular attention to mortality, blood transfusion and reports of dark urine. The objectives of the efficacy analysis are to quantify the change in gametocyte prevalence and density and to assess the infectivity of gametocytes to mosquitoes as measured by membrane feeding, following administration of SLD PQ. The impact

of age, sex, and G6PD status on the efficacy and safety of PQ given at single doses  $\leq 0.75$  mg/kg will be evaluated and modelled to generate a therapeutic dose range to inform safe deployment in Africa. Results will be presented from analyses on a pool of 13 studies, representing a total of 5,017 participants.

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### INVESTIGATION OF G6PD ENZYME ACTIVITY IN NORMAL SUBJECTS FOR ENTRY INTO TAFENOQUINE CLINICAL STUDIES

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Tafenoquine (TQ) is an investigational 8-aminoquinoline (8-AQ) in development as a single dose treatment for the radical cure of *P. vivax* malaria. However, 8-AQs cause hemolysis in glucose-6-phosphate dehydrogenase deficient (G6PDd) individuals. Although TQ will be contra-indicated in G6PDd individuals, it is important to exclude subjects with insufficient levels of G6PD from participation in clinical studies and beyond. We undertook TAF115226, a G6PD assay validation study as part of an integrated phase IIb/III program with the primary objective to establish a centre specific median value for G6PD activity in healthy normal males, since they are truly normal or deficient unlike females. 70% of the median was used as entry criteria in TQ trials. A total of 824 subjects were screened, resulting in 808 enrolled from 19 centres across 3 continents. A blood sample was obtained to confirm eligibility criteria based upon reticulocyte count ( $\leq 2.5\%$ ) and hemoglobin level ( $\geq 120$  g/L). A second sample was analysed for G6PD quantitative activity using a commercial spectrophotometric assay (Trinity Biotech, Ireland). The secondary objective was to determine the intra-laboratory variability of the G6PD assay. To further understand intra-laboratory variability, after completion of Phase IIb, blinded samples with known G6PD activity were sent for G6PD analysis by United Kingdom National External Quality Assessment Service. A number of steps were taken to improve accuracy and precision of assay measurements and to harmonise methodology in absolute values at future sites in Phase III resulting in reduced inter-and-intra laboratory variability. Median G6PD activity did not subsequently vary greatly between centres (range 7.17-10.51 IU/gHb). Furthermore, we have determined a global median G6PD activity of 8.16 IU/gHb, (70% of which is 5.71 IU/gHb) in G6PD normal males. In conclusion, we have been able to harmonise a reproducible quantitative G6PD assay across three continents to support TQ clinical studies and set global G6PD values to assist potential future real world use of TQ.

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### TRIAL COMPARING TWO ARTEMISININ BASED COMBINATION THERAPIES FOR THE TREATMENT OF PLASMODIUM FALCIPARUM MALARIA IN RWANDA

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Artemisinin-based combination therapies (ACTs) have been prescribed in Rwanda as first-line treatments for malaria since 2006. Although these drugs have contributed substantially in the battle against malaria, recent data from sub-Saharan Africa suggest that *Plasmodium falciparum* are beginning to develop resistance against some forms of ACTs. The aim of this study was to compare the effectiveness of two ACTs, Coartem and Duo-Cotexin, for the treatment of uncomplicated *P. falciparum* malaria among children in Rwanda. Five hundred thirty-six patients aged 1 to 14 years with uncomplicated *P. falciparum* malaria were randomized

to receive treatment with Coartem (n=267) or Duo-Cotexin (n=269) between September 2013 and December 2015. Chi-squared tests were used to compare rates of adequate clinical and parasitological response (ACPR) for the two ACTs at three and 28 days after initiating treatment. These preliminary data are not genotype-corrected and therefore do not differentiate between treatment failure due to recurrent parasitaemic episodes versus re-infections. ACPR at three days was recorded for 265 patients treated with Coartem and for 266 patients treated with Duo-Cotexin ( $\chi^2 = 0.194$ ;  $p = .659$ ). At 28 days, the ACPR was significantly lower for patients treated with Coartem (86.5%) compared to those treated with Duo-Cotexin. (97.4%) ( $\chi^2 = 21.03$ ;  $p < .0001$ ). In conclusion, unadjusted ACPR data indicated that Coartem and Duo-Cotexin were both highly effective at treating children diagnosed with uncomplicated malaria at three days post-treatment; ACPR was 99% for both ACTs. At 28 days, a higher proportion of children who received Duo-Cotexin remained asymptomatic and parasite free compared to those treated with Coartem. This difference between the three- and 28-day results suggests that Duo-Cotexin provides protection against reinfection for longer compared to Coartem. Genotype-corrected data will allow for more robust conclusions to be drawn. Malaria control programs must enact resistance surveillance and mitigation plans to ensure treatments remain effective.

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#### MOLECULAR DETECTION METHODS TO ESTIMATE *PLASMODIUM FALCIPARUM* GAMETOCYTE CARRIAGE IN NORTHWESTERN CAMBODIA

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Gametocyte carriage and density are critical factors in malaria transmission from human to anopheline hosts, though defining precise transmission risk remains challenging to study. While molecular methods have greatly improved understanding of submicroscopic gametocyte carriage, it is unclear how much these low-density infections contribute to clinical transmission. We compared the burden of microscopic vs. submicroscopic gametocytemia in *Plasmodium falciparum* malaria patients in a randomized, open label clinical trial of atovaquone-proguanil (AP) vs. artesunate + atovaquone-proguanil (ASAP). All patients received a 3 day course of oral therapy with their assigned regimen, as well as a single low dose of 15 mg primaquine on the first day of treatment. Light microscopy (LM) and a nested reverse transcriptase PCR assay (nRT-PCR) targeting Pfs25 RNA expression indicative of mature gametocytes (stage V) was performed on blood samples from 205 volunteers daily for 3 days, and then weekly to estimate gametocyte density and duration of carriage. At screening, *P. falciparum* gametocyte positivity was 25% (51/205) by LM compared to 28% based on pfs25 RNA detection (57/205). At enrollment, 23% of volunteers had detectable gametocytemia by LM which was reduced to 1% by day 14 in the ASAP group but only 8% in AP group ( $p = 0.03$ ). Volunteers in the AP only group had a longer duration and higher density of gametocyte carriage by LM than those receiving ASAP. Follow-up submicroscopic parasitemia assessments in follow-up remain pending at the time of submission. *P. falciparum* gametocyte carriage at baseline by LM was higher than the rate typically seen in other recent studies performed in this area (approximately 10%). While those receiving

ASAP had substantial reductions in risk as well as carriage, 8% of those receiving AP alone remained gametocytemic, even by LM despite receiving primaquine. This suggests that additional studies to more carefully evaluate the efficacy of single low dose primaquine on gametocyte reduction are warranted.

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#### TOWARDS MALARIA ELIMINATION: ANALYSIS OF MALARIA SURVEILLANCE DATA AMONG UNDER FIVES IN OYO STATE, NIGERIA (2010 - 2014)

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In Nigeria, malaria among under fives remains a major public health problem in terms of morbidity and mortality. We conducted a secondary data analysis to determine the morbidity, mortality and seasonal variations of malaria among under fives in Oyo State, Nigeria. We reviewed and analysed data obtained from the Integrated Disease Surveillance and Response System for the period; 2010 to 2014. Abstracted data were number of malaria cases among under-5 children disaggregated by Local Government Areas (LGA), year of reporting and season. Incidence, proportion of malaria morbidity and number of deaths across months per LGA were determined. Data were analysed using descriptive statistics while trigonometric regression was used to examine seasonal variations. A total of 404,216 malaria cases were reported between 2010 and 2014. The incidence of malaria among under-5 children per 100,000 population was 5,602 in 2010; 14,005 in 2011, 18,938 in 2012; 14,005 in 2013 and 7674 in 2014. The highest incidence recorded in 2012 accounted for 31.4% of the total malaria morbidity. Incidence was highest from the month of July to August 2013 with an increase from 1764 cases per 100,000 in July to 3,129 cases per 100,000 population in August. The total number of malaria deaths was 15. A sinusoidal pattern was observed in the monthly distribution with malaria peak rates in June to August and lowest rates in October to December. Seasonal index showed that the peak number of malaria cases was in the second quarter of the year. The annual trend of malaria among under-5 children in Oyo state showed a gradual rise in incidence from 2010 to 2014 in spite of the scale up of interventions. The findings also suggest that there exists a significant monthly variation in malaria rates in Oyo State, Nigeria.

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#### USE OF THE IMMUNO-EPIDEMIOLOGICAL BIOMARKER OF HUMAN EXPOSURE TO ANOPHELES BITES IN THE MONITORING OF MALARIA TRANSMISSION IN (PRE) ELIMINATION AREAS

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The burden of malaria is gradually declining in many parts of Africa, and is characterized by spatial and temporal variability that presents new and evolving challenges for malaria control programs. New, sensitive and large-scale monitoring indicators that measure the actual risk of malaria transmission/infection over time and space need to be prioritized in a context of elimination. Here, we present the use of an indicator based on the human IgG antibody responses to the gSG6-P1 peptide of the

Anopheles saliva as a pertinent tool for monitoring malaria transmission in very low malaria transmission areas, which can be considered as a "picture" of a malaria pre-elimination area. Two longitudinal studies that cover different seasons of malaria transmission were carried out in northern and central Senegal. At each visit, entomological, parasitological and sociological data were collected. Parasitological and clinical data were correlated with the quantitative level of IgG responses to the gSG6-P1 salivary peptide in children. In northern Senegal, the biomarker of exposure to Anopheles bites indicated that some children were exposed to Anopheles bites during the dry season that has no or very low Anopheles density. Interestingly, children with *P. falciparum* infection in the dry season had higher levels of anti-gSG6-P1 IgG responses than non-infected ones ( $P < 0.01$ ). This biomarker even seemed to discriminate non-infected children from asymptomatic carriers of the parasite ( $P < 0.01$ ). In central Senegal area, the level of specific IgG level increased significantly within the exposure season in area with very low exposure to Anopheles, determined by classical entomological methods ( $P < 0.01$ ). This increase was observed in 69% of children. The biomarker of exposure to Anopheles bites appears to be a sensitive and relevant tool for detecting a risk of *P. falciparum* transmission and assessing the level and heterogeneity of malaria transmission (hot spots). The use of such an immunological indicator may be essential for monitoring malaria and assessing the effectiveness of vector control strategies in (pre)elimination malaria areas.

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#### RISK FACTOR ASSESSMENT FOR MALARIA AMONG FOREST-GOERS IN A PRE-ELIMINATION SETTING, PHU YEN PROVINCE, VIETNAM

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Progressing from malaria control to elimination requires understanding and targeting interventions to populations at high risk. In Vietnam, forest-goers are often not reached by health services. These mobile populations are difficult to test, treat, and track via routine measures. If undiagnosed, forest goers can maintain parasite reservoirs and contribute to ongoing transmission. A case-control study was conducted to identify malaria risk factors associated with forest-goers in Dong-Xuan District, Phu Yen Province. A case was considered anyone residing in the target area with malaria, confirmed by rapid diagnostic test (RDT) or microscopy and had slept overnight in the forest. Controls were healthy neighbors of cases and negative for malaria by RDT. Participants were interviewed face-to-face using a standard questionnaire. Logistic regression was used to calculate odds ratios (ORs) and 95% confidence intervals (CI) for risk factors after adjusting socio-demographic characteristics. In 2015, we found 81 cases of which 66.7% were positive for *Plasmodium falciparum*, 29.6% for *P. vivax*, and 3.7% had a mixed infection. The majority of cases were male (88%) with a mean age of 34.2 years. Cases were less likely to use treated nets (OR=0.3; 95% CI 0.1-0.8), but more likely to sleep in a hut without walls (OR=5.1; 95% CI 1.6-16), work after dark (OR=2.7; 95% CI 1.3-5.6), bathe in the stream after dark (OR=2.6; 95% CI 1.1-6.0) and collect water after dark (OR=2.0; 95% CI 1.1-3.8) as compared to 94 neighborhood controls. Risk factors for malaria among forest-goers in Vietnam are similar to risk factors for malaria in other areas. As Vietnam moves toward malaria elimination, targeted education and malaria prevention strategies are needed for this hard-to-reach group.

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#### ESTABLISHING NEW LINE OF *PLASMODIUM FALCIPARUM* INFECTED CLONAL STRAINS IN SUPPORT OF CONTROLLED HUMAN MALARIA INFECTION STUDIES

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Malaria causes 212 million new cases and approximately 429 000 deaths in 2015. This far, antimalaria drug resistance has developed for all classes of drugs and no licensed vaccine is in place. However, there are efforts to develop new and/or improved anti-malarial drugs and vaccines. Controlled human malaria infection (CHMI) studies have shown some correlation between natural and experimental infections. Conversely, these experimental infections and challenges are mostly done using laboratory clones obtained > 30 years ago. This limits data interpretation because experimental and natural infections might not correlate because field parasites are highly genetically and phenotypically diverse. The field displays a wide genetic diversity which currently is not represented by available laboratory strains for CHMI. Other strains including the South American 7G8 *Plasmodium falciparum* clone of Brazilian strain IMTM22 have been used in limited volunteers. This study will provide an opportunity to have a new clone with different characteristics that will offer an opportunity for protectiveness, which can lead to further optimization of the vaccine or the candidate drug. Forty field isolates from different regions in Kenya underwent limiting dilution to generate single clones. For each of the 40 parent parasites, 3-10 clones were obtained, generating a total of 212 clones. Of the 212 clones, 80 lines were confirmed to be single clones based on neutral microsatellites. After successful limiting dilution assays, the next phase of super-cloning of these for subsequent single clones is underway.

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#### TIMELINESS AND COMPLETENESS OF MALARIA CASE NOTIFICATION AND RESPONSE IN ZANZIBAR, 2013-2015

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Zanzibar has made significant progress toward malaria elimination over the past decade and now considered a low transmission setting with the potential to achieve elimination, but challenges still exist. Malaria surveillance plays a key role in identifying new malaria cases rapidly to reduce transmission. This study aimed to assess timeliness and completeness of malaria case notification and response. Timeliness and completeness were assessed using an evaluation tool developed by the University of California, San Francisco. Data collected from 2013 to 2015 through individual case reporting system, the Malaria Case Notification (MCN). Each District Malaria Surveillance Officer (DMSO) was equipped with tablet. Once a new case is notified, a household follow up will be guided through an active case response protocol and data transmitted through the system. Additional case data are entered into the tablet at the facility and household. Each household member is tested and new cases are treated immediately. Timeliness was defined as the number of reactive case detection (RACD) events followed up within 48 hours of notification divided by the total number of RACD events reported within 48 hours. Completeness was defined as the number of individuals screened during the RACD divided by the total number of individuals living in the

index household. In 2013, on average 91% of notified malaria cases were followed up at the household level within 48 hours of occurrence. This number improved to 93% in 2014 and 93.1% in 2015. In 2013, on average 84% of case household members was screened during RACD activities. The average proportion of household members screened in 2014 and 2015 was 84.4% and 90% respectively. The case follow up and household members' screening for malaria fall short of the national target of 100% due to heavy workload among DMSOs, incomplete patient information and technical malfunctions. Our results suggest that while the MCN system has improved malaria case reporting from health facilities, efforts should also focus on improving data quality and timely household level follow-up to eliminate malaria in Zanzibar.

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### EVALUATING *PLASMODIUM* HRP2 PLASMA CONCENTRATION FOR DEVELOPMENT OF HIGHLY SENSITIVE PLASMA-SPECIFIC RAPID DIAGNOSTIC TEST IN UGANDA

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HRP2-specific rapid diagnostic tests (RDTs) form the primary mode for malaria diagnosis in most resource-limited settings in Africa. However, sensitivity is hampered by products of hemolysis, which increase background and limit the volume of blood which can be evaluated. This limitation is partly responsible for the inadequacy of current malaria RDTs in detecting low-density (asymptomatic) parasitemia, of importance in elimination programs. Despite the 2-decade history of targeting HRP2 for malaria diagnosis, its distribution within clinical samples has not been well defined; if sufficient concentrations of HRP2 are present in the plasma in low-density infections, larger volumes of blood could be evaluated by excluding cellular components of sample, increasing assay sensitivity. We measured by ELISA the relative distribution of HRP2 within whole blood and plasma samples from patients with asymptomatic *Plasmodium falciparum* parasitemia as determined by quantitative PCR. Our findings demonstrate that a significant proportion of the distribution of HRP2 in whole blood is found in plasma, and this proportion increases with reducing parasite density, with HRP2 concentrations approaching that of whole blood at parasite densities below 1 parasite/μL. Representative data include: 1) mean HRP2 concentration of 10<sup>1</sup> ng/mL vs. 5<sup>1</sup> ng/mL in whole blood vs. plasma at 1 parasite/μL for a ratio of ~ 2:1 whole blood HRP2: plasma HRP2; and 2) mean HRP2 concentration of 10<sup>2</sup> ng/mL vs. 1 ng/mL in whole blood vs. plasma at 10<sup>4</sup> parasite/μL for a ratio of ~ 100:1 whole blood HRP2: plasma HRP2. This finding offers scope for greatly improving the sensitivity in detecting *P. falciparum* by separating cells and plasma prior to testing, particularly in low-density infections, if the gains accrued through a plasma-only assay can be made to outweigh the loss of the intracellular component of HRP2. The authors are currently developing non-centrifuge-based methods to achieve this.

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### UTILIZATION OF INSECTICIDE-TREATED BED NETS AMONG OVER-FIVES IN LAGOS, NIGERIA

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Insecticide-treated bed nets are an important preventive measure for malaria. Over-fives, although less vulnerable to malaria than under-fives, also need to be protected as they are also at risk of the disease and are an important reservoir for malaria infection. The objective of this study was to assess the utilization of insecticide treated bed nets by over-fives (5-14 years) in a rural community of Lagos, Nigeria. This was a community-based, descriptive, cross-sectional study. A multi-stage sampling technique was used to select the households included in the study. All children living in each selected household were enumerated and for each child, information was elicited on if the child slept under a bed net the previous night. Utilization among over-fives was compared with that of under-fives in the household. The data was analyzed using SPSS version 20 statistical software. A total of 773 children were listed across 242 households giving a mean of 3 children in each household. Eighty-nine (36.8%) households owned at least 1 insecticide treated bed net. Over-fives constituted a sizable proportion (68.7%) of children. A lower proportion (29.6%) of over-fives slept under nets compared to under-fives (32.6%). Over-fives were less likely to sleep under an insecticide treated bed net compared to under-fives. Utilization of insecticide treated bed nets for older children was relatively low among over-fives. This is not completely unexpected as control interventions have been targeted mainly at younger children. Efforts should be made to ensure that this sub-population is covered with the life saving intervention.

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### THE USE OF ANTIBODY MEASUREMENTS TO SUPPORT MALARIA ELIMINATION ACTIVITIES IN HAITI

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Measuring and monitoring malaria is challenging in elimination settings. Antimalarial antibody measurements are a unique metric as they reflect historical and recent exposure to malaria. We describe antimalarial antibody patterns by age group and malaria infection status using multi-antigenic responses in Haiti, a country considered suitable for elimination with low *Plasmodium falciparum* prevalence, parasites sensitive to chloroquine, an inefficient vector and low risk of importation. Data were collected from easy-access-groups across all ages (schools, health facilities and churches) in areas with recent or ongoing transmission in Haiti (n~6500). Participants were tested with malaria highly-sensitive (HS-) rapid diagnostic tests (RDT) and conventional RDTs, and samples were assayed for presence of IgG against twenty *P. falciparum* antigens, including markers of short and long-term exposure, using a bead-based assay. Results from the initial collections in the southern peninsula of Haiti detected fourteen positives by either RDT (3.8%; 14/368). Preliminary results suggest a greater breadth of antibody responses in those with

positive RDTs (median 17, interquartile range 10 - 20) than those with negative RDTs (3, 1 - 7),  $p < 0.001$ . Multiple linear regression showed that the breadth of antibody responses increased with age (0.1 per year,  $p < 0.001$ ) and was higher in males than females (2.4,  $p < 0.001$ ). Our results show the potential of using multi-antigenic responses to describe exposure to *P. falciparum* malaria in an elimination context and may help to determine how to incorporate antibody measurements to guide elimination activities in Haiti.

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### PLASMODIUM FALCIPARUM GAMETOCYTE CARRIAGE BEFORE AND AFTER TREATMENT WITH ARTEMISININ COMBINATION THERAPIES

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Gametocytes play a crucial role in promoting malaria transmission and its surveillance is significant in transmission-blocking interventions. Lack of an approved malaria vaccine leaves antimalarial drugs as one of the best tools towards malaria parasite control. However, with the limited investigation of gametocytocidal properties of antimalarial drugs in the current era, efforts to reduce malaria transmission and elimination could be in jeopardy. Here, we sought to determine *falciparum* gametocyte carriage before and following treatment with different artemisinin-based combination therapies. We analysed a set of 133 isolates in time-points collected from 19 participants in a study to examine *in-vivo* and *in-vitro* efficacy of artemisinin combination therapy in Kisumu County, Western Kenya in 2013. We genetically determined gametocyte carriage using reverse transcription real-time PCR to detect both early and late stage gametocytes through different time-point from Day 0 to Day 42. Subsequently, gametocyte carriage for a subset of the samples will be correlated to parasite clearance rates. Finally, comparison of malaria episodes in our isolates as either recrudescence or re-infection will be done. 63% (12/19) of the participants in this study received Artesunate-mefloquine (ASMQ) whereas 37% (7/19) received Artemisinin-lumefantrine (AL). Prior to treatment, gametocyte carriage was 100% (12/12) and 85.7% (6/7) among participants in the ASMQ and AL arms respectively. Following treatment, gametocyte carriage was significantly lowered by the two arms of treatment at Day 14 (8% (ASMQ) & 0% (AL)). Subsequent time-points reveal an increase in gametocyte carriage which stood at 33% and 28% for ASMQ and AL arms respectively. The increase in gametocyte carriage observed after Day 14 could be attributed to either parasite recrudescence or re-infection which we aim to determine in our subsequent assays. Some antimalarial drugs have been shown to instigate gametocytogenesis but so far this has not been associated with artemisinin derivatives. The results presented here are preliminary pending analysis of more isolates.

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### VULNERABILITY AND ELIMINATION OF MALARIA AND LYMPHATIC FILARIASIS IN THE DOMINICAN REPUBLIC: A NATIONWIDE BATEY SURVEY

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Identifying and reaching vulnerable groups is crucial to malaria and lymphatic filariasis (LF) elimination. The island of Hispaniola, shared by Haiti and the Dominican Republic (DR), is the last malaria-endemic island

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in the Caribbean and accounts for >90% of LF cases in the Americas. In the DR, malaria outbreaks occur in areas historically dependent on migrant labor from Haiti. A legacy of political exclusion and stigma against migrants and their descendants in the DR may exacerbate vulnerability to disease. The goals of this study were to: 1) determine malaria and LF prevalence in *bateyes*, or company towns historically home to migrant laborers; 2) characterize illness experiences, treatment-seeking, and malaria knowledge and intervention practices; and 3) measure stigma and its association with illness and access to care. In 2016, a cross-sectional survey was conducted in three major agricultural regions of the DR. 780 individuals completed household questionnaires and 1418 provided blood samples. No cases (0%) of *Plasmodium* infection were detected by RDT or microscopy. Six (6) individuals (0.4%) were positive for LF antigen. The sample population was composed of three distinct social groups: Haitian-born persons (33.1%), persons of Haitian descent (29.4%), and those without Haitian descent (37.5%). Only 10.5% were migrants (from Haiti or within the DR). Vulnerability trended strongly across social groups: recent fever, forgoing care, limited knowledge, not owning a mosquito net, and stigma were highest among Haitian-born persons, followed by persons of Haitian descent, and lastly by those without Haitian descent. Stigma was not associated with fever or care-seeking after controlling for social group, suggesting that disease-related stigma is less important than stigma due to poverty, origin, or skin color. In this low-transmission setting, social groups that have been historically marginalized remain vulnerable to disease. As elimination efforts try to improve surveillance, access-to-care, and uptake of knowledge and interventions, these findings can contribute to more holistic framings of risk and outreach strategies.

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### HARNESSING DHIS2 AND MHEALTH TOOLS TO STRENGTHEN PRIVATE SECTOR MALARIA SURVEILLANCE IN THE GREATER MEKONG SUBREGION

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Governments in the Greater Mekong Subregion have ambitious targets to eliminate malaria by 2030. While 20% of positive malaria cases in the region are detected through private sector channels, data from the private health care sector are not readily available in national surveillance systems. National systems are set up to store caseload data; they lack functionality to combine program data with epidemiological data to deliver effective intervention monitoring and resource allocation. Since 2016 Population Services International has used DHIS2 as an integrated health management information system for malaria in Cambodia, Laos and Myanmar. DHIS2 and its complementary Android-based mHealth tools have been leveraged to monitor data on caseload, quality of care and stock management at private providers and worksites with the goal of integrating data with national surveillance systems. Data is available to program officers in actionable DHIS2 dashboards. Our experience has produced valuable lessons learned: 1) mobile tools reduce the lag time between data collection and actionable data analysis by 50%, 2) data triangulation on malaria caseload, quality of care and stock management in DHIS2 can be used to prioritize program interventions, and 3) complex DHIS2 configuration and custom coding of mHealth tools highlight the challenges of creating standardized tools for malaria surveillance that can be adapted across countries, and the flexibility of DHIS2 to synthesize data from multiple sources into automated dashboards. Our results provide actionable evidence for transforming surveillance into a core intervention: 1) appropriately designed mHealth tools increase accuracy and timeliness of surveillance data, 2) integration of multiple data sources in a single system like DHIS2 enables triangulation of data for supporting decision making processes and effective resource allocation, and 3) there is a need for standardized and customizable malaria surveillance tools. Learnings

should inform the development of standard system design principles for strengthening national surveillance systems to become fit for malaria elimination.

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#### EXPANDING THE ANTIMALARIAL PIPELINE: THE DISCOVERY OF PYRIMIDINEDIONES, A NEW SERIES TO CURE AND BLOCK MALARIA TRANSMISSION

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In 2010 GSK published the Tres Cantos antimalarial set (TCAMS) which comprises over 13,533 hits derived from whole cell screening of 2M compounds from the GSK corporate collection against *Plasmodium falciparum*. [1] Identification of a new class of anti-malarial agents that possess dual activity and are able to inhibit the asexual blood-stage as well as to block transmission was initiated in our group. [2] Quinazolindione series was identified as a very promising family presenting submicromolar potency against both schizonts and gametocytes, efficacy in an *in vivo* model and a promising physico-chemical profile. Quinazolindiones were found to target *Plasmodium falciparum* ATP4. During lead optimization program a novel related series containing a pyrimidinedione core was found. Medicinal chemistry efforts lead to an advanced molecule with an *in vivo* potency comparable with assets in clinical development, a physico-chemical profile compatible with an oral administration and no cross-resistance with current antimalarials in the market. The strategy to optimize this novel scaffold as well as the profiling of the most advanced compound will be disclosed in this communication.

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#### A CASE FOR INVESTMENT IN THAILAND'S MALARIA ELIMINATION PROGRAM: A COST-BENEFIT ANALYSIS STUDY

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In the past decade Thailand had remarkable success in reducing malaria morbidity and mortality. The country's new National Malaria Elimination Strategy (2017–2026) supports a programmatic shift from malaria control to elimination. To advocate for full programmatic funding for the national malaria elimination operational plan, a cost-benefit analysis was conducted to monetize the value of expected benefits from malaria financing to total investment costs over a 10-year investment period (2017–2026). The overall analysis spanned 20 years (2017–2036) to ensure that projections extend into a post-elimination timeframe, during which the malaria program can fully be integrated into the national health system. Analyses assessed two investment scenarios: the first assumed full financing for all elimination interventions included in the 2017–2026 National Malaria Elimination Strategy, while the second assumed no commitment to fully fund elimination interventions. Programmatic data collected from decades of Thai malaria prevention and control efforts were used to project the impact of differing levels of financing in different sectors for malaria elimination; historical epidemiological trend data were used to model potential worse-case resurgence. The analyses estimated that approximately 3,000,000 malaria cases could be averted if all elimination interventions were supported. The financial support required to acquire and maintain malaria-free status would be substantially lower than the projected amount lost in a no investment scenario. Our analyses also projected that it would cost 2.9 billion Thai Baht to eliminate malaria by 2024, and stay malaria-free for 20 years, whereas a no or

limited investment scenario would ultimately result in a cost of 19.4 billion Thai Baht to the national health system. The economic impact of inadequate funding for malaria would be felt at both micro and macro levels; for example, households would experience a decline in income and productivity, and tourism would be affected due to changes in perceptions of Thailand as an attractive travel destination.

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#### AUTODISSEMINATION OF PYRIPROXYFEN FOR CONTROLLING SELF-SUSTAINING CAPTIVE POPULATION OF ANOPHELES ARABIENSIS

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Autodissemination of pyriproxyfen (PPF), i.e. co-opting adult female malaria vectors to transfer PPF, a synthetic juvenile hormone to their aquatic habitats has proven effective in controlling malaria vectors, *Anopheles arabiensis* under semi-field settings. This approach has potential to amplify treatment coverage of mosquito's habitats that are hard to locate via conventional larviciding. This study demonstrated the impact of PPF intervention in crashing self-sustaining population of *An. arabiensis* under semi-field conditions in rural Tanzania. A stable population of *An. arabiensis* mosquitoes were established inside a semi-field systems from laboratory reared larvae. The larvae were fed on natural occurring food in the 12 habitats installed in each study chamber, and the emerging adults were fed on cattle. Before and after introducing clay pots treated with PPF, population was monitored using emergence traps and supervised HLC. All collected mosquitoes were counted, identified and released back into the chamber. At 38th generations, four treated clay pots with 0.2gAI PPF were introduced in the treatment chamber and its impact on established colonies was monitored in parallel with untreated chamber. In conclusion, the emergence between control and treatment chamber were similar with average catch of 14 and 13 mosquitoes respectively before PPF treatment. The numbers of adult *An. arabiensis* mosquitoes emerging from the aquatic habitats declined gradually reaching zero on week 11 post treatment, which was done only once. Similarly, numbers of mosquitoes attempting to bite HLC volunteers inside treatment chambers also decreased gradually, reaching zero at week 14. The numbers of adults in the control chamber however continued to rise. These results form the basis for designing autodissemination field trial to control wild population of *An.arabiensis*.

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#### SPATIO-TEMPORAL DETERMINANTS OF SUCCESS AND FAILURE OF ANTI-MALARIA INTERVENTIONS IN HIGH ENDEMIC AREAS: A MODEL BASED RE-EXAMINATION OF THE GARKI PROJECT

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While malaria control efforts have greatly reduced its burden over the past two decades in Africa, the Sahel remains one of the most challenging regions for effective control where transmission is mostly confined to the short rainy season. The Garki project provides a comprehensive dataset of the entomology and parasitology of malaria as well as the effect of a combination of intervention techniques on the control of the disease in the Sahel between 1969 and 1975. While vector populations were greatly suppressed, parasitological prevalence rates didn't respond as well to intervention, and even rebounded to higher baseline levels after control

interventions were halted. Incomplete coverage, migration of people and vectors into, out of and within the region, and poorly administered and ineffective intervention techniques are among the most cited reasons for failure. More importantly, a lack of understanding of the spatial dynamics of malaria in the region seems to be one of the strongest underlying themes for failure. Here, we take advantage of the demographic, entomological, and parasitological data from the project, as well as weather and more recent topographical data from the region to construct a spatiotemporal map of vector and human populations within the region to infer migration trends, and to study the spatiotemporal efficacy of interventions that were carried out. With the use of a mathematical model of malarial and intervention dynamics that has been calibrated to match vector and parasitological dynamics observed in the Garki region, the map helps answer more nuanced questions in connected areas on both seasonal and multi-year timescales about permanent vector habitats and vector migration impact effect sizes of vector control interventions, and the magnitude of human mobility on sustaining gains from effective interventions. Additionally, it aids in the selection of intervention combinations and deployment strategies, and highlights potential failure modes and vulnerabilities in elimination operations.

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#### THE IMPACT OF MASS DRUG ADMINISTRATION ON SUBMICROSCOPIC MALARIA INFECTION: A PILOT STUDY ON NGodhe ISLAND IN LAKE VICTORIA, KENYA

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The 2017 WHO framework for malaria elimination calls for all endemic countries to work towards the ultimate goal of elimination, regardless of their current malaria burden. In this context mass drug administration (MDA) has received renewed interest, however evidence supporting its use in moderate to high transmission settings is missing. Our study site, Lake Victoria basin in Kenya, has heterogeneity in malaria prevalence, high in main land and moderate to low on islands, with high percentage of asymptomatic and submicroscopic infection. On Ngodhe, one of the small islands, we conducted two rounds of MDA with a 35-day interval in January-March 2016, before the start of the long rainy season. Pre-intervention parasite rates were 3% by microscopy and 10% by PCR. Artequick (artemisinin and piperazine) and a single low-dose primaquine were administered under direct supervision to cover all islanders (N~500) by house and school visits. Compliance, defined as the proportion of population that completed treatment, was 91% and 87% for rounds 1 and 2, respectively. No serious adverse events were observed, except for a few minor side effects such as dizziness (N=7) and abdominal pain or cramp (N=6). MDA succeeded in suppressing malaria prevalence to 0% by microscopy and to 2% by PCR. However, malaria prevalence rebounded to 9% by PCR two months after MDA. This post-MDA resurgence was largely owed to imported cases (62%), of which 80% were submicroscopic. The beach survey on Ngodhe Island conducted prior to MDA which characterized the arrival on the beach suggested returning residents, such as students and church visitors, as a possible risk group of parasite importation rather than visitors from outside. Our findings indicate that while MDA provided short-term suppression of malaria prevalence to undetectable level by microscopy, measures against imported cases from surrounding endemic areas and local vector mosquitoes are crucial for sustainable malaria elimination. This is the first report showing the effect of MDA on submicroscopic infections and provides detailed information for the future expansion of MDA in moderate to high transmission settings.

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#### EVALUATING THE EFFECTIVENESS AND FEASIBILITY OF REACTIVE TARGETED PARASITE ELIMINATION VS. REACTIVE CASE DETECTION, WITH AND WITHOUT REACTIVE VECTOR CONTROL, AS A COMMUNITY LEVEL INTERVENTION IN RESPONSE TO CONFIRMED, PASSIVELY IDENTIFIED MALARIA CASES IN ZAMBEZI REGION, NAMIBIA: PRELIMINARY RESULTS FROM A CLUSTER RANDOMIZED CONTROLLED TRIAL

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Reactive case detection (RACD), testing and treating individuals around passively detected cases is a strategy commonly used to reduce or interrupt malaria transmission though its effectiveness may be limited by low sensitivity of RDT for low density infections and logistical challenges. Other interventions such as reactive-Targeted Parasite Elimination (TPE), a form of presumptive treatment, or reactive vector control (RAVC) with Actellic CS may be feasible and more effective. We are conducting a cluster randomized controlled trial with 2x2 factorial design to compare TPE vs. RACD, and RAVC vs. no RAVC in the surrounding 500m of index cases. The primary outcome is cumulative malaria incidence. Secondary outcomes include seroprevalence and infection prevalence both to be measured in a post-intervention cross-sectional survey, intervention coverage, safety, acceptability, adherence, and cost-effectiveness. As of April 3rd 30, 30, 26 and 45 index case interventions have been conducted in the TPE, TPE+RAVC, RACD and RACD+RAVC arms respectively. Among TPE intervention events (N=2018), 83% of individuals received the intervention, 5% were ineligible, 11% were missed, and 1% refused. In RACD intervention events (N=2269), 92% of individuals were tested (3% RDT positive), 18% were missed, and 0.3% refused. Reasons for TPE ineligibility include use of contraindicated medication (39%), allergy to the study drug (18%), and age under 6 months old (21%). Eight adverse events have been reported, none was serious. 60 RAVC interventions have been conducted, in which 739 sleeping structures in 446 households were sprayed. This resulted in 87% of households achieving the minimum target of 85% sleeping structures sprayed (N=446). The intervention has been safe and acceptable. Responding to cases during an ongoing regional outbreak has been a challenge. Data collection and analysis on primary and secondary outcomes are in progress and will be shared.

### SHORT-TERM CHANGES IN ANAEMIA AND MALARIA PREVALENCE IN CHILDREN UNDER-FIVE YEARS DURING ONE YEAR OF REPEATED CROSS-SECTIONAL SURVEYS IN RURAL MALAWI

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Pre-school-aged children bear the highest proportion of anaemia, mainly in Africa. In stable transmission areas, malaria accounts for the highest proportion of anaemia. Haematocrit as an indicator of anaemia is easy to measure in the field and has been proposed as an additional and sensitive indicator of changes in malaria prevalence in communities. We report short-term temporal variations of malaria, and anaemia prevalence in children in rural Malawi. We repeated five cross-sectional surveys over one year in rural communities in Chikwawa district, Malawi. Different households were sampled per survey; all children, 6-59 months, in the household were tested for malaria parasitaemia and haemoglobin levels using rapid diagnostic tests (mRDT), and Hemocue 301, respectively. Anthropometric measurements and sociodemographic details were also recorded. Adult mosquitoes sampling, using odour-baited traps, was done in two-thirds of households; ambient temperature and rainfall were recorded using a HOBO® weather station. A total of 894 children were included from 1568 households. Overall, mRDT-positive and anaemia (Hb < 11g/dL) prevalence were 33.8 and 58.7%, respectively. Relative risk of anaemia in mRDT-positive children was 1.45 (95% CI: 1.22, 1.72 p <0.001). Mean haemoglobin was higher in parasitaemic [9.9 g/dl (95% CI: 9.7, 10.1)] than children recently treated for malaria [9.2 g/dl (95% CI: 8.7, 9.7)] p=0.0106. Temporal anaemia and parasite prevalence varied differently. Mean z-scores for weight-for-age, weight-for-height and height-for-age were all less than 0. In conclusion, anaemia prevalence is an unsuitable indicator for short-term changes in malaria prevalence, although malaria is an important factor in anaemia. High burden of undiagnosed malaria, anaemia and malnutrition in rural communities requires integrated control strategies.

### DIFFERENCES IN TREATMENT-SEEKING RATE FOR MALARIA-ATTRIBUTABLE FEVER AND NON-MALARIAL FEBRILE ILLNESS IN AFRICAN CHILDREN

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The rate at which febrile individuals seek care is a critical determinant of health system effectiveness for febrile illnesses including malaria. For malaria, understanding the fraction of cases that seek care is also crucial for correctly interpreting data on reported cases as part of burden estimation processes. Currently, data on care seeking for fever is used as a proxy for malaria, but this ignores that treatment seeking rates are likely to differ for malaria versus non-malaria febrile causes. Here, we use household survey data on fever positivity rate and treatment seeking behaviour in African children with a multinomial modelling approach to generate treatment-seeking rates for malaria-attributable and non-malarial febrile illness. The national estimates of treatment-seeking rate for malaria-attributable fever presented here can be used to generate more accurate predictions of the burden of *Plasmodium falciparum* malaria in Africa.

### THE IMPACT OF URBANIZATION AND POPULATION DENSITY ON CHILDHOOD *PLASMODIUM FALCIPARUM* PARASITE PREVALENCE RATES IN AFRICA

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Although malaria has been traditionally regarded as less of a problem in urban areas compared to neighbouring rural areas, the risk of malaria infection continues to exist in densely populated, urban areas of Africa. Despite the recognition that urbanization influences the epidemiology of malaria, there is little consensus on urbanization relevant for malaria parasite mapping. Previous studies examining the relationship between urbanization and malaria transmission have used products defining urbanization at global/continental scales developed in the early 2000s, that overestimate actual urban extents while the population estimates are over 15 years old and estimated at administrative unit level. This study sought to discriminate an urbanization definition that is most relevant for malaria parasite mapping using individual level malaria infection data obtained from nationally representative household-based surveys. Boosted regression tree (BRT) modelling was used to determine the effect of urbanization on malaria transmission and if this effect varied with urbanization definition. In addition, the most recent high resolution population distribution data was used to determine whether population density had significant effect on malaria parasite prevalence and if so, could population density replace urban classifications in modelling malaria transmission patterns. The risk of malaria infection was shown to decline from rural areas through peri-urban settlements to urban central areas. Population density was found to be an important predictor of malaria risk. The final boosted regression trees (BRT) model with urbanization and population density gave the best model fit compared to the models with urbanization only. Given the challenges in uniformly classifying urban areas across different countries, population density provides a reliable metric to adjust for the patterns of malaria risk in densely populated urban areas. Future malaria risk models can, therefore, be improved by including both population density and urbanization which have both been shown to have significant impact on malaria risk in this study.

### HIGH MALARIA TRANSMISSION INTENSITY IN A REMOTE PERUVIAN AMAZON VILLAGE: THE ACHILLES HEEL OF MALARIA ELIMINATION

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A prospective cohort study was conducted in a remote rural village in the Peruvian Amazon named Santa Emilia. Microscopy and a species-specific qPCR assay were used to assess the presence of parasitemia. Plasmas were

obtained for genome-scale protein microarray analysis using a combined Pf500/Pv500 chip containing a down-selected list of the 500 most seroreactive antigens. Considering qPCR as gold standard, microscopy was able to detect 40.2% and 22% of any Pv and Pf infection, respectively. Considering passive surveillance, microscopy detected 48.1% and 54.5% of the Pv and Pf cases, respectively; while considering active surveillance, microscopy was detected 38.3% and 19.6% of the Pv and Pf cases, respectively. This study was able to detect *Plasmodium* infections through very active surveillance and qPCR that otherwise would go undetected under current surveillance and diagnostic tools. Protein microarray analysis was performed during high and low (September) malaria seasons: 324 samples for protein microarray analysis. The top 200 most seroreactive antigens were selected for comparison. Seroreactivity increased with age, and in response to documented malaria infection, but was lower in September than in March for all age groups. While there was a gradual increase in seroreactivity associated with age, the differential seroreactivity between high and low transmission season was greatest in youngest and this difference decreased with age. Monitoring changes in transmission intensity and identification of malaria foci is mandatory for best intervention efforts. Without better strategies accounting for remote underserved villages, asymptomatic, and submicroscopic infections, and better diagnostic and surveillance tools, it will not be sufficient to decrease the continued malaria transmission due to a high reservoir of infection impeding progress towards attaining a sustainable control and even to progress into elimination of malaria transmission. Elimination efforts will most likely need a more sensitive assay such as a q-PCR or other field deployable molecular based assay.

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#### FINE SCALE MAPPING OF MALARIA INFECTION CLUSTERS BY USING ROUTINELY COLLECTED HEALTH FACILITY DATA IN URBAN DAR ES SALAAM, TANZANIA

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This study investigated whether passively collected routine health facility data can be used for mapping spatial heterogeneities in malaria transmission at the level of local government housing cluster administrative units in Dar es Salaam, Tanzania. From June 2012 to Jan 2013, residential locations of patients tested for malaria at a public health facility were traced based on their local leaders' names and georeferencing the point locations of these leaders' houses. Geographic information systems (GIS) were used to visualize the spatial distribution of malaria infection rates. Spatial scan statistics were deployed to detect spatial clustering of high infection rates. Among 2,407 patients tested for malaria, 46.6% (1,121) could be traced to their 411 different residential housing clusters. One small spatially aggregated cluster of neighborhoods with high prevalence was identified. While the home residence housing cluster leader was unambiguously identified for 73.8% (240/325) of malaria-positive patients, only 42.3% (881/2,082) of those with negative test results were successfully traced. It was concluded that recording simple points of reference during routine health facility visits can be used for mapping malaria infection burden on very fine geographic scales, potentially offering a feasible approach to rational geographic targeting of malaria control interventions. However, in order to tap the full potential of this approach, it would be necessary to optimize patient tracing success and eliminate biases by blinding personnel to test results.

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#### EVALUATION OF MALARIA URBAN RISK BY USING AN IMMUNO EPIDEMIOLOGICAL BIOMARKER OF HUMAN EXPOSURE TO ANOPHELES BITES

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Urban malaria represents an underestimated serious health concern in African countries. This study aims to evaluate the risk of malaria transmission in urban area by evaluating the level of human exposure to *Anopheles* bites using an *Anopheles* salivary biomarker (gSG6-P1 peptide). Two multidisciplinary cross-sectional studies were undertaken in five sites of Bouaké city (three urban districts and two surrounding villages, used as control; Côte d'Ivoire) during the rainy season and the dry season. Blood samples were obtained from children aged 6 months to 14 years-old for immunological tests. The level of anti-gSG6-P1 IgG antibodies was significantly higher in rainy season compared to dry season ( $p < 0.0001$ ) in the urban sites. Interestingly, children with the highest anti-gSG6-P1 IgG responses in rainy season were infected by *P. falciparum*. Surprisingly, no difference of anti-gSG6-P1 IgG level was observed between urban and rural areas, whatever the season. The *Anopheles* salivary biomarker is a suitable tool for accurately assessing the risk of malaria transmission in urban areas. It shows that urban population could be as highly exposed to *Anopheles* bites as children living in rural area. This study highlights, highlighting the high risk of malaria transmission in African urban settings.

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#### PREVALENCE OF MIXED-SPECIES MALARIA INFECTIONS IN UGANDA

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In sub-Saharan Africa malaria is principally caused by *Plasmodium falciparum*, and contributions of other plasmodial species are uncertain. As part of a drug resistance surveillance program, we collected blood samples from 50 children aged 6 months - 10 years presenting with malaria at each of 10 diverse sites around Uganda in 2016. Children presenting with fever were diagnosed by clinic personnel by either Giemsa-stained blood smear (176 subjects) or HRP-2-based rapid diagnostic test (RDT; 323 subjects). To assess infecting species, DNA was extracted from blood spots with Chelex, species-specific primers were used to amplify 18S ribosomal RNA genes, and species were discriminated based on amplicon size using electrophoresis. Of 499 studied samples, 474 (95.0%) demonstrated plasmodial infection. *P. falciparum* was identified in 472 samples, *P. malariae* in 22, *P. ovale* in 15, and *P. vivax* in 4. 435 were pure *P. falciparum*, 2 did not contain *P. falciparum* (one pure *P. malariae*, one *P. malariae/P. vivax*), and the remainder were mixed infections including *P. falciparum*. Non-falciparum species varied geographically, with 0-13 samples demonstrating *P. malariae*, 0-9 demonstrating *P. ovale*, and 0-2 demonstrating *P. vivax* at different sites. No pure non-falciparum infections were identified in RDT-positive samples. Results were complicated by use of two different diagnostic tests, but 41/474 (8.7%) samples with molecular identification of plasmodial infection included non-falciparum infections. Five of 10 study sites underwent regular indoor residual spraying of insecticide (IRS) programs either from 2010-14 or from 2014 to

the present, with consequent decreases in malaria prevalence. Stratifying samples based on history of IRS at collection sites, mixed species or non-falciparum infections were seen in 27/189 (14.8%) samples from IRS sites and 13/285 (4.6%) samples from non-IRS sites ( $p = 0.0013$ ). Our results demonstrate that non-falciparum malaria infection is common in Uganda and they suggest that decreased prevalence of malaria due to IRS may be associated with increased relative prevalence of non-falciparum infections.

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#### TOWARD DECREASING MALARIA TRANSMISSION IN RURAL COMMUNITIES IN MADAGASCAR RESULT FROM A BASELINE SURVEY FOR A CLUSTER RANDOMIZED TRIAL IN MANANJARY DISTRICT

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Community-based interventions may facilitate reducing malaria infection in high-transmission areas. In Madagascar, febrile patients are routinely screened by malaria rapid diagnostic test (RDT) and patients with positive RDTs are treated by artemisin-based combination therapy (ACT). We initiated a cluster-randomized study to compare active malaria infection case detection by RDT and routine case management. We randomized 22 villages (11 per arm) that met the study criteria. During December 2016–February 2017, we conducted a baseline survey in which all study participants were screened by RDT and completed a questionnaire including demographic and fever ( $>37.5^{\circ}\text{C}$ ) information. Following survey completion, community health workers in intervention villages began fortnightly household visits to perform RDT on participants and treat those with a positive RDT using ACT; visits will continue for eight months. The study was designed to detect a decrease in the RDT-positivity from 10% to 5% in the intervention arm. We present the results of the baseline survey. A total of 27,076 participants (95.2% of the eligible population) from 6,340 households were screened: 14,263 (56.2%) and 12,868 (47.4%) in the intervention and control villages, respectively. Participants in the intervention group were older (mean age = 21.1 years CI95% [20.8–21.4] vs 19.5 years CI95% [19.2–19.8];  $p = >10^{-5}$ ) and more likely to be female (55% CI95% [54.1% - 55.7%] vs 53% CI95% [51.7% - 53.5%];  $p = 10^{-3}$ ) than those in the control arm. A total of 2,016 (7.4% CI95% [7.1% - 7.7%]) participants with positive RDTs were treated by ACT; 1141 (8.0%) in intervention and 875 (6.8%) in control villages ( $p < 10^{-3}$ ); 287 (28.5% CI95% [25.7% - 31.3%]) were febrile. There was no difference in RDT positivity among febrile participants ( $n=1005$ ) between intervention and control villages ( $p=0.6$ ). Participation in the baseline survey was nearly 100% suggesting a strong interest among villagers to identify interventions to reduce malaria. Over 85% of RDT-positive participants were afebrile indicating that asymptomatic malaria infection may be an important contributor to parasite transmission.

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#### THE WEEKLY ASSOCIATIONS BETWEEN CLIMATIC FACTORS AND *PLASMODIUM VIVAX* AND *P. FALCIPARUM* MALARIA IN CHINA 2005-2014

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Malaria, a deadly parasitic disease of tropics is transmitted by Anopheles mosquitoes, which is sensitive to the environment. While many environmental factors influence malaria transmission, the role of climatic factors is crucial. However, there are few in-depth epidemiological studies on the effect of short-term variation of climatic factors on transmission of *Plasmodium vivax* and *P. falciparum* malaria in China. **Methods:** We modelled the national malaria data collected between 2005 and 2014 to examine the effect climatic factors, including weekly temperature, rainfall, relative humidity, hours of sunshine, wind speed and atmospheric pressure on *P. vivax* and *P. falciparum* malaria. We combined the distributed Lag Non-linear Model (DLNM) with quasi-Poisson regression. DLNM is a modelling framework which simultaneously describe exposure-response relationship with a potential of non-linearity and delayed effects. The significant effect of temperature first appeared at 2 week lag and consistently increased up to 10 weeks for *P. vivax*, and at a lag of 3-7 week for *P. falciparum*. The effect of relative humidity was significant at a lag of 1-6 week with the highest effect at 3 weeks for *P. vivax*, and at a lag of 5-6 week for *P. falciparum*. Rainfall was significantly associated with *P. vivax* at a lag of 3-7 week, but no association with *P. falciparum* at any lag. The significant effect of hours of sunshine was observed at an early lag of 0-5 weeks for *P. vivax*, and at 1-3 weeks for *P. falciparum*. Wind speed and atmospheric pressure were also associated with *P. vivax* at a lag of 0-7 week, and 5-8 week, respectively. In conclusion, overall climatic factors play a crucial role in transmission of *P. vivax* and *P. falciparum* malaria in China, at different lag periods. The delayed time estimated overlaps with the time required for development of mosquitoes and *Plasmodium*. Health authorities can use this as an evidence base for malaria early-warning system.

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#### SEROSURVEILLANCE TO INFORM MALARIA ELIMINATION PROGRAMS IN SOUTHEAST MYANMAR

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Malaria prevalence in Myanmar has declined dramatically over the past decade. *Plasmodium* cases, however, are still highest in Myanmar compared to other countries within the Greater Mekong region. The presence of artemisinin-resistant *P. falciparum* in the region prompted the WHO and National Malaria Control Programs to strive for malaria elimination by 2030. Accurate surveillance of *Plasmodium* transmission in Myanmar is therefore imperative to achieving this goal. The objective of this study was to explore the use of serosurveillance to identify populations at high risk of infection in Southeast Myanmar, and to longitudinally observe changes in the immune response to *Plasmodium* spp. in a region that has recently achieved a marked decline in malaria transmission ( $> 90\%$  reduction in disease incidence between 2005 - 2014 in some provinces). We performed a longitudinal study of 114 villages across South-East Myanmar from April 2015 to June 2016. Rapid diagnostic tests and ELISAs were performed on  $> 15,000$  participant dried blood

spots to determine current infection and exposure levels. Prevalence of *Plasmodium* spp. infection by RDT was extremely low (<1%), however, serology revealed high levels of IgG specific for *P. falciparum* schizont extract. IgG seroprevalence was greater in high-risk populations (migrant workers (41%) and forest dwellers (50 %) compared to village residents (36%). IgG responses were sustained (>40% seroprevalence) throughout the 14 months of the study, peaking in high malaria transmission season each year despite the extremely low incidence of infection. Through measuring serological responses to malaria, we have revealed that antibodies are being maintained in a pre-elimination setting despite recent large reductions in malaria incidence. Antibodies were also capable of identifying high-risk populations, and the maintenance of high IgG levels over time may be indicative of an undetected parasite reservoir in the region. Combined with molecular diagnosis, serosurveillance could prove to be an important surveillance tool to inform the ambitious target of malaria elimination by 2030 in the Greater Mekong region.

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#### ADAPTIVE GEOSTATISTICAL SAMPLING ENABLES EFFICIENT IDENTIFICATION OF MALARIA HOTSPOTS IN REPEATED CROSS-SECTIONAL SURVEYS IN RURAL MALAWI

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Adaptive geostatistical designs (AGD) allow collection of geostatistical data over time to depend on information obtained from previous information to optimise data collection towards the analysis objective. AGDs offer exciting opportunities as spatial mapping is increasingly used in health programmes, particularly in poor resource settings where uniformly precise mapping may be unrealistically costly but identification of critical areas could help optimise use of available interventions and resources. Two AGD constructions are *singleton* and *batch* sampling. In singleton sampling, locations  $x_i$  are chosen sequentially and at each stage,  $x_{k+1}$  depends on data obtained at locations  $x_1, \dots, x_k$ . In batch sampling, locations are chosen in batches of size  $b > 1$ , allowing a new batch,  $x(kb+1), \dots, x(kb+b)$ , to depend on data obtained at locations  $x_1, \dots, x_{kb}$ . We assessed the efficiency of specific batch AGD relative to their singleton adaptive and non-adaptive counterparts using simulations. We used batch AGD guided sampling design to determine malaria prevalence in children aged 6-59 months in the communities living around Majete Wildlife Reserve in Chikwawa district, Malawi using a rolling cross-sectional survey with five sampling rounds over a 1 year period, and fitted a geostatistical model to predict malaria prevalence in the area. Simulations suggested a significant gain in predictive efficiency using AGD approaches. While batch adaptive sampling proved (necessarily) less efficient statistically than singleton sampling, the suggested loss in efficiency was limited, and is more realistic in most practical settings because of the associated field-work costs. Among 876 recruited children from 1,377 households over a 12-month period, malaria prevalence was associated with age, socio-economic status and ownership of insecticide-treated mosquito nets, as well as additional spatially structured stochastic component. Predictive maps identified *hotspots* defined as a predicted malaria prevalence over 30%. AGD sampling approaches can be implemented successfully in surveys, and support fine scale mapping to guide intervention strategies.

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#### INSECTICIDE-TREATED NETS AND PROTECTION AGAINST INSECTICIDE-RESISTANT MALARIA VECTORS IN WESTERN KENYA

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Insecticide resistance might reduce the efficacy of malaria vector control. In 2013 and 2014, malaria vectors from 50 villages, of varying pyrethroid resistance, in western Kenya were assayed annually for resistance to deltamethrin. Long-lasting insecticide-treated nets (LLIN) were distributed to households at universal coverage. Children were recruited into cohorts, cleared of malaria-causing parasites, and tested fortnightly for reinfection. The infection incidence rates in our 2 cohorts were 2.2 (95% CI 1.9–2.5) infections/person-year and 2.8 (95% CI 2.5–3.0) infections/person-year. LLIN users had lower infection rates than non-LLIN users in both low resistance (rate ratio 0.61, 95% CI 0.42–0.88) and high resistance (rate ratio 0.55, 95% CI 0.35–0.87) villages ( $p = 0.63$ ). The association between insecticide resistance and infection incidence was not significant ( $p = 0.99$ ). Although the incidence of infection was high among net users, LLINs provided significant protection ( $p = 0.01$ ) against infection with malaria parasite regardless of vector insecticide resistance.

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#### INCREASING PREVALENCE OF *PLASMODIUM OVALE* DURING IMPLEMENTATION OF ARTEMISININ COMBINATION THERAPY IN KENYA

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The predominant malaria species in Kenya, *Plasmodium falciparum* (Pf), occurs sympatrically with *P. ovale curtisi* (Poc), *P. ovale wallikeri* (Pow), and *P. malariae* (Pm). Though studies show that diagnosis of non-*falciparum* strains using microscopy or rapid diagnostic kits is inconclusive, accurate information on temporal malaria species prevalence across Kenya, determined using molecular methods is rare. 1294 surveillance study samples collected from five locations across Kenya, between 2013 and 2015, were diagnosed for malaria and *Plasmodium* species composition using polymerase chain reaction (PCR) method. Out of the 1294 samples, there were 80% ( $n=1044$ ) positive for malaria comprising 689 (66%) Pf mono-infections, 38 (4%) single non-*falciparum* species mono-infections while 355 (34%) were multiple species infections. 1% ( $n=6$ ) comprised four species namely Pf, Poc, Pow and Pm while the remainder comprised either two or three species. Pf/Poc was the most prevalent two species infection 64% ( $n=228$ ) followed by Pf/Pm 8% ( $n=27$ ) whereas Pf/Pow was at 4% ( $n=16$ ). Notably, the prevalence of non-*falciparum* malaria rose to 53% from 33% with Pf/Poc increasing by two-fold to 22% in 2015 from 13% in 2013. The study reveals significant high burden of the non-

*falciparum* parasite species than previously reported in periodic malaria indicator surveys. Similar studies need to be undertaken in other regions of Africa, across longer periods in order to provide evidence to improve intervention strategies.

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#### BIOMARKER DEVELOPMENT AND PRIORITIZATION OF GLOBALLY SUITABLE *PLASMODIUM FALCIPARUM* MEROZOITE-STAGE VACCINE CANDIDATE ANTIGENS

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*Plasmodium* species antigens accessible at the time of merozoite release are likely targets of biologically functional antibodies. Since, most of the immune-epidemiological correlates of protection against leading malaria vaccine candidates have been developed in African settings, it is important for these parameters to be tested in other malaria endemic settings in order to assess the global suitability of these vaccine candidates. Naturally acquired Immunoglobulin G (IgG) antibodies against fifteen *Plasmodium falciparum* merozoite-stage vaccine candidate antigens were analyzed in the plasma of Indian residents from a longitudinal cohort. The same antibodies were quantified and their functionality was assessed using flow cytometry-based immunofluorescence and opsonic phagocytosis of merozoites (OP) assays, respectively. Antibody levels against each antigen were significantly associated with protection against clinical malaria in a univariate analysis. However, only merozoite surface protein 1 (MSP-1), glutamate-rich protein (GLURP) and Pf12 antibody responses remained significantly associated with protective immunity, when all antibody measures together with age (confounder) were fitted in a single multivariate model. Opsonic phagocytosis mechanism previously shown to be protective in Africa showed a similar effect (hazard ratio [HR] = 0.36; 95% confidence interval [CI] = .19–.70; P = .002) in this study. Of the antimerozoite-specific antibodies, only IgG3 was significantly associated with both OP and protection (HR = 0.50; 95% CI = .27–.91; P = .02). These findings support previous studies in Africa that found OP of merozoites and IgG3 to be associated with protection against malaria and further shows MSP1, GLURP and Pf12 are strong vaccine candidates, thus giving further impetus for the prioritization and development of globally successful vaccines.

#### 343 BURDEN OF ASYMPTOMATIC MALARIA IN CHILDREN 2-17 YEARS FROM MALARIA ENDEMIC REGIONS OF KENYA

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In the last decade, preschool and school age children have been identified among the groups at the highest risk of asymptomatic malaria irrespective of the transmission setting. Persistence of parasitaemia in this age group negatively impacts on their health and school performance. We estimated the burden of asymptomatic malaria in children aged 2-17 years residing in four sites located in the two malaria endemic regions of Kenya (western and coastal Kenya). The four sites comprised a rural and urban site each

in coast and western Kenya. Four cross-sectional surveys were conducted during a 24 month period (2014-2016). The average interval between the initial survey and the first follow up, the first follow up and second follow up, and the second and third follow up was 8.8, 7.3, and 5.9 months, respectively. A total of 3446 apparently healthy children were recruited in the study. Parasitaemia presence was determined by microscopy, and all parasitized children were treated with artemether-lumefantrine. On average the prevalence of asymptomatic malaria was 18.5% and

ranged between 14% and 19% during the four cross-sectional surveys. Asymptomatic malaria was significantly higher among boys (P=<0.01), in the western region (P=<0.0001), and in the rural sites (P=<0.0001). Of the 1391 children who participated in all four surveys, 41% (572) were parasitized at least once: 14 had asymptomatic malaria during all four surveys, 77 during three surveys and 170 during two surveys. In the children who participated in all four surveys, asymptomatic malaria increased with increasing age from 15.8% during the initial survey to 22.1% during the fourth survey. These results show the burden of asymptomatic malaria in Kenyan children is substantial; therefore, as malaria control programs move towards elimination, both symptomatic and asymptomatic cases must be targeted.

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#### A FRAMEWORK FOR MALARIA SURVEILLANCE IN TANZANIA

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Tanzania is entering a new era of malaria control, with a realistic possibility of reducing malaria prevalence to less than 1% by 2020. As prevalence across the country continues to decline and heterogeneity in transmission increases, a timely and accurate surveillance system will become increasingly important in the transition from control to potential elimination. A suitable malaria surveillance-response system must include key indicators relating to the disease that can be used to trigger additional investigations and direct targeted response action. Due to the changing epidemiology, particularly the increasing heterogeneity of malaria, the surveillance approaches of the past may no longer be adequate. The complex context of malaria transmission which includes the parasite, the vector, the human host, as well as multiple factors related to the environment and health system context, requires a systematic and operationally feasible surveillance approach. For this purpose, an innovative malaria surveillance framework proposed for Tanzania will be presented. It contains three major elements: (i) disease surveillance, (ii) programmatic surveillance, and (iii) transmission surveillance. Data will be collected through several complementary approaches, such as: passive routine disease reporting, routine programmatic data reporting, parasitological and entomological surveys, and climatic surveillance. This surveillance framework is based on three key pillars and is strictly linked to targeted response action. Each element of the framework will generate an alert if an abnormal situation or disruption of equilibrium occurs. Malaria disease surveillance, one of the major elements of the framework, can indicate possible outbreaks. In such cases, a full investigation is initiated, and appropriate responses are implemented. The system will explore the role of various drivers of trends in malaria incidence. It will offer useful information to guide decision-makers in outbreak detection, risk assessment, investments for malaria control and intervention choice.

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#### SUBMICROSCOPIC MALARIA INFECTIONS IN PREGNANT WOMEN FROM SIX DEPARTMENTS IN HAITI

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Hispaniola is the last island in the Caribbean where malaria transmission remains endemic, with the majority of infections occurring in Haiti. Though pregnant women are a high-risk group for malaria infections, surveillance or intervention programs targeting pregnant women in Haiti are rudimentary at best. Thus, data on malaria in pregnancy (MIP) in Haiti are sparse and risk factors for infection have not previously been

investigated. A cross-sectional study was conducted among pregnant women in six departments of Haiti. After obtaining informed consent, whole blood samples and demographic surveys were collected to investigate malaria prevalence, anemia and socio-behavioral risk factors for infection, respectively. A total of 311 pregnant women were screened for *Plasmodium falciparum* infection using a rapid diagnostic test (RDT), microscopy, and a novel, quantitative reverse-transcriptase polymerase chain reaction methodology (qRT-PCR). Overall, 1.2% (4/311) of pregnant women were positive for malaria infection by both microscopy and RDT. However, using the qRT-PCR, 16.4% (51/311) of pregnant women were positive. The prevalence of malaria infection varied with geographic locations ranging between 0% to 46.4%. Additionally, 53% of pregnant women had some form of anemia; however no significant association was found between anemia and submicroscopic malaria infection. The socio-behavioral risk factors identified to be protective of malaria infection were marital status ( $P < 0.05$ ) and travel within one month prior to screening ( $P < 0.05$ ). In conclusion, this study is the first to document the high prevalence of submicroscopic malaria infections among pregnant women in Haiti and identify social and behavioral risk factors for disease transmission.

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#### FREQUENCIES OF *PLASMODIUM FALCIPARUM* GENE MUTATIONS IN ASYMPTOMATIC INFECTIONS: EVALUATING MALARIA TRANSMISSION REDUCTION IN AN ENDEMIC AREA

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Malaria transmission in endemic areas remains high due to a circulating reservoir of asymptomatic parasites which are usually undetected or untreated. Determining inherent traits in asymptomatic parasite isolates transmitted from one infection to another accounts for parasite susceptibilities to drugs when infection is treated. Artemisinin-based combination treatments have proved to be effective in clearing resistant parasites but only from treated infections. This study determined malaria transmission rates in asymptomatic individuals and frequencies of multidrug resistance 1 gene mutation in *Plasmodium falciparum* (*Pfmdr1*) isolates from a pool of asymptomatic and symptomatic infections from two communities in southwest Nigeria, using RFLP-PCR specific for mutation detection at codon 86 and 1246. Of the 165 healthy children and adults evaluated, 39 (23.6%) had asymptomatic parasitemia and similar proportion of isolates harbored the mutant 86Y and 1246Y alleles compared to isolates from symptomatic infections with higher parasitemia. Strategies to reduce transmission of infection from asymptomatic carriers is limited in endemic areas due to drug resistance, because most parasites harbor mutant genes. Other strategies to reduce asymptomatic infection are needed such as mass drug administration should be advocated to eliminate the reservoir of malaria infection.

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#### SEROLOGICALLY DEFINED HETEROGENEITY IN *PLASMODIUM VIVAX* MALARIA TRANSMISSION USING A NOVEL CHIMERIC RECOMBINANT PROTEIN IN RIVERINE VILLAGES OF THE PERUVIAN AMAZON

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Serological tools for stratifying malaria risk are needed to guide and monitor control efforts. Here, we determined the antibody levels and seroconversion rates to a *Plasmodium vivax* novel chimeric recombinant protein in individuals living in riverine villages of Mazan district in the Peruvian Amazon, and related those serological measures with malaria incidence rates. Population-based surveys were conducted in June 2015, collecting dried blood spot samples from participants in Gamitanacocha (GC), Libertad (LI) and Primero Enero (PE) (villages along Mazan River), and Urco Miraño (UM) (Napo River). ELISA was used to detect IgG responses to a chimeric protein containing the Apical Membrane Antigen 1 (AMA-1) and the Merozoite Surface Protein 1-19 (MSP1-19), expressed in a *Pichia pastoris* culture system. Age-specific seroprevalence (sPrev) was analysed using a reversible catalytic model that estimated seroconversion rates (SCR,  $\lambda$ ). *P. vivax* incidence rates (IR) were obtained from the malaria surveillance data (i.e. passive and active case detection). Due to the proximity between LI and PE, joint sPrev, SCR, and IR estimates were calculated for these villages. Local Moran's I with an empirical Bayesian rate method was used to detect spatial clusters of serology-positive individuals. A total 812 individuals participated in surveys (87.0% of census population): GC (90 individuals; 89.1% of population), LI-PE (391; 83.5%), UM (331; 90.9%). sPrev and SCR by village were: GC (sPrev= 26.7%;  $\lambda = 0.038$  [95%CI: 0.022-0.057]), LI-PE (38.9%; 0.066 [95%CI: 0.054, 0.080]), and UM (30.8%; 0.048 [95%CI: 0.038-0.060]). SCR estimations strongly correlated with *cP. vivax* malaria incidence rates in 2015 ( $r = 0.99$ ,  $p = 0.02$ ): GC (IR=0.346 confirmed cases/person/year), LI-PE (0.423), UM (0.371). The spatial analysis identified well-defined clusters of *P. vivax* seropositive individuals in all villages. In conclusion, antibody responses to the chimeric protein PvAMA1-PvMSP1-19 has efficiently and accurately characterized the heterogeneity in *P. vivax* malaria transmission at micro-geographical level in the study area.

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#### ASSOCIATION OF MALARIA AND ANEMIA WITH MALNUTRITION IN CHILDREN FOLLOWING A SEASONAL MALARIA CHEMOPREVENTION PROGRAM IN A RURAL AREA OF BURKINA FASO

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The burden of undernutrition and malaria or undernutrition and anemia co-morbidities is unwell known in West Africa. A pre-implementation study on seasonal malaria chemoprevention (SMC) plus azithromycin in Burkina Faso, provided suitable circumstances to deepen knowledge on the association between malnutrition and both malaria and anemia

in under five year old children. Three consecutive annual cross-sectional studies were conducted in a rural area in the health district of Houunde a month after the end of SMC drugs administration from 2014 to 2016. At each survey anthropometric measurements (weight, height) were realized. Clinical malaria, diagnosed using RDTs and blood smears, and hemoglobin concentration were recorded from 2000 randomly selected children. Data were pooled and malnutrition status were determined and classified as stunting (HAZ<-2), underweight (WAZ<-2) and wasting (WHZ<-2), parameters which were also categorized into moderate (-3< Z score < -2) and severe (Z score <-3). Bivariate analysis and multivariate logistic regression were used to estimate the relationship between undernutrition and clinical malaria as well as anemia. The overall prevalence of undernutrition remained high (60.39%). Underweight was the most prevalent type of undernutrition (52.44%) representing 31.67% of the study population. The prevalence of clinical malaria and moderate anemia were low among children, 4.6% and 3.0% respectively. There was no significant association between undernutrition overall and clinical malaria ( $p>0.05$ ). In addition, there was no evidence of association between severe and moderate aspects of undernutrition and clinical malaria ( $p>0.05$ ). Severely underweighted and moderately stunted children were more at risk of moderate anemia  $aOR=2.85$  (95%CI (1.79; 4.54),  $p<0.001$  and  $aOR=3.37$  (95% CI (2.30; 4.95),  $p<0.001$ . After three consecutive annual SMC drugs administrations, clinical malaria and moderate anemia were rare. No association was found between clinical malaria and undernutrition. Moderate anemia was associated with severe underweight and moderate stunting.

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#### SPANNING THE ELIMINATION SPECTRUM: EVALUATING THE MALARIA SURVEILLANCE SYSTEM IN MOZAMBIQUE

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Malaria transmission throughout Mozambique is extremely variable, with prevalence ranging as high as 68% in the north, to below 3% in the south. As a result, the country is simultaneously pursuing elimination in the south of the country and addressing high burden areas in the north. Reorienting the routine health information system (HIS) for malaria such that it addresses the needs of the different transmission levels is a challenge, yet one that is vital to eventually achieving elimination. In order to help the country tailor their system to the unique epidemiology of the country, an assessment of the passive surveillance systems in the country was conducted. This assessment consisted of both qualitative and quantitative methods involving documentation review, key stakeholder interviews from the central to the community level, and data analysis to evaluate the performance of the system including key indicators such as timeliness and completeness of the system. Although the study found high acceptability at health facilities in the country, a number of weaknesses were also identified. Specifically, a lack of standardized reporting tools was found at the health facility and district levels, along with poor integration of other malaria indicators into the HIS. Availability and timeliness were also low, due to poor internet connectivity and long delays (> 3 months) in reporting, respectively. Data quality issues, including accuracy and completeness, were also found to be major gaps, as was poor data use and the lack of a case-based system to support elimination efforts. In response to this assessment, it was recommended that the country ensure the use of a standardized set of collection tools and that the availability of these tools is guaranteed, and that the IT infrastructure and data management capacity of districts and provinces be strengthened to improve data quality. Finally, in order to support ongoing elimination efforts in the country, it was recommended that the program

create a case-based surveillance system with timely case notification, investigation and classification, active case detection when required, and foci investigation.

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#### WHAT PROPORTION OF *PLASMODIUM FALCIPARUM* AND *P. VIVAX* MALARIA INFECTIONS ARE IN MOSQUITOES?

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To design effective malaria intervention strategies, it would be beneficial to know where the infections are before and after implementation. The proportion of infections that are in mosquitoes is generally not known. In some sites, some component measures are available such as the number of infections detected in humans using high-resolution genotyping or the number of infected host-seeking mosquitoes per person. However, frequently all the component measures together are not available, are restricted to younger age-groups or suffer from low detectability. We use simulation models to predict the proportion of *Plasmodium falciparum* and *P. vivax* infections that are in mosquitoes. We use OpenMalaria, an established comprehensive simulator of *P. falciparum* malaria epidemiology and control which has been extensively fitted to data, and a recent simulation model of *P. vivax*, validating where possible. We predict the proportions by transmission intensity, seasonality, mosquito species, and after the introduction of long-lasting insecticidal nets (LLIN) and mass drug administration (MDA). The predicted proportion of *P. falciparum* infections that are in mosquitoes is approximately 60% for a setting with 200 infectious bites per person per year (EIR) and decreases to a floor of roughly 15% when the EIR is lower than 10. A similar pattern is predicted for *P. vivax*, but the floor is slightly lower since humans harbour a higher number of infections. The patterns stem from saturation in the number of infections in humans at high transmission intensities, chiefly driven by the relationship between EIR and the force of infection. A mass campaign with LLIN covering 80% of the population decreases the proportion of infections in mosquitoes with the proportion slowly recovering. For *P. falciparum*, MDA with a blood-stage drug effective for one month and 80% coverage resulted in a sharp spike in the proportion of infections in mosquitoes in the following five-day time-step. For *P. vivax*, MDA with liver-stage or both blood- and liver-stage drugs produced a sharp spike. Predictions of where the infections are may inform the timing and combination of interventions.

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#### TEMPORAL TRENDS OF PARASITEMIA IN UNCOMPLICATED *FALCIPARUM* INFECTIONS IN KENYA DURING THE PERIOD OF ARTEMISININ COMBINATION THERAPY USE IN 2008 TO 2016

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*Plasmodium falciparum* parasitemia correlates with clinical features and prognosis, aiding clinical decision-making depending on malaria endemicity. World health organization identifies high parasitemia as an indicator of severe malaria infection. However there is little information about periodical malaria parasitemia in symptomatic individuals with uncomplicated malaria during the deployment of artemisinin combination therapy (ACTs) in Kenya. This study determined parasitemia in uncomplicated *falciparum* infections in Kenya during the era of ACTs. Malaria parasitemia prevalence in 6 different demographical regions in Kenya was assessed between 2008 and 2016 using microscopy and polymerase chain reaction (PCR). A total of 3738 samples were analyzed consisting of Kisumu site (1,697), Kombewa (569), Kisii (989), Kericho (322), Marigat (51) and Malindi (110). Overall median parasitemia was 1.55 interquartile ranges (IQR) 0.25 to 3.62, 95% confidence interval).

Median parasitemia was lowest in 2013 with median of 1.13,  $n=550$  (IQR 0.13 to 3.10) and highest in 2016 2.2,  $n=243$  (IQR 0.25 to 4.5). Parasitemia was lowest at Marigat and highest at Kombewa and Kisii. Parasitemia fluctuated during the entire period of study. Kisumu site had varied median 1.35  $n=258$  (IQR 0.18 to 3.03) and 3  $n=81$  (IQR 1.35 to 5.15) as the lowest and highest median for the year 2013 and 2016 respectively. Kericho district hospital had steady increase in median parasitemia 0.30  $n=59$  (IQR 0.1 to 2.20) year 2008 to 1.9  $n=19$  (IQR 0.90 to 5.75) in 2011 thereafter a decline in median parasitemia. PCR results are underway. These findings show parasitemia among symptomatic individuals with uncomplicated malaria during deployment of ACTs. Despite significant regional variation in parasite density, there were no periodical changes to imply the effect of ACT usage during the study period. Since ACTs are still effective in Kenya, it is useful to continue monitoring changes in parasitemia to assess if it would presage resistance.

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#### MALARIA SURVEILLANCE DATA ANALYSIS IN SOUTHERN ETHIOPIA

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Ongoing analysis of surveillance data is important for detecting outbreaks, monitoring disease trends, evaluating the effectiveness of disease control programs and policies and to determine efficient allocation of public health resources. The purpose of this analysis is to assess three (2011-2013) years of malaria morbidity and mortality data collected through PHEM and HMIS sections of Halaba Woreda. We conducted descriptive cross-sectional study using Microsoft Excel to describe the occurrences and distributions of malaria cases by person, place and time. A total of 123,344 (22.4%) malaria cases were reported by PHEM and HMIS. Proportion of falciparum cases reported to PHEM and HMIS were 48.2% and 43.4%; *P. vivax* was 51.8% and 56.6% respectively. The average total malaria incidence was 247 cases per 1000 person. The average annual incidence of total malaria was 390 and 247 per 1000 persons per year in PHEM and HMIS data respectively. Total malaria cases declined by 11.1% in all ages and by 10.1% in <5 year children over the three years. However, admissions increased by 301 in <5 children and by 1788 in all ages of malaria inpatient cases. The average mortality per 100,000 persons during the study period was 2.8. Overall malaria positivity rate was 55.6% from parasitological tests received. Eighty-three percent of cases were reported from health centres. In conclusion, malaria morbidity is declining but admissions are increasing. Also, a shift in the proportion of plasmodium species was observed. Increase in malaria admissions could be due to the increasing number of new health facility. Due to the discrepancies in PHEM and HMIS data, data quality is in question which should be evaluated and action be taken.

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#### FACTORS ASSOCIATED WITH SEEKING TREATMENT FOR FEBRILE CHILDREN IN HEALTH CENTERS IN MALI

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Fever in children is a common reason for outpatient visits to health centers. Malaria is the main cause evoked in Mali. According to Mali's 2015 Malaria Indicator Survey (MIS), among children less than five years of age who had fever the two weeks before the survey only 32.4% sought medical care at health facilities. The goal of this study was to analyze factors associated with care seeking for children with fever using data from the 2015 Mali MIS. A total of 2055 children who had fever in the two weeks preceding the survey were included in a multiple logistic regression analysis. After adjusting for urban/rural residence, region and mother's age, mother's educational level and household wealth quintile were significantly

associated with care seeking for febrile children. Care was less likely to be sought for children whose mothers had no educational level compared to children whose mothers had secondary or higher level of education (AOR=0.68; 95% CI [0.48-0.97]). Children living in households in the middle wealth quintile or higher were more likely to seek care for fever than those in the lower wealth quintiles (AOR=0.61; 95% CI [0.45-0.84]; AOR=0.60; 95% CI [0.42-0.84]). Mother's age, region and urban/rural residence were not significantly associated with care seeking for fever in adjusted models. However seeking traditional healers was associated with non-seek to medical treatment in health centers when children has fever (AOR=19.75 95% CI [7.78-50.13]). But a significant association was not observed with the age category and place residence to seek medical care for febrile children. The results suggest that more educated mothers and households with middle or higher wealth index were likely to seek care for their febrile children. The seeking traditional healers were associated with non-seek to medical care in health centers in Mali.

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#### MALARIA AND ANEMIA AMONG PREGNANT WOMEN IN MALI, WEST AFRICA

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Malaria and anemia in pregnancy remains a major health problem in Sub-Saharan Africa. To investigate the effect of increased control efforts on pregnancy-associated malaria, we carried out this study over three consecutive annual transmission seasons in Niore du Sahel, located in a Sahelian area in the northwest of Mali. Our goal was to determine the prevalence of malaria and anemia in pregnant women and assess the coverage rate of both intermittent preventive treatments using sulphadoxine-pyrimethamin and insecticide-treated bednets (ITN) among pregnant women A cross-sectional study at the Reference Health Centre of Niore-du-Sahel was conducted to recruit 478 pregnant women during antenatal visit. Capillary blood was collected for malaria parasitemia using microscopy and for hemoglobin levels using HemoCue 301+. Malaria infection and anemia proportion (Hb<11g/dL) was 13.8% and 49.6%, respectively. Self-reported ITN use rate was 80.3% while IPTp – SP all 3 doses proportion was 55.4%. The prevalence of malaria (17.7%;  $P=0.548$ ) and anemia (68.9%;  $P=0.009$ ) was higher among age group 14 – 17 years compared to >27 years. Anemia proportion was higher in primigravid (59.6%) compared to multigravid women (47.1%;  $P=0.05$ ). Malaria was also more prevalent during the third trimester (18.3%) comparing to the first trimester (11%;  $P=0.071$ ). Anemia increased from the first trimester (29.9%) to the third trimester (62.5%;  $P<0.05$ ). Malaria was higher in pregnant women not using any preventive measure (28.8%; OR = 6.85 [2.78 – 16.86];  $P<0.0001$ ), followed by those using ITN alone (23.9%; OR = 5.31 [2.32 – 12.11];  $P<0.0001$ ), and IPTp – SP alone (16.7%; OR = 3.37 [0.82 – 13.83];  $P=0.074$ ) compared to those using ITN and IPTp – SP (5.6%). In conclusion, malaria and anemia are still common in young primigravid women and most prevalent during the third trimester. Additional control interventions are needed for malaria elimination in Niore du Sahel.

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#### NATURALLY ACQUIRED ANTIBODY RESPONSE TO PLASMODIUM FALCIPARUM DESCRIBES HETEROGENEITY IN TRANSMISSION ON ISLANDS IN LAKE VICTORIA

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As markers of exposure anti-malaria antibody responses can help characterise heterogeneity in malaria transmission. In the present study antibody responses to *Plasmodium falciparum* AMA-1, MSP-119 and CSP were measured to describe transmission patterns and heterogeneity in meso-endemic settings in Lake Victoria. Two cross-sectional surveys were conducted in Lake Victoria from January to August 2012. The study area comprised of three settings: mainland (Ungoye), large island (Mfangano) and small islands (Takawiri, Kibuogi, and Ngodhe). Individuals provided a finger-blood sample to assess malaria infection by microscopy and PCR. Antibody response to *P. falciparum* was determined in 4,112 individuals by ELISA using eluted dried blood from filter paper. The overall seroprevalence was 64.0% for AMA-1, 39.5% for MSP-119, and 12.9% for CSP. Within settings, seroprevalences for merozoite antigens were similar between Ungoye and Mfangano (AMA-1; 68.6% vs. 66.3%, MSP-119; 43.4% vs. 45.4%), but higher when compared to the small islands ( $P < 0.01$  for all comparison). The overall serological outcomes generally increase with age ( $P < 0.001$ ). For AMA-1 antigen, the seroconversion rates (SCRs) ranged from 0.121 (Ngodhe) to 0.202 (Ungoye), and are strongly correlated to parasite prevalence. No evidence was found of an increase of antibody levels with parasite densities and sub-microscopic parasite carriage in our study areas. Enlarged spleen was independently associated with antibody levels to all the antigens tested in children  $\leq 12$  years old in all study areas. We observed heterogeneity of parasite prevalence and serological indices across study sites in Lake Victoria. These data suggest that AMA-1 and MSP-119 sero-epidemiological analysis may provide further evidence in assessing variation in malaria exposure and evaluating malaria control/elimination efforts in high endemic area.

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#### SPATIOTEMPORAL EPIDEMIOLOGY OF MALARIA IN MYANMAR 2012-2015

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Malaria in Myanmar has greatly reduced by 53% from 2012 (391,679 confirmed cases) to 2015 (182,616 confirmed cases) but numbers remain much higher than in other countries in the Greater Mekong Subregion. Reducing the disease burden has been a constant challenge in populations in hard to reach areas and among migrant populations and seasonal workers travelling across the country. The National Strategic Plan for malaria elimination in Myanmar 2016-2030 states that the country aims to eliminate Malaria in three phases. Elimination by 2020 in areas with multidrug resistance including artemisinin resistance, reduction of overall incidence by 60% and mortality by 75% from 2014 values by 2020 and elimination from all areas by 2025 with prevention of reintroduction where it has been eliminated. To support achieving these goals it is essential to have an accurate picture of malaria incidence over space and time. Malaria Surveillance data were obtained from the National Malaria Control Programme, Department of Public Health, Myanmar from 2012 to 2015. A detailed analysis of routine incidence data over space and time, including annual parasite index, annual examination rates and positivity rates was done to examine the spatial and temporal trends in malaria cases in Myanmar. Township level analyses on numbers of confirmed cases were produced and analysis were performed on spatial temporal variations, seasonality and correlated with non-disease variables such as

temperature, rainfall and humidity. Testing rates increased in 2015 with annual blood examination rates from 2.1 in 2012 to 5.1 in 2015. In 2015 more tests were done in vulnerable populations and as a result more than 30,000 additional positive cases were identified than in 2014. Several high risks areas were identified including in Rakhine State where 100% of the population are at risk, with 90% in Kayin and 89% in Kachin States. The peak season of positive cases was found to be in June and July during the wet season. Myanmar has made great progress towards the goal of malaria elimination and ongoing improvements in surveillance and increased testing will greatly assist the NMCP in achieving this.

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#### INCREASE IN PFDHFR AND PFDHPS MUTATIONS AFTER DISCONTINUATION OF COTRIMOXAZOLE PROPHYLAXIS FROM HIV-1 INFECTED INDIVIDUALS IN MALARIA ENDEMIC AREAS

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The WHO recommends cotrimoxazole (CTX) prophylaxis for HIV-1 infected individuals in regions with high prevalence of infectious diseases. However, with scale-up of antiretroviral therapy (ART), the usefulness of CTX is not well defined especially since there is debate that its usage might increase risk of developing cross-resistance to closely related sulfadoxine-pyrimethamine (SP) that is recommended for Intermittent preventive treatment in pregnancy/infants (IPTP/i). We conducted a non-blinded non-inferiority randomized controlled trial in Homabay, western Kenya to assess the effect of stopping CTX prophylaxis among HIV-1 infected adults. The subjects had to be on ART for >18 months with CD4 >350 cells/mm<sup>3</sup>. 500 subjects were enrolled; 250 in STOP-CTX arm and 250 in CTX arm. Blood samples were collected at time points 0, 3, 6, 9 and 12 months. Prevalence of mutations associated with SP-resistance was determined in the STOP-CTX arm at 3.2% (85/2625) and the CTX arm at 0.6% (16/2625) in the malaria positive cases. The triple mutant haplotype (*pfdhfr* 511/59R/108N) was the most prevalent at 52.9% (n = 45/85) in the STOP CTX arm and 6.3% (n = 1/16) in the CTX arm whereas the double mutant (*pfdhps* 437G/540E) was the most prevalent at 57.6% (n = 49/85) in the STOP CTX arm and 25% (n = 4/16) in the CTX arm. The prevalence of quintuple haplotype (511/59R/108N/437G/540E) was 51.8% (n = 44/85) in the STOP-CTX arm and 6.3% (n = 1/16) in the CTX arm. The triple mutant haplotype (511/59R/108N) increased from 14.3% (1/7) in M0 to 55.6% (20/36) in M12 in the STOP-CTX arm. Similarly, there was a two-fold increase in prevalence of double mutant haplotype (437G/540E) in *pfdhps* gene from 28.6% (2/7) to 69.4% (25/36) in M0 and M12 respectively. These findings suggest that cotrimoxazole is protective against malaria infections among HIV-infected individuals and does not select for SP-resistant parasites. In the context of malaria elimination, a decision to use cotrimoxazole for mass drug administration could reduce malaria carriage and therefore lower transmission without jeopardizing IPTP/i.

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#### PFHRP2 GENE MUTATION IN BANGLADESH

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More than 90% of symptomatic malaria cases in Bangladesh are caused by *Plasmodium falciparum*. Species-specific diagnoses are crucial to case management, because severity and treatment varies between species. Diagnoses can be made using rapid diagnostic tests (RDTs) which are used in field sites to rapidly detect the presence and type of malaria. *P. falciparum* causes the most virulent cases of malaria, and *P. falciparum*

histidine-rich protein 2 (PfHRP2) is a common target of falciparum malaria RDTs. Here we report a case in which a blood sample from malaria patient in Bangladesh which tested negative on PfHRP2-based RDTs. Three different brands of RDTs were used to reconfirm failure of diagnosis: InTec® Pan/Pf Combo and Pf/Pv Combo (InTec® Products Inc., Xiamen, China), SD Bioline Pf/Pv Combo (Standard Diagnostics, Yongin, Republic of Korea), and Falcivax Pv/Pf Combo (Falcivax; Zephyr Biomedicals, India). The sample was confirmed to be a *Plasmodium falciparum* infection by PCR and microscopy. A parasitemia of 5120 parasites/μl was counted in a thin blood film, corresponding to 0.1024% of erythrocytes infected. Low parasitemia resulting in false negative result was ruled out because the parasitemia was 50 times of that normally required for RDTs. The negative results is attributed to a deletion of part of the *Pfhrp2* gene. The exon1/intron1 part of gene was found to be deleted and the initial portion of exon 2 was found to be deleted by sequencing. The missing regulatory upstream region accounts for the negative results on PfHRP2-based RDTs. This is a first report of *hrp2* gene deletion in Bangladesh. This finding may have implications for malaria diagnostics and case management in Bangladesh and other regions of South Asia.

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#### GENETIC VARIATIONS IN *PLASMODIUM FALCIPARUM* APICAL MEROZOITE PROTEIN PF34 FROM CENTRAL INDIA

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The genetic diversity and evolutionary plasticity are the major obstacle for malaria control. Due to diversity in the *Plasmodium* genes, new forms of drug resistance have emerged and it is also a major challenge to malaria vaccine development. There are challenges for development of a new biomarker for rapid diagnostic test (RDT) as deletion in already existing target for RDT, histidine rich protein (HRP2), has been reported. In this study genetic diversity of *P. falciparum* GPI anchored protein (Pf34) was assessed. Previous studies have shown that Pf34 is expressed later during erythrocytic stage as well as sporozoite stage of parasite and localized in the rhoptry neck of merozoite apex. It is encoded by a single exon gene (978 bp) located on chromosome 4 and having a molecular weight of ~34 kDa. Rhoptry proteins, that play important role in the invasion of host cell and formation of parasitophorous vacuole, are considered as potential vaccine candidate. Amino acid sequence analysis of Pf34 was done from reference strain of *P. falciparum* (PF3D7\_0419700) and it was found that Pf34 is having amino acid repeats (NNI, NND, NNN). Samples were collected from Darbha CHC hospital, district Bastar, Chhattisgarh, India. Amplification of *Pf34* gene was done using designed primers through polymerase chain reaction (PCR). Sequencing of PCR products was done by Dideoxy chain termination method using 3130 XL genetic analyzer. Out of 160 samples, 140 samples were successfully sequenced and analyzed. Among these 140 samples 5 types of nucleotide substitutions (A160G, G179T, C229A, G279T, T483A) and one insertion (272\_273 ins TAA) were observed. All these substitutions and insertion lead to change in the amino acids (Non-synonymous substitutions). Total six types of variants of Pf34 protein (N54D, G60V, H77N, N90\_K91 ins N, Q93H and S161R) were observed and about 8% of isolates were harbouring variant Pf34. This study may help in estimating the potential of Pf34 as a vaccine candidate or biomarker for further studies to develop malaria control methods.

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#### GENETIC RELATEDNESS ANALYSIS OF *PLASMODIUM FALCIPARUM* INFECTIONS IN SPATIALLY CLUSTERED COMMUNITIES OF WESTERN KENYA

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Establishing genetic relatedness in parasites of index malaria cases and reactive case detection outcomes ensures intervention targeting by identifying locations and risk groups driving transmission. We hypothesized that limited parasite types yield transmission of highly related parasites and proceeded to investigate genetic relatedness in reactive case detection. Between October, 2015 – August, 2016, index household members, five neighbouring and control households within 100m radius and ≥ 500m of the index case compound were respectively identified. Using MSP-1 and MSP-2, *Plasmodium falciparum* isolates were genotyped for genetic relatedness in 136 (22 index cases, 11 index household members, 76 neighbours, 27 controls) and 190 (36 index cases, 18 index household members, 101 neighbours, 35 controls) samples respectively. Of these, 254 (77.9%) were positive for MSP-1 (116) and MSP-2 (138) with K1, RO33 and FC27 as the predominant allelic families. Multiplicity of infection was highest in neighbours (1.21 and 1.36) for MSP-1 and MSP-2 respectively with no polyclonality in MSP-2 controls. A significant heterozygosity was observed between index case and control ( $P < 0.02$ ). 36.3% of index household members showed relatedness with index case, 17.1% of neighbours with index case and 11.1% of controls with index case in MSP-1. However, MSP-2 showed 11.1% of index household members with genetic relatedness to index case, 15.8% of neighbours with index case and 14.8% of controls with index case. Results indicate highest genetic relatedness in index case and its household members and neighbour in MSP-1 and MSP-2 respectively.

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#### VARIABILITY OF MALARIA PARASITES FROM NON-HUMAN PRIMATES IN THE BRAZILIAN ATLANTIC FOREST

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Non-human primates (NHPs) as a source for *Plasmodium* infections in humans are a challenge for malaria elimination. Autochthonous malaria in the Brazilian Atlantic Forest seems to be related to non-human primates infected with *Plasmodium simium* and *Plasmodium brasilianum*. These parasites are similar to the human parasites *Plasmodium vivax* and *Plasmodium malariae*, respectively. This study was motivated by the report of 932 autochthonous cases in the Atlantic Forest between 2007 and 2013. Of importance, an outbreak was reported in 2015 and 2016 with 49 autochthonous cases in Rio de Janeiro State. We studied 280 captive NHPs housed in the Primatology Center of Rio de Janeiro State (CPRJ) in the Atlantic forest. The samples were collected from 2011 to 2016 and they were screened for the presence of *Plasmodium* using optical microscopy and nested PCR for detection of 18S SSU rRNA gene. Here, we investigate the phylogenies and genetic diversity among these parasite species using DNA sequencing and microsatellite genotyping. The result of the molecular diagnosis identified an infection rate of 30% (9/30) in 2011, 25% (7/27) in January/2015 and 18% (2/11) in July/2015 and 6.6% January/2016. The sequencing of a small fragment of the 18S SSU

rRNA encoding gene showed a high genetic similarity between *P. vivax* and *P. simium*, and between *P. malariae* and *Plasmodium brasilianum*, as demonstrated by other authors. This high identity is also observed in the phylogenetic reconstruction of isolates studied here. Three *loci* of microsatellite and two polymorphic blocks of PvMSP-1 showed that there is a great genetic diversity among the circulating parasites. The data from microsatellites will be also very useful for comparing recurrent infections in the same animal, to verify if they are the same chronic infection or a different one. The present results provide evidence that non-human primates may act as reservoirs for parasites of the genus *Plasmodium*, highlighting the potential of zoonotic transmission of the parasite in areas of the Atlantic forest, hampering the elimination of malaria in the country.

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### DRAMATIC CHANGES IN MALARIA POPULATION GENETIC COMPLEXITY IN DIELMO AND NDIOP, SENEGAL REVEALED USING GENOMIC SURVEILLANCE

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The challenges of calculating traditional estimates of malaria transmission in low transmission areas emphasize the need for exploring innovative strategies, including harnessing genomics. A simple genotyping tool comprised of 24 independent single nucleotide polymorphisms (SNPs) revealed changes in *Plasmodium falciparum* transmission dynamics across multiple years of intervention application in Thiès, Senegal, including declines and rebounds in transmission through epidemiological modeling of *R0*. We applied this tool to an extensive longitudinal collection in Dielmo and Ndiop Senegal, where determinants of malaria infection have been conducted for decades. Using blinded samples from two distant time-points in this longitudinal cohort, we applied the molecular barcode tool to detect changes in parasite genotypes related to changes in transmission intensity. The goal of this genetic surveillance study was to validate the molecular barcode as a tool to assess parasite population diversity changes related to transmission dynamics and to track parasite genotypes across space and time. We observed a striking difference in the genetic diversity between the two parasite populations. In one population, we detected a high percentage (50%) of polygenomic infections, no shared genotypes, and no previously detected genotypes. In the alternate population we detected only monogenomic infections, three shared parasite genotype clusters representing two-thirds (67%) of the population. Upon unblinding it was revealed that the first population was from 2001-2002 where EIR was high (~350 in Dielmo and 79 in Ndiop) and that the second population was from 2014 where EIR was low (26 in Dielmo and 0 in Ndiop). Using neighbor-joining tree analysis we found no strict clustering of barcodes suggesting that parasite genotypes were shared between Dielmo and Ndiop. However, when we compared these genotypes with over 1000 other Senegal parasite genotypes from distinct locales, we detected that one of the 2014 genotypes from Dielmo had been previously detected in Thiès, Senegal in 2007 and again in 2010, suggesting possible importation of malaria.

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### SURVEILLANCE OF PFMDR1, PFATPASE SINGLE NUCLEOTIDE POLYMORPHISM (SNP) PREVALENT AMONG PLASMODIUM FALCIPARUM UNCOMPLICATED MALARIA CASES OF NORTHEAST INDIA (YEAR 2015) AS ANTIMALARIAL DRUG RESISTANT MARKER

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North east India is corridor to south east asian countries that historically remained first to report antimalarial drug resistance. Though Artemisinin combined therapy (ACT) is still effective in region but there is serious concern about progressive decline in parasite clearance rate to therapy. Study districts Mizoram, Meghalaya, Tripura are endemic with high proportion of *Plasmodium falciparum* malaria cases. Membrane transporter Pfm<sup>dr1</sup> and Pfatp6 polymorphism is reported to be associated with lumefantrine and artemisinin tolerance respectively worldwide. Pfm<sup>dr1</sup> 86,184, 1246 codons and Pfatp6 codons 402, 431 polymorphism were studied by nested PCR, restriction fragment length polymorphism (RFLP) and Sanger sequencing techniques. There observed significant trend towards predominance of Pfm<sup>dr1</sup> N86 allele among population since introduction of Artemether-Lumefantrine in year 2013. Pfatp6 codon L402V, E431K polymorphism was observed among 3/73 and 7/73 samples respectively. Predominance of Pfm<sup>dr1</sup> N86 allele in malaria parasite isolates of north East India establishes its increased tolerance to lumefantrine. Insufficient pfatp6 polymorphism based reports from region limits to determine its efficacy as Artemisinin resistant marker. Continued molecular surveillance should take place to develop robust markers to track disease and to take timely action measures.

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### PREVALENCE OF PFMDR1 AND PFK13 POLYMORPHISMS IN THREE PROVINCES IN ANGOLA, 2015

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Artemisinin-based combination therapy is the first-line anti-malarial treatment for uncomplicated *Plasmodium falciparum* infection in Angola. With the recent emergence of artemisinin resistance in the Greater Mekong sub-region and suspected lumefantrine resistance in Angola, it is important to characterize the background prevalence of polymorphisms in *pfk13*, associated with artemisinin resistance, and *pfmdr1*, associated with lumefantrine resistance. DNA was isolated from 506 dried blood spots collected at enrollment during the 2015 round of therapeutic efficacy studies in Benguela, Zaire and Lunda Sul Provinces in Angola. The *pfk13* propeller domain and *pfmdr1* segments were sequenced using the Sanger method and analyzed for polymorphisms. Additionally, *pfmdr1* copy number was assessed using a real-time PCR method. The majority of samples, 99% (413/416), were wildtype for *pfk13*, and all three non-wildtype samples (1%) carried the A578S mutation commonly observed in Africa and not associated with artemisinin resistance. The *pfmdr1* wildtype NYD haplotype (N86Y, Y184F, D1246Y) was predominant in all three provinces, with a frequency of 63% (133/209) of isolates in Benguela, 46% (105/227) in Zaire and 62% (69/109) in Lunda Sul, counting mixed infections as single infections. The NFD (N86Y, Y184F, D1246Y) haplotype, was found in 23% (49/209) of isolates in Benguela, 31% (72/227) in Zaire and 31% (35/109) in Lunda Sul. A total of 98% (497/506) samples were

successfully analyzed for *pfmdr1* copy number, all of which carried one copy of *pfmdr1*. The lack of *pfk13* mutations associated with artemisinin resistance support the clinical outcome data showing greater than 99% Day 3 clearance rates in Angola. Our results indicate no molecular evidence of emergence of artemisinin resistant K13 alleles in Angola's therapeutic efficacy sites, but reveal a high prevalence of N86, a potential marker for lumefantrine resistance. A 2017 follow-up study is underway.

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#### COMPARATIVE LANDSCAPE GENETICS OF *PLASMODIUM FALCIPARUM*, *ANOPHELES ARABIENSIS*, AND *AN. GAMBIAE* IN KENYA

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Malaria parasite dispersal patterns are complex, as they are affected by both the movement of mosquitoes and humans. Mosquito dispersal distance ranges from less than one to several kilometers throughout a lifetime, whereas humans can carry malaria parasites over immense distances. Previous population genetic studies suggest that the highlands surrounding the Rift Valley are barriers to mosquito gene flow (dispersal) across Kenya. However, the arid landscape and sparse human densities between Western and Eastern Kenya may also restrict gene flow. Here, we disentangle the effects of elevation, land cover type, and human population density on gene flow of malaria parasites and mosquitoes across Kenya using a landscape genetics approach. Landscape genetics uses techniques in population genetics, landscape ecology, and spatial statistics to quantify the effects of landscape factors on genetic differentiation. We genotyped *Plasmodium falciparum*, *Anopheles arabiensis*, and *An. gambiae* specimens collected from 14 sites across Kenya at 10 microsatellite loci. We created landscape resistance surfaces in ArcGIS and optimized them using ResistanceGA. ResistanceGA optimization increases the ability to detect the effect of landscape and is based on a genetic algorithm, pairwise genetic distances (FST), and pairwise landscape resistance distances (measured in Circuitscape). Lastly, mixed effect models were fit by maximum likelihood. Initial analyses indicate that low human population densities primarily restrict gene flow of *An. arabiensis* across Kenya. Analyses for *An. gambiae* and *P. falciparum* will be similarly conducted. As many countries are approaching malaria elimination, it is critical to understand the underlying factors that influence the dispersal of malaria parasites and vectors in order to effectively sustain local control and elimination. Moreover, knowledge of how malaria parasites and vectors disperse is also important to predicting how insecticide and antimalarial resistance spread.

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#### PREVALENCE OF HUMAN GENETIC POLYMORPHISMS ASSOCIATED WITH PROTECTION FROM MALARIA IN REGIONS OF UGANDA WITH DIFFERENT LEVELS OF MALARIA ENDEMICITY

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Mutations in several human genes have been associated with protection against malaria. In particular, the sickle hemoglobin mutation (E6V in the globin gene), the  $\alpha$ -thalassemia 3.7kb deletion, a common African variant of the gene encoding glucose-6-phosphate dehydrogenase (G6PD A-), and the CD36 T188G mutation have been associated with protection against different clinical presentations of malaria. We hypothesized that the prevalence of genetic polymorphisms that are protective against malaria would be lower in regions of Uganda with lower, compared to higher malaria endemicity. To test this hypothesis, we compared the prevalence of these polymorphisms in 1,344 subjects enrolled in cohorts from Kanungu,

Jinja, and Tororo Districts, which are historically believed to have had low, moderate, and high malaria endemicity. DNA was purified from buffy coat samples using a Qiagen column. Genes of interest were amplified with nested PCR, amplicons were subjected to mutation-specific restriction endonuclease digestion, reaction products were resolved by agarose gel electrophoresis, and genotypes were determined based on the sizes of reaction product. For simplicity, heterozygous and homozygous mutations were considered together in our analysis; in all cases most non-wild type results were heterozygous. The prevalences of the sickle hemoglobin mutation (28% Tororo, 25% Jinja, 7% Kanungu), the  $\alpha$ -thalassemia 3.7kb deletion (53% Tororo, 45% Jinja, 18% Kanungu) and G6PD A- (29% Tororo, 18% Jinja, 8% Kanungu) were significantly greater in Tororo and Jinja compared to Kanungu (chi square  $p < 0.0001$  for all 3 alleles). For the CD36 T188G mutation (27% Tororo, 17% Jinja, 18% Kanungu), the prevalence was significantly greater in Tororo compared to Kanungu and Jinja ( $p < 0.0001$ ). These results are consistent with our hypothesis, and suggest that human malaria-protective genetic polymorphisms were selected by evolutionary pressure from longstanding endemic malaria.

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#### AN OVERVIEW OF *PLASMODIUM VIVAX* GENOME STRUCTURE FROM A DUFFY NEGATIVE PATIENT AND ITS RELEVANCE TO ERYTHROCYTE INVASION

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*Plasmodium vivax* invasion depends on the Duffy that recognizes and interacts with the Duffy antigen. Our previous study has shown that mutations in *P. vivax* Binding Protein 1 (*PvDBP1*) do not explain how the parasite invades Duffy negative human erythrocytes. While expansion of *PvDBP1* may allow low affinity binding to another receptor on Duffy null erythrocytes, there are potentially other protein ligands such as *PvDBP2/EBP*, *PvRBP1* and *PvRBP2* that can recognize host cell receptors prior to erythrocyte invasion. Here, we used an Illumina Hi-Seq sequencing platform to obtain whole genome sequence of *P. vivax* isolated from a Duffy-negative patient in Ethiopia. Between 16 and 322 million pair-end 150bp reads were generated for 11 Ethiopian *P. vivax* samples, including one from a Duffy-negative and 10 from Duffy-positive patients. A range of 10-95% of the reads was mapped to PvP01, a new *P. vivax* reference genome sequence generated from an Indonesian isolate. Average *P. vivax* genome coverage was 28-582x and over 95% of the genome was covered by at least 15 reads for all 11 samples. Over 80 million reads were generated for the Duffy-negative *P. vivax* sample, and 63% of which were mapped to the *P. vivax* reference genome resulting in 106x average coverage. Comparisons of five erythrocyte-binding genes obtained by whole genome sequencing across these samples indicated that *PvRBP2* is most polymorphic between the Duffy-negative and Duffy-positive *P. vivax*, as well as among the Duffy-positive isolates. Such diversity may suggest the presence of different binding domains that are under differential selective pressure. Polymorphisms in genome structure and position will be discussed among the Ethiopian *P. vivax* isolates in the context of erythrocyte invasion.

### STATISTICAL INFERENCE OF *PLASMODIUM FALCIPARUM* TRANSMISSION NETWORKS BASED JOINTLY ON GENETIC AND EPIDEMIOLOGICAL DATA

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Decisions about how to target resources for malaria surveillance and control depend on accurate characterization of spatiotemporal variability in transmission. For viral and bacterial pathogens, recent advances in genetic sequencing and statistical methods have made this possible through the estimation of transmission linkages between individual infections. To date, no method has offered a similar capability for malaria parasites, given distinct features of their genetics as compared to viruses and bacteria. To provide such a capability, we developed a new statistical method for making inferences about transmission linkages between individual cases of *Plasmodium falciparum* malaria in low-transmission settings. This method makes use of the multi-locus genetic composition of an individual's parasites, their home location, and their detection date. We make explicit assumptions about the processes that generate and erode genetic variation, including parasite importation and stochastic loss during transmission, and we explicitly account for three distinct sources of genotyping error. Using Bayesian inference techniques, we validated the method with simulated data and made inferences about individual-level transmission networks with empirical data from low-transmission settings in Africa. In analyses of simulated data, we found that >80% of individual-level transmission linkages were correctly identified by our method. Compared to spatial and temporal data, genetic data contributed significantly more information to transmission network estimates, although the combination of all three data types improved accuracy and reduced uncertainty. Using transmission network estimates based on empirical data, we derived spatiotemporal estimates of key epidemiological metrics, such as the reproduction number under control,  $R_c$ , and the proportion of known infections that were imported from elsewhere or that derived directly from imported infections. In combination, our analyses of simulated and empirical data show that this new method provides robust estimates of localized metrics to inform malaria elimination efforts.

### DELETION OF *PFDXR* REVEALS ESSENTIAL ISOPRENOID FUNCTIONS IN *PLASMODIUM FALCIPARUM* CYTOSTOME FORMATION AND MAINTENANCE OF DIGESTIVE VACUOLE

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Isoprenoids comprise a large and diverse class of biomolecules synthesized from two basic components, isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP), which perform many essential cellular functions. Prokaryotes and plastid-containing eukaryotes, such as Apicomplexan parasites, synthesize isoprenoids via a mevalonate-independent metabolic pathway involving methylerythritol phosphate (MEP), which is not present in humans. The enzyme 1-deoxy-D-xylulose-5-phosphate reductoisomerase (DXR) catalyzes the first committed step in the MEP pathway. In this study, we generated *Plasmodium falciparum*

DXR (PF3D7\_1467300) deletion mutants (*PfΔdxr*), using the zinc-finger nuclease technology for targeted genome editing. *PfΔdxr* parasites reveal an absolute requirement for an exogenous supply of IPP for viability, firmly establishing the lack of alternative routes to isoprenoid precursor synthesis in this parasite. Using the *PfΔdxr* strain, we characterize the biological effects resulting from loss of *de novo* isoprenoid biosynthesis. Compared to wild-type *P. falciparum*, the *PfΔdxr* parasites accumulated the DXR substrate DOXP. All metabolites downstream of the disruption were below the limit of detection in the *PfΔdxr* strain, thereby confirming that the MEP pathway is non-functional downstream of DXR. *PfΔdxr* parasites also exhibited marked abnormalities in cellular architecture, including dramatically altered cytostomes. As compared to the parental strain, the number and size of cytostomes were significantly higher in *PfΔdxr* parasites, probably indicating the inability of these cytostomes to fuse to digestive vacuoles. *PfΔdxr* parasites also appeared to be devoid of digestive vacuolar membrane, as indicated by the absence of an electron-lucent region surrounding hemozoin crystals. All these morphological features were reverted by the addition of IPP. Taken together, our data suggest that the MEP pathway, which generates IPP for downstream cellular processes, is critical for preserving the normal cellular architecture of *P. falciparum* asexual blood stage parasites.

### VAR CODE: A NEW MOLECULAR EPIDEMIOLOGY TOOL FOR MONITORING *PLASMODIUM FALCIPARUM* IN A HIGH TRANSMISSION AREA OF GHANA, WEST AFRICA

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*Plasmodium falciparum* Erythrocyte Membrane Protein 1 (*PfEMP1*) is encoded by the *var* multi-gene family and is a major target of naturally acquired immunity. Variation in *PfEMP1* underlies parasite fitness, measured as its ability to evade the host immune response and establish a chronic infection to optimize transmission. We investigated the extent of *PfEMP1 var* (DBL $\alpha$ ) sequence diversity and population structure in the asymptomatic *P. falciparum* reservoir in an area with seasonal transmission from Bongo District, Ghana. We sampled individuals across all ages with asymptomatic *P. falciparum* infections detected by microscopy and, for the first time, those with sub-microscopic infections to examine *var* genetics of the entire parasite reservoir. Our analysis of 1284 *P. falciparum* isolates revealed 45,513 DBL $\alpha$  variants. Strikingly, this extensive diversity was uniquely structured into DBL $\alpha$  repertoires that had minimal overlap, with most isolate repertoires sharing <10% of their DBL $\alpha$  variants. The absence of related parasites was demonstrated by pairwise type sharing (PTS) regardless of whether we looked at single (median PTS=0.02), multiple (median PTS=0.04), microscopic (median PTS=0.03) or sub-microscopic (median PTS=0.02) infections. Here we define an isolate repertoire as a *var* code as it is frequently composed of >1 parasite genome. The extensive diversity and lack of relatedness of *var* codes allowed us to gain valuable information from multiple infections, often excluded from traditional population structure analyses with neutral SNPs and microsatellite markers, with orders of magnitude lower diversity than *var*. Fingerprinting parasite isolates using this *var* code revealed a transmission system composed of a large parasite population of 1283 distinct *var* codes in a relatively small human population of 1284 individuals where only two people shared the same *var* code (PTS=0.97). Using computational experiments, this large effective parasite population with essentially non-overlapping *var* codes was shown to have profound implications for understanding the epidemiology and control of malaria.

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### THE STUDY OF MULTIPLE EDITING OF *PLASMODIUM FALCIPARUM* GENES USING A TANDEM SGRNAS EXPRESSION CASSETTE

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This study was undertaken to edit multiple genes by utilizing a transfectant vector carrying a tandem sgRNAs expression cassette. Two sgRNA which from the *Plasmodium falciparum* K13 (kelch protein) and NUP116 (Nucleoporin) gene respectively tandem in a clone expression framework, while the corresponding directed mutation of homologous recombination sequences were cloned into the same expression vector, the vector and the Cas9 expression plasmid together with electroporation into the 3D7 strain of *Plasmodium falciparum*. Transgenic strains were obtained by drug screening. The *Plasmodium falciparum* of double site-mutations of k13 and nup116 genes were successfully obtained. In conclusion, the tandem sgRNAs expression vector based on CRISPR/Cas9 editing technique can be used to edit different genes of *Plasmodium falciparum* simultaneously.

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### PREREQUISITE EMERGENCE OF BACKGROUND MUTATIONS FOR *KELCH13*-RELATED ARTEMISININ-RESISTANT *PLASMODIUM FALCIPARUM* ISOLATES

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Spread of resistant *Plasmodium falciparum* to artemisinin derivatives (ART) is a great concern in Greater Mekong subregion. So far, six SNPs in *kelch13* gene have been validated to confer ART resistance. Recent genome-wide association study revealed that the *kelch13* mutations often arise in combination with mutations in some specific genes, hereafter referred as background mutations. These mutations might augment the level of artemisinin resistance or compensate for the potential disadvantage induced by *kelch13* mutation(s). The aim of this study is to clarify evolutionary processes for the acquirement of ART resistance in the context of *kelch13* and background mutations. We sequenced *kelch13* and genotyped six background genes (V127M in *arps10*, D193Y in *fd*, T484I in *mdr2*, C1484F in *pibp*, I356T in *crt*, and V1157L in *nif4*) in 587 global *P. falciparum* isolates taken before the introduction of artemisinin combination therapy. Results showed that background mutations were prevalent in the Greater Mekong sub-region, whereas scarcely distributed in Melanesia, South America and Africa, and which was well correlated with the prevalence of mutant-*kelch13*. In some background genes, the mutant allele reached high frequencies prior to the increase in *kelch13* mutations. These results suggest that the background mutations are prerequisite for the emergence of ART resistance accompanied with the *kelch13* mutations.

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### THE ROLE OF COMPLEMENT IN ANTIBODY-MEDIATED IMMUNITY AGAINST MALARIA IN PREGNANCY

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Malaria in pregnancy is a leading cause globally of poor maternal health, and low birth weight and premature delivery of neonates leading to greatly increased mortality and morbidity. Malaria in pregnancy (MiP) is characterised by the accumulation of *Plasmodium falciparum*-infected red blood cells (pRBCs) in the placenta. Infection is less prevalent with successive pregnancies partly due to the acquisition of antibodies directed against parasite antigens that mediate pRBC sequestration. However, effector mechanisms of antibodies are incompletely understood. Antibody responses are predominantly IgG1 and IgG3 subtypes, supporting a potential role for complement, but this has not been defined. Using sera collected from a longitudinal cohort of 350 pregnant women from a malaria-endemic province in Papua New Guinea we have established that acquired antibodies among pregnant women promote the deposition of complement on the surface of placental-binding pRBCs through binding C1q, and activate complement leading to fixation of C3b. Interestingly, complement fixation does not lead to cell lysis or parasite killing. Instead, antibodies with complement inhibit binding of pRBCs to placental vascular receptors and enhance phagocytosis by monocytes, mechanisms likely to provide protection against MiP. Using genetically-engineered *P. falciparum*, we identified the parasite antigen *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) as the major target of complement-fixing antibodies. Analysis of longitudinal data among pregnant women supports a role for complement fixation by acquired antibodies in protection against placental infection and adverse pregnancy outcomes. These findings provide new insights into the mechanisms mediating immunity to malaria in pregnancy to advance vaccine development.

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### AN IMMUNOSUPPRESSIVE ROLE FOR LAG3 IN TR1 CELLS DURING MALARIA AND VISCERAL LEISHMANIASIS

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Immunoregulatory mechanisms determine the balance between immune pathology and disease resolution. Many factors contribute to these mechanisms, but IL-10 production has been shown to be critical in a number of inflammatory diseases. Recently a Foxp3- regulatory CD4+ T (Tr1) cell subset has been identified as key producers of IL-10 in many diseases. They are prominent in visceral leishmaniasis (VL) patients, and their frequency was positively correlated with chronic malaria exposure in Ugandan children. In mouse models of VL and malaria, we recently reported that Tr1 cells suppressed anti-parasitic immune responses, but also played a key role in preventing immunopathology. Hence, if Tr1 cells can be appropriately manipulated, they have the potential to be used as immunotherapeutic tools. To be able to do this, we need to be able define them more clearly and identify unique immune checkpoint molecules they express that can be targeted for functional effects. Here we used RNAseq to compare gene signatures in Tr1 and Th1 cells from human volunteers participating in controlled human malaria infection (CHMI) studies with *Plasmodium falciparum*, as well as from mice with experimental VL caused by infection with *Leishmania donovani* to define a Tr1 profile which is independent of host and infection type. Furthermore, we functionally validated a number of the molecules identified, which included a variety of chemokine receptors, immune checkpoint inhibitors, and transcription factors. Lymphocyte activating gene 3 (LAG3) was one such molecule upregulated in Tr1 cells at both the RNA and protein level, and we showed that blockade of this molecule modulated Tr1 cell function. During experimental VL, both *ex vivo* and *in vivo* blockade of LAG3 reduced Tr1 cell numbers, which corresponded with reduced parasite burden *in vivo*. Additionally, LAG3 blockade increased parasite-specific IFN $\gamma$  production

in VL patient whole blood assays, as well as antigen-specific IFN $\gamma$  and TNF by murine splenocytes. Together these data identify LAG3 as a potential immune checkpoint target for modulation during malaria and VL.

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### NEUREGULIN-1 ATTENUATES MALARIAL MORTALITY ASSOCIATED WITH EXPERIMENTAL CEREBRAL MALARIA

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Cerebral malaria (CM) is a neurological manifestation of the infection caused by *P. falciparum*. *P. falciparum* causes endothelial dysfunction through parasite sequestration and expression of adhesion molecules which in turn disrupts blood brain barrier (BBB) integrity leading to high inflammation. Recently associated with CM is a neurotrophic growth factor Neuregulin-1 (NRG-1). NRG-1 has shown to protect against several brain injuries related to neurotoxin exposure and acute ischemic stroke. However, whether NRG-1 has a potentially inhibiting role in BBB disruption and attenuates CM associated mortality remain unknown. Neuregulin-1 will improve survival and reduce ECM-associated inflammatory response in mice with late stage ECM. The effects of NRG-1 were tested on ECM-associated mortality and brain inflammation in *P. berghei* ANKA (PbA)-infected mice. These effects were then compared to current treatment used today, artemether (ARM). Results show ARM treatment (25mg/kg/day) was effective in clearing parasites and reducing mortality in PbA-infected mice by 82%. Despite the increase in parasite level with NRG-1 treated mice, NRG-1 therapy (1.25 ng/kg/day) increased survival against ECM by 73%. Additionally, NRG-1 therapy lowered levels of systemic and brain pro-inflammatory factors IL-6, IL-1 $\alpha$ , TNF $\alpha$ , and CXCL10. NRG-1 therapy increased anti-inflammatory factors IL-5 and IL-13 while simultaneously inhibiting leukocyte accumulation in brain parenchymal vessels of PbA infected mice. Results suggests that NRG-1 treatment attenuates ECM-associated brain injury and inflammation. NRG-1 may serve as a novel therapeutic addition for the treatment and management of CM.

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### THE TRANSCRIPTION FACTOR T-BET SUPPRESSES GERMINAL CENTRE DEVELOPMENT AND HUMORAL IMMUNITY TO BLOOD-STAGE PLASMODIUM INFECTION

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Natural immunity to *Plasmodium* parasites is long-known to require years of repeated infections to develop, and only provides non-sterilizing protection from clinical disease. The reasons for this are elusive to date, however, increasing evidence from immuno-epidemiological studies in malaria endemic areas indicate that parasite-specific antibodies and memory B cells, which mediate protection, are acquired inefficiently and short-lived. Conversely, individuals exposed to infrequent symptomatic infections have been shown to generate stable memory B cells in the absence of frequent boosting. These findings suggest that acute malaria infections impede the establishment of B cell-mediated humoral immunity. In support of this, recent evidence revealed that pro-inflammatory pathways that drive malaria pathology can impair the humoral immune response to murine malaria infection, by inhibiting the differentiation of T follicular helper (Tfh) cells and impairing germinal centre (GC) responses. Using the *P. berghei* ANKA murine model, we investigated the role of the pro-inflammatory T helper 1 transcription factor T-bet in regulating the development of GC responses during blood-stage malaria. Genetic

ablation of T-bet significantly restored Tfh cell differentiation and the formation of GCs in the spleen, which led to an enhanced production of parasite-specific antibodies following infection. We found that T-bet expression by CD4<sup>+</sup> T cells inhibited Tfh cell differentiation, and compromised the development of the extrafollicular plasma cell and GC response to infection. Furthermore, B cell-specific expression of T-bet during infection affected the maturation of the GC B cell response by modulating the cellular dynamics of the GC reaction. Together these findings show that pro-inflammation mediated by T-bet, suppresses the development of the GC response and the generation of protective humoral immunity to blood-stage malaria.

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### NOVEL PLASMODIUM VIVAX DUFFY BINDING PROTEIN VACCINE CANDIDATE ARE ASSOCIATED STRONG AND PERSISTENT NATURALLY ACQUIRED IGG AND BINDING-INHIBITORY ANTIBODIES RESPONSE, IN LONG-TERM EXPOSURE POPULATION

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In natural infections, the *Plasmodium vivax* Duffy binding protein II (DBPII), which is a leading *vivax* vaccine candidate, is weakly immunogenic and induces strain-specific immunity. DEKnull2 is an engineered DBPII vaccine with modified strain variant surface residues that can elicit broadly neutralizing, strain-transcending anti-DBP inhibitory antibodies. DEKnull2 evolved from proof-of-concept studies that demonstrated that a synthetic DBPII allele, DEKnull, which lacked the dominant variant Bcell epitope can induce in mice more broadly inhibitory antibodies. Here, we investigated whether DEKnull and DEKnull2 are immunogenic in individuals naturally exposed to malaria in the Brazilian Amazon region. ELISA-detected IgG antibody responses to non-mutated DBPII variants (Sal1 and Brazil) and to synthetic DBPII immunogens (DEKnull, DEKnull2) were evaluated in a native community with long-term exposure to malaria. The study design included four cross-sectional surveys, three carried-out during the first year (baseline, 6 and 12 months), and two carried-out 6 years later, at the time that malaria cases had declined dramatically in the area. During the first year, consecutive serological surveys demonstrated that while few exposed-individuals responded to the original DEKnull, similar frequencies of responders (~50%) were obtained by using either enhanced DEKnull2 immunogen or native DBPII variants; of interest, the levels of DEKnull2 IgG-antibodies was much more higher than those obtained from native DBPII-variants. Six years later, the number of responder to native proteins decreased significantly, however, the levels and frequency of responders to the DEKnull2 remained relatively stable. Remarkable, long-term DEKnull2 responders with high levels of antibodies (reactivity index >20) were individuals able to produce broadly reactive binding-inhibitory antibodies (as detected by COS cell assay). DEKnull2 is highly immunogenic in naturally exposed-individuals, suggesting that the engineered vaccine could be a promising candidate towards the development of protective blood-stage *vivax* malaria vaccine.

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**INFLUENCE TO MOSQUITOES BITES ON ANTIBODY RESPONSES SPECIFIC TO MALARIA ANTIGENS**

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In malaria endemic areas, populations are frequently exposed to salivary components of blood-sucking mosquitoes that could modulate the immune response of the human host by their immunomodulatory properties. Consequences on pathogen-specific immune responses are not well known. Thus, we studied the influence of exposure to the main mosquitoes on the development of antibody (Ab) responses specific to *Plasmodium* antigens acquired during natural infection according to 1/ individual level of exposure to *Anopheles* bites and 2/ the presence of other mosquitoes (*Aedes* and *Culex*). A study was carried out in Bouaké (Côte d'Ivoire) where entomological data and blood samples from children (0-14 years) were collected. We evaluated the Ab responses (IgG, IgG1, IgG3) to 2 blood-stage antigens of *P. falciparum* (AMA-1, MSP-1) by ELISA. The individual level of child exposure to *Anopheles* bites was evaluated by quantifying specific IgG responses to the *Anopheles* gSG6-P1 salivary peptide. Immunological profiles of the anti-*Plasmodium* Ab responses in children were different according to the *Plasmodium* antigens. The comparison of immunological profiles according to the individual exposure level to *Anopheles* bites showed that anti-*Plasmodium* Ab responses were higher in children with low exposure level compared to those highly exposed to *Anopheles* bites. High and low exposed children to *Anopheles* but highly exposed to *Culex* bites have a low level of anti-*Plasmodium* immune responses. These findings suggest that anti-*Plasmodium* immune responses are modulated with the level of exposure to *Anopheles* bites but also to other hematophagous mosquito species. The immunosuppressive effect of the saliva of the mosquitoes has been highlighted. The immunomodulatory properties of mosquitoes' saliva on anti-plasmodial immune responses should be taken into account in epidemiological studies and especially in vaccine trials.

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**NATURALLY ACQUIRED ANTIBODIES TO *PLASMODIUM FALCIPARUM* AND THEIR ASSOCIATION WITH REDUCED MALARIA RISK: DISCERNING BETWEEN EXPOSURE AND PROTECTION**

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A main criterion to identify vaccine candidates against malaria is the proof that acquired immunity against them is associated with protection from disease. We aimed to study the protective role of IgG/IgM antibody responses against 23 pre-erythrocytic and erythrocytic antigens from *Plasmodium falciparum*, including members of the EBA and Pfrh families, and novel antigens merely or not previously studied in malaria-exposed populations (AARP, CyRPA, P41). We measured antibody levels by Luminex® in plasma from Mozambican children (n=261) who were monitored from birth until 3 years of age for clinical malaria. Aiming for a better approach to find correlates of protection against clinical malaria and overcome heterogeneity in malaria exposure that can confound our results, we compared classical cox regression models to models estimated after excluding children unexposed during the follow-up, defined by clinical and serological data. Also, to measure the antibody direct effect on the malaria episode, we limited the follow-up time to the time the specific IgG drop to 25% of their levels at first contact, that ranged from 78 days (PTRAMP) to 550 days (P41). Either excluding the non-exposed individuals or limiting the follow-up time in the analyses was useful to avoid antibodies' associations with higher risk of clinical malaria (which are exposure markers). Protective associations were found for IgG (and IgG breadth) at 24 months old (and not at younger ages), although the antibody levels were not necessarily higher than at younger ages. Together, these findings show the importance of age in the acquisition of protective immunity and of either excluding non-exposed population or limiting the follow-up time according to antibody decay when studying correlates of protection.

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**THE IMPACT OF CONCURRENT EXPOSURE TO *PLASMODIUM FALCIPARUM* ON THE DEVELOPMENT OF NATURALLY ACQUIRED IMMUNITY TO MALARIA IN YOUNG MALAWIAN CHILDREN**

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Antibodies targeting the blood-stage of malaria and variant antigens expressed on the surface of infected erythrocytes are important indicators of naturally-acquired immunity against malaria. To identify their role in development of immunity in infancy, the levels of antibodies against merozoite surface proteins (MSP1 19kD, MSP2), erythrocyte binding antigen 175 (EBA175), reticulocyte binding protein homologue 2A (Pfrh2), schizont extract and variant surface antigens for parasite lines E8B, R29, 3D7 overexpressing Var A were measured in plasma from 18 month old Malawian infants in a randomised controlled trial of nutrient supplements. Children were actively followed from birth and had malaria testing for febrile episodes. Of 601 children, 144 experienced malaria episodes before 18 months. Antibody prevalence was higher in children who had experienced episodes of clinical malaria, or who were parasitaemic at the time of sample collection than children with no malaria history. Children who had experienced clinical malaria were significantly more commonly seropositive for MSP1, MSP2, schizont extract and E8B parasite-line. Antibody prevalence did not differ between children having single and multiple episodes. Antibody levels also differed with history of malaria episodes or parasitaemia. Children who were parasitaemic at sample collection had significantly higher levels of antibodies for all the antigens except Pfrh2. Children with malaria episodes had higher antibody levels

than those who had no episodes, but multiple episodes did not increase antibody levels compared to single episodes. Antibody levels at 18 months were similar in children who had episodes before 6 months of age or between 6 and 18 months. We observed boosting of malaria antibodies following clinical malaria and in children with current parasitaemia episodes in early childhood, but neither the number of episodes or time since malaria episode influenced antibody levels or prevalence. The results suggest that some merozoite antigens may be useful biomarkers of malaria exposure in infancy.

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### PFEMP1 SPECIFIC IGG ANTIBODIES PROFILES FROM BIRTH TO TWELVE MONTHS OF AGE IN BENINESE INFANTS

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The cytoadherence properties of *Plasmodium falciparum* infected erythrocytes (IE) represent a major contributor to the pathogenesis of malaria through interactions with various endothelial cell surface receptors. These interactions are mediated by members of the highly variable *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) expressed on the IE surface. One particular component of PfEMP1 proteins, the cysteine-rich interdomain region (CIDR), is known to play a very important role in the adhesive interactions between IE and endothelial receptors, making this region a potential vaccine target of interest. Here, we investigated the dynamics of maternally-transferred IgG antibodies targeting the CIDR of a panel of different PfEMP1 proteins, as well as infants' own acquisition of antibodies with the same specificities during the first year of life. We used plasma samples collected longitudinally from the offspring of a cohort of pregnant women who had themselves been followed closely through pregnancy. We show that the levels of all anti-CIDR antibodies quantified declined to the point of disappearing over the 6 first months of life. Antibodies with specificity for the CIDR predicted to adhere to selected receptors (CD36, EPCR) or for the CIDR associated with the unknown phenomenon on were subsequently acquired by infants between 7-12 months of age, their levels being a function of *P. falciparum* history during infancy. Infected infants developed stronger antibody responses to the CIDR associated with either EPCR binding or unknown compared to uninfected infants. Parasites infecting children before 6 month of age preferentially transcribed *var* gene belonging to the group B and those encoding DBLb containing PfEMP1.

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### IMMUNE RESPONSE AGAINST A NOVEL *PLASMODIUM VIVAX* ERYTHROCYTE BINDING PROTEIN IN A BRAZILIAN NATURALLY EXPOSED POPULATION

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Recently, a new member of the Duffy binding protein family was discovered in *Plasmodium vivax* – named Erythrocyte binding protein (EBP2) -- and this protein may play a role as a ligand mediating an alternative invasion pathway for young Duffy-positive reticulocytes (Ntumngia et al., MBio. e01261-16, 2016). In individuals naturally exposed to malaria in the Amazon region, we investigated the antibody response against EBP2 and their relationship to antibodies induced by the Duffy binding Protein (DBPII), the major ligand involved in *P.vivax* reticulocyte invasion. An open cohort study was carried among 425 volunteers from a native Amazonian community with long-term *P.vivax* exposure. ELISA-detected IgG antibodies were evaluated by using recombinant proteins based on ligand-domain of EBP2 and DBPII. The study design included five cross-sectional surveys, three carried-out during the first year (baseline, 6 and 12 months), and two 6 and 7 years later. Taken together, the results

demonstrated that (1) during the first follow-up year, the frequencies of anti-EBP2 (50-60%) was higher than frequencies of anti-DBPII (30-36%); (2) Six to 7 years later, at the time that malaria transmission had declined dramatically in the study area, the profile of EBP2 antibody response remained relatively stable while DBPII antibodies dropped significantly. Conclusion: As compared with DBPII, EBP2 is highly immunogenic and induces a long-term immune response. Further studies should investigate whether antibodies against EBP2 are able to block reticulocytes *P.vivax* invasion.

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### IMMUNE RESPONSE IN PATIENTS WITH DIFFERENT PARASITIC PROFILES IN FIVE PROVINCES OF GABON, CENTRAL AFRICA: CROSS-SECTIONAL STUDY

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Malaria, blood filariasis (*Loa loa* and *Mansonella perstans*) and intestinal parasites (helminths and protozoan) are endemic in Gabon. This geographical codistribution leads to polyparasitism with for consequences the possible interaction between parasites. It is possible that intestinal protozoan and helminths could modulate malaria immunity increasing susceptibility to malaria. A cross-sectional study was conducted between September 2013 and November 2016. Blood and stool samples were collected in five of the nine provinces of Gabon. Parasitological diagnosis were performed to detect malaria parasites, blood filariasis and intestinal parasites. Then, six groups were constituted presenting either malaria only, helminths only, intestinal protozoan only, helminths and malaria coinfection, protozoan and malaria coinfection and no parasites. Flow cytometer was used to measure IL-10, IL-6 and TNF-alpha inflammatory cytokines levels and ELISA sandwich, to detect total IgG directed against GLURP R0, GLURP R2, MSP1-19, MSP2 3D7 and FC27, AMA-1 surface proteins of *falciparum* merozoite asexual form. Globally, 843 subjects were enrolled and 414 returned stool samples. Malaria, filariasis and intestinal parasites prevalence were respectively 21.7, 13.3, and 37.7%. IL-10 and IL-6 levels were higher in malaria group. Moreover, IL-10/TNF-alpha ratio was higher among patients without parasites ( $p < 0.0001$ ). Specific IgG directed against MSP3, GLURP R2 and AMA-1 were elevated in helminths group (respectively  $p_{MSP3}$ ,  $AMA-1=0.01$ ,  $p_{GLURP R2}=0.002$ ). Correlation analysis showed an inverse relation between specific IgG level to MSP2 FC27 and TNF-alpha concentration ( $r=-0.3$ ;  $p=0.03$ ). Strong Th2 response was observed in all patients in this study and IgG directed against surface protein were predominant in patients with helminths infection.

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### ANTIBODIES PROMOTE COMPLEMENT ACTIVATION AGAINST *PLASMODIUM FALCIPARUM* SPOOROZOITES, PROVIDING A NOVEL MECHANISM OF ANTI-MALARIAL IMMUNITY

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At present, we lack an in depth understanding of the immune mechanisms against *Plasmodium falciparum* sporozoites, and consequently lack reliable correlates of protective immunity. Without this fundamental knowledge, the development and evaluation of existing or candidate malaria vaccines is severely impaired. Antibodies to sporozoites and their major surface

antigen, circumsporozoite protein (CSP), have been loosely associated with protection in studies of naturally acquired and vaccine-induced immunity, but it remains unclear how they function, and there is limited knowledge of key functional epitopes. Here we investigated the functional role of antibodies to CSP and sporozoites in activating the complement system via the antibody-dependent classical pathway. We have established that antibodies form malaria-exposed individuals can fix the complement protein, C1q, to recombinant CSP, which is an essential interaction that initiates the classical activation pathway, and leads to formation of the membrane attack complex. These C1q-fixing antibodies were strongly correlated with IgG1, IgG3 and IgM antibody types, and could recognise multiple CSP epitopes. Malaria-specific antibodies mediated C1q-fixation and subsequent classical activation on the surface of *P. falciparum* sporozoites, which consequently inhibited parasite migration through cells and could lead to cell death *in vitro*. Therefore complement activation may be an important antibody mechanism to inhibit sporozoite infection and pre-erythrocytic development. Effective C1q-fixing antibodies were poorly acquired though natural malaria exposure in children and only a minority of highly exposed adults had anti-CSP antibodies with strong complement-fixing activity. In summary, we identified a novel function of malaria-specific antibodies in activating the human complement system and consequently inhibiting sporozoite function and leading to cell death, which provides important insights for vaccine development and evaluation.

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#### EFFECT OF ALLELIC POLYMORPHISM ON MALARIA PARASITE SPECIFIC *EX VIVO* INTERFERON-GAMMA RESPONSE TO APICAL MEMBRANE ANTIGEN 1 IN A MALARIA EXPOSED REGION.

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Despite extensive genetic diversity in leading candidate antigens, vaccines have been and continue to be formulated using recombinant antigens representing only one or two strains. However, narrowing the focus to immunologically relevant polymorphisms would enhance the identification of relevant antigenic diversity. The effect of allelic polymorphism on malaria parasite specific *ex vivo* IFN- $\gamma$  response to Apical Membrane Antigen 1 (AMA 1) in a malaria exposed population was investigated in this study. Five study subjects with known HLA A and/or B super types were recruited and peripheral blood mononuclear cells (PBMCs) isolated from their venous blood samples. Bioinformatically selected MHC class I-specific AMA1 epitopes from the 3D7 parasite strain were aligned with corresponding sequences from 7G8, FVO, tm284, FC27 and AAN35928 AMA1 strains and peptides with amino acid variability were selected. A total of 133 peptides (62 allelic sets of 3 or 2 peptides each) were selected and synthesized for stimulation of subjects' PBMCs in IFN- $\gamma$  ELISpot Assays. Out of the 62 allelic set sequences, 17.74% were partially positive, and were of the HLA A01, A02, A03 and B44 specific alleles. Variation in peptide responses among allelic sets were common among alleles with amino acid alterations at the peptide binding sites. Further broad-based research in this area may help inform the design of an effective malaria vaccine using recombinant antigens.

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#### VALIDATION AND OPERATIONAL FEASIBILITY OF THREE STRATEGIES FOR GEOLOCATING MALARIA INFECTIONS DETECTED AT HEALTH FACILITIES, SCHOOLS AND CHURCHES IN HAITI

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With a total number of 21,167 confirmed cases in 2016, malaria prevalence is estimated to be <1% in Haiti and infections are likely to be spatially clustered. Due to this heterogeneity and the need to target elimination interventions to such areas, it is essential to identify the location of cases detected through the passive surveillance system. This is a challenge in rural areas where formal household addresses are unavailable. This study will be conducted in the Artibonite Department in Haiti, where we will investigate the accuracy of three geolocation strategies: the self-reported nearest landmark, the location of residence identified by the participant on a digital map, and the location of residence of school children by using a GPS data logger. Landmarks will be catalogued during parallel census activities and include schools, markets, and health facilities. The likely area of participants' residence will be triangulated by the overlapping catchment areas of these landmarks based on the Euclidian distance. Digital maps will be displayed on a tablet screen (6 x 3.5 inch) using free Landsat imagery with 50-foot resolution. These strategies will be tested by recruiting 2100 randomly selected individuals per venue type during an easy access group study to be conducted in 25 primary schools, 8 churches, and 10 health facilities. A random sample of 25% of all participants from schools and health facilities will be traced to their households with a Garmin GPS receiver to serve as a gold standard for validation purposes. Accuracy will be assessed by the proportion of households correctly identified within given tolerance zones around the true location as well as the straight-line distance between the identified and true locations. The precision of these geolocation strategies will be compared along with their respective costs, feasibility and expected usefulness for malaria passive surveillance in health facilities and in sentinel populations. This study will provide evidence with respect to the accuracy and operational feasibility of different geolocation strategies in resource-poor settings where malaria elimination efforts are implemented.

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#### IDENTIFYING THE COMPONENTS OF SEVERE MALARIA ACIDOSIS BY METABOLOMICS

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Metabolic acidosis is common in severe malaria and predicts mortality in all age groups. During severe infection, a large parasite biomass sequesters in the microvasculature resulting in acidosis and subsequent clinical deterioration. We hypothesized that previously uncharacterized acids besides lactic acid contribute to acidosis. We enrolled 108 adult patients with *P. falciparum* malaria and 48 healthy controls in an observational study in 2014-2015 in Bangladesh. Out of 108 malaria patients, 61 had severe malaria and 23 had WHO defined severe malaria acidosis (base deficit >8mEq/L); the severe malaria case fatality rate was 33%. In severe malaria acidosis, lactate only explained on average 49% (95%CI; 11%) of the base deficit. We applied ultra-high performance liquid-chromatography mass spectrometry to measure the complete plasma acid load and detected 355 unique metabolites. In addition to lactate, we identified 12 acids and 6 additional metabolites associated with standard base deficit in severe malaria patients (false detection rate (FDR) <20%, adjusted  $p < 0.05$ ). Metabolic pathway analysis revealed enrichment of arginine and proline metabolism (FDR 1.5%,  $p < 0.001$ ) and glycine and glyoxylate metabolism (FDR <1%,  $p < 0.01$ ). Increases in metabolite levels were associated with mortality in univariate regression ( $p < 0.05$ ), including dimethylglycine, pipercolic acid, homoserine, and glutamine. Metabolites implicated in host-acidosis were further evaluated using an *in vitro* Pf3D7 culture and analyte secretion was assessed using the same analytical platform. A total of 3 out of 12 acids were found to be secreted by Pf3D7 *in vitro*, including proline, pyruvic acid, and lactic acid, potentially contributing to hyperlactataemia. Our results show acidosis in severe malaria is associated with circulating arginine and glycine metabolites.

Arginine metabolites are likely accumulating due to its catabolism in severe malaria; Glycine metabolites indicate dysregulated energy metabolism. These results further characterize acidosis in severe malaria, and may provide markers of infection and potential targets for adjunctive therapy.

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#### TARGETING *PLASMODIUM* SPOROZOITE LIVER INVASION WITH A PHAGE DISPLAY LIBRARY

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After inoculation by the bite of an infected mosquito, the *Plasmodium sporozoite* enters the blood stream. To initiate a productive infection, sporozoites must exit the blood vessels in the liver by preferentially traversing liver macrophages (Kupffer cells). Using a phage display library we previously identified peptides which bind to Kupffer cells and by doing so, inhibit sporozoite invasion. We determined that the peptides bind to the Kupffer cell receptor CD68 and that during invasion, sporozoite surface GAPDH interacts with CD68. Importantly we showed that one of the CD68-binding peptides serves as a pre-erythrocytic vaccine candidate. We screened the same phage display library to investigate *P. berghei* sporozoite interaction with mouse hepatocytes. Our candidate hepatocyte-binding peptides competitively inhibit sporozoite hepatocyte invasion *in vitro*. We are now seeking the identification of a sporozoite ligand, which presumably competes with the peptides for hepatocyte binding and invasion. These experiments may lead to the identification of an additional pre-erythrocytic vaccine candidate.

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#### ADVANCED MEDICAL IMAGING IN IN EARLY MALARIA: CAN IT HELP US UNDERSTAND WHERE THE PARASITES GO AND ORGAN-SPECIFIC HOST RESPONSES?

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Functional imaging to study biological changes is being increasingly utilized. We hypothesized that non-invasive imaging in human experimental malaria will provide insights into both parasite location and disease pathogenesis. We performed whole body 18F FDG-PET/MRI at baseline, and just prior to treatment in 5 participants undergoing induced blood stage malaria (IBSM) infection with *P. falciparum* (n=2) and *P. vivax* (n=3). At the time of imaging participant parasitemia were between 2,753 and 27,704 parasites/mL. MRI sequences were performed to assess cerebral vascular and blood brain barrier integrity. MRI data was qualitatively reported, including review of cerebral imaging. PET data was qualitatively reported to assess for changes by a nuclear medicine physician. Semi-quantitative standardized uptake values were reported for the liver, spleen, bone marrow, skeletal muscle and brain. Quantitative analysis of FDG uptake the spleen by PATLAK is underway. Preliminary review of MRI data did not identify any gross changes from baseline, including specific neuroimaging reporting. Preliminary quantitative PET analysis found increased post-inoculation 18F FDG uptake in the liver and spleen of *P. falciparum* participants. Increased uptake was found in the liver, spleen, bone marrow, skeletal muscle and cortex of *P. vivax* participants, with reduced uptake in the cerebellum. These findings indicate early activation of the reticuloendothelial system in subpatent malaria infection, and present material for further *in vivo* analysis, including investigation for sequestration of *P. vivax* in non-endothelial

lined compartments. Refined imaging sequences will be applied to a further population of *P. falciparum* and *P. vivax* participants in experimental malaria infection studies.

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#### DEVELOPMENT OF A NOVEL AMPLICON DEEP SEQUENCING MARKER AND DATA ANALYSIS PIPELINE FOR GENOTYPING OF MULTI-CLONAL *PLASMODIUM FALCIPARUM* INFECTIONS

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Amplicon deep sequencing permits sensitive detection of minority clones and improves discriminatory power for genotyping multi-clone *Plasmodium falciparum* infections. Currently used genotyping methods based on length polymorphic markers such as merozoite surface proteins 1 and 2 (*msp1*, *msp2*) may suffer from competition of templates in PCR. Diversity in amplicon sequencing relies on haplotypes created from several SNPs. In search for highly diverse markers 3'411 genomes from 23 countries, published by the *Plasmodium falciparum* Community Project (MalariaGEN), were screened for high diversity within a stretch of 500 bp. A 430 bp fragment of PF3D7\_0104100 (conserved *Plasmodium* membrane protein, *cpmp*) was identified. Targeted *cpmp* sequencing was conducted in duplicate on mixtures of parasite culture strains and on 153 archived field samples from 34 children aged 1-5 years from a cohort study conducted in Papua New Guinea (PNG). A protocol to multiplex up to 384 samples in a single sequencing run was applied. Software "HaplotypR" was developed for data analysis with the aim to distinguish true minority clones from sequencing errors. HaplotypR was validated using experimental mixtures of *P. falciparum* *in vitro* culture strains and tested on field samples. Expected heterozygosity (He) of *cpmp* was 0.93 in the worldwide MalariaGEN dataset and 0.96 in field samples from PNG. Results from amplicon sequencing were compared to those obtained from length polymorphic marker *msp2*, which had a He of 0.95 in field samples. Highly multiplexed amplicon sequencing of marker *cpmp* displayed greater sensitivity in detecting minority clones, which was reflected in a mean multiplicity of infection (MOI) 2.48 for *cpmp* compared to 2.03 for *msp2*. False haplotype calls owing to sequencing errors were observed below a frequency of 1%.

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#### AN OPTIMIZED METHOD FOR LARGE-SCALE PRODUCTION OF SYNCHRONIZED STAGE V *PLASMODIUM FALCIPARUM* GAMETOCYTES FOR USE IN HIGH-THROUGHPUT ANTIMALARIAL ASSAYS

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In a global effort for malaria elimination, there has recently been renewed interest in studying ways to reduce transmission of the parasite by targeting mature sexual stage gametocytes. Recent drug studies have shown that current antimalarial drugs act on *Plasmodium falciparum* gametocytes differently than asexual stages and are usually less efficacious. Therefore, it is essential that drug discovery efforts include finding transmission-blocking compounds that are effective at eliminating metabolically quiescent Stage V gametocytes. Experimental Therapeutics' (ET) mission to develop a once a week prophylactic drug is aided by knowledge of candidate compounds' anti-gametocyte activity to discriminate against similar structures and help align with potential partners target product profile. One of the major obstacles with researching gametocytes is the length of time it takes to prepare

an adequate amount of these transmissible forms for high-throughput screenings. Although there have been several protocols recently published on obtaining large amounts of synchronized gametocytes, current approaches involve very laborious bulk-up procedures that include multiple rounds of time-consuming synchronization and enrichment techniques over the course of several weeks. Therefore, we sought to improve current protocols by comparing various synchronization, enrichment, and cryopreservation techniques to ultimately create a simplified, time-saving method of producing large amounts of viable gametocytes to be included in high-throughput drug screening. Using a combination of traditional and novel methods, we have greatly reduced bulk-up time and increased in-house capacity. Using a 384-well plate format, we currently have the capability to screen approximately 140 compounds and prescreen over 700 compounds in duplicate per assay run, with the potential to further increase throughput. This optimized and validated assay will serve as an important tool to access candidate compounds' transmission blocking ability for Experimental Therapeutics' ET's plasmodium drug evaluation paradigm.

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#### TRACKING LONG-LASTING INSECTICIDE-TREATED NETS DISTRIBUTED THROUGH SCHOOLS IN A MALARIA ENDEMIC REGION OF NORTHERN ZAMBIA

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Zambia has adopted mass campaigns every 3 years as the main distribution channel of long-lasting insecticide-treated nets (LLINs). Models, however, show that household LLIN coverage following mass distribution drops to 65% and 35% after 2 and 3 years, respectively. To ensure sustained coverage above the target of 100%, a program was piloted in Luapula Province, northern Zambia, using primary schools as points of distribution. The Southern Africa ICEMR project sought to track nets distributed in Nchelenge District of Luapula Province to determine whether the objectives of increasing household coverage and use of LLINs were met. From 12 primary schools 2,225 nets were distributed in October 2016. Monthly surveys of nets were conducted in 25 randomly selected households in the catchment area of these schools alternating between cross-sectional and longitudinal cohorts from December 2016 to March 2017. A total of 76 households were sampled. Sixty-seven nets of different origins were found in 46 households; 29 households had only one LLIN, 13 had two and 4 households had three nets. Twenty-five percent of nets were obtained through mass campaigns, 16.4% came from schools, 10.4% from community health workers and 14.9% were purchased. Of all nets found, 84% (56 out of 67) were hanging and being used. Of the 11 nets obtained from schools, 8 were in use at the time of the visits, all being occupied by school-aged children. Despite only sampling 0.25% of households across the district (76 out of 31,000), 32 of these had primary school-going children likely to be in grades that received nets, yet only 10 households had a school-distributed net. Across all households, school-net distribution increased the household coverage of LLINs from 56.6% (43 households) to 60.5% (46 households) and the mean number of LLINs per household from 1.22 to 1.46 nets in the 5 months post-distribution. Usage, however, did not increase as nets already present in the house were used; three school nets were still wrapped. In addition to helping understand utility, studies like this need to be expanded to help better understand mechanisms of getting interventions (LLINs) into communities.

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#### COMPLETENESS OF MALARIA INDICATORS REPORTED THROUGH THE DISTRICT HEALTH INFORMATION SYSTEM IN KENYA, 2011-2015

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The adoption of District Health Information Systems (DHIS2) has contributed to improved availability of routine health facility-based data in many countries. We conducted a 5-year retrospective, longitudinal assessment of DHIS2-reported malaria data from January 2011-December 2015 in Kenya. Malaria data were analyzed for 6,235 public and 3,143 private facilities. Completeness of reporting was measured as the percentage of data values reported over the expected number in a given year. Between 2011 and 2015, completeness of reporting in the public sector increased significantly for confirmed malaria [26.5% to 41.8% in children aged <5 years and 30.6% to 51.2% in persons aged ≥5 years [both p<0.0001] and for new antenatal care (ANC) clients [53.7% to 70.5%, p<0.0001] but decreased for intermittent preventive treatment in pregnancy (IPTp) doses 1 and 2 [64.6% to 53.7% and 64.6% to 53.4%, respectively, both p<0.0001]. The number of people tested for malaria was not available in DHIS2 from 2011 to 2014, although 59%-91% of facilities had malaria diagnostics available. In 2015, only sparse malaria-test data for microscopy [11.5% for <5 years; 11.8% for ≥5 years] and rapid diagnostic tests (RDT) [8.1% for all ages] was reported. In the private sector, completeness of reporting increased significantly for confirmed malaria [16.7% to 23.1% in <5 years; 19.4% to 28.6% in ≥5 years], new ANC clients [16.2% to 23.6%], and IPTp doses 1 and 2 [16.6% to 20.2% and 15.5% to 20.5%, respectively] [all p<0.0001]. In 2015, <3% of malaria-test data was reported. Key malaria indicator reporting completeness improved since DHIS2 implementation in 2011 in Kenya. The major gaps identified were in malaria-testing data in both sectors and overall low reporting of malaria indicators in the private sector. Integrating malaria-test data into DHIS2 by including total number tested and number of test-positives will improve the data available to determine Kenya's progress towards meeting the test, treat, track initiative. Providing standard data reporting forms and training on DHIS2 implementation are recommended to improve private-sector reporting.

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#### IMPROVING ADHERENCE TO THE KENYA NATIONAL MALARIA DIAGNOSIS AND TREATMENT GUIDELINES; AN OUTREACH TRAINING AND SUPPORT SUPERVISION (OTSS) APPROACH IN VIHIGA COUNTY, WESTERN KENYA

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The Kenya national malaria diagnosis, treatment and prevention guidelines fifth edition May 2016 recommends the following: All people with suspected malaria should have a parasitological test to confirm the diagnosis, a rapid diagnostic test or microscopy, treatment should be administered to confirmed malaria cases, recommended first line for uncomplicated malaria is Artemether Lumefantrine and severe malaria treatment first line is Artesunate. The 2015 Kenya Malaria Indicators Survey report, 72% of under 5 years with fever who sought treatment, 39% were tested for malaria, instead of 100% confirmation as per the guidelines, Malaria Care Kenya health facility assessment baseline Final

report, October 2015 There were 153 observations of post-test diagnosis and treatment of the 81 negative malaria diagnostic test results recorded, 11 (14%) were prescribed an antimalarial, this is against the national malaria treatment guidelines. In Vihiga County of the 5 health facilities sampled in this assessment 40% of these facilities patients with a negative malaria test result received an antimalarial treatment. Outreach training support supervision is an approach implemented by Malaria Care project a USAID partnership whose aims is to scale up high-quality diagnosis and case management services for malaria and other febrile illnesses. Implemented by a team of skilled supervisors a clinician and laboratory technologist trained on case management and advanced malaria microscopy, this team visited 45 public health facilities through scheduled visits in Vihiga County western Kenya, for the period November 2015 to December 2016 to assess malaria core indicators of Clinical Observation, Laboratory observation, RDT Competence, Adherence to testing prior to treatment, Negative and positive test adherence and general using an electronic checklist, they provided onsite mentorship, based on the outreach training support supervision report their was improvement of the Malaria Care core indicators in adherence to the Kenya national guidelines fifth edition 2016 across the three rounds of outreach support supervision conducted in Vihiga County.

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#### **HISTORICALLY-SHAPED ATTITUDES AND PERCEPTIONS ON HEALTH RESEARCH MAY DETER PREGNANT WOMEN FROM ACCEPTING MALARIA RESEARCH AND PREVENTION: A QUALITATIVE INQUIRY IN MONROVIA, LIBERIA**

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Liberia is one of the countries with a lowest public spending as a % of GDP allocated to health. Two civil wars and a recent Ebola outbreak have contributed to a health system that needs vast investment to see its capacity to lead infectious diseases research strengthened. Malaria is highly endemic. It affects 45% of the 6-59 months children according to the last Malaria Indicator Survey. Due to the diversion of resources for health during the Ebola crisis, a 62% increase of malaria-attributable mortality was estimated. In spite of these constraints, there is a paucity of epidemiological research. With the aim to map barriers to engage pregnant women in malaria prevention and research, we conducted a qualitative inquiry nested in a malaria prevalence study at the St Joseph Catholic Hospital in Monrovia. Grounded theory guided data collection and analysis. We sought for hospital staff, community leaders, and pregnant women participants to the prevalence study as key informants. Recruitment started in November 2016 and ended in February 2017. Thirty-nine informants partook in twenty-six in-depth interviews and in three focus group discussions. Common agreement was that cultural beliefs and widespread corruption deter people from adhering to prevention advices. Witchcraft and vectors other than mosquitoes as perceived causes of malaria impel people to first seek care from African herbalists. So does lack of trust in health staff, experiences of bribery, low awareness of vulnerabilities, and the introduction of cost recovery in some clinics. The informants suggested that researchers should design prevention tools inspired on the ointments and concoctions provided by the herbalists, and that community-tailored education may help allay fears induced from previous exposure to clinical trials that may discourage women from engaging in any academia-led activity. We conclude that these identified deterrents shaped by recent historical events must be addressed, in partnership with local leaders, at the outset of any future program, as these are drivers that may influence Liberians deciding not to accept take part in malaria research and prevention.

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#### **DO DEMOGRAPHIC AND HEALTH SURVEYS ACCURATELY MEASURE MALARIA DIAGNOSIS AND TREATMENT RECEIVED BY CHILDREN UNDER FIVE YEARS? A TREATMENT RECALL VALIDATION STUDY IN MALI**

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Access to and treatment with artemisinin-based combination therapy (ACT) is monitored periodically through large-scale, nationally representative household surveys. These estimate the proportion of children under five years (U5s) with a fever in the past 2 weeks who received an ACT, among those who received any antimalarial. However, this indicator is subject to a caregiver's recall of events during the fever episode and ability to differentiate between drugs. In Mali, it is hypothesized that estimates of reported treatment received by U5s during a fever episode contain information bias due to seasonal malaria chemoprevention (SMC), generic local language terms for antimalarials, and low caregiver literacy. The 2015 Mali Malaria Indicator Survey (MIS) showed that only 32% of children receiving antimalarials reportedly received ACT, while 26% received amodiaquine and 21% sulfadoxine-pyrimethamine. A prospective case control study was designed to validate the ability of caregivers to recall diagnosis and treatment received by U5s when seeking care for a fever in Mali, replicating a study previously performed in Zambia. Details of the consultation (if diagnostic test was done, test result, drugs recommended and received) were recorded at the facility during or immediately after the consultation and serve as the gold standard to which caregiver recall was compared. Consenting caregivers received a follow-up interview at home within 14 days of the consultation, comprising standard questions from MIS and Demographic and Health Survey. 1600 caregivers of febrile U5s seeking care from urban public and private facilities, rural public facilities, and community health workers were recruited. Sensitivity and specificity of caregivers' recall of ACT treatment is presented, along with description of factors associated with incorrect recall of treatment. This evidence will help determine the reliability of standard DHS questions regarding reported malaria treatment in a West African setting with ongoing national SMC, and inform global ACT coverage estimates generated from nationally-representative household surveys such as DHS.

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#### **CLINICAL RISK FACTORS FOR MORTALITY IN UGANDAN CHILDREN WITH SEVERE MALARIA**

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Few studies have assessed risk factors for mortality in severe malaria since artesunate replaced quinine as first-line therapy for severe malaria in most African countries. We assessed clinical risk factors for mortality in Ugandan children admitted to a regional (Jinja) or national (Mulago) referral hospital with one or more of the five most common manifestations of severe malaria: cerebral malaria (CM), respiratory distress (RD), malaria with

complicated seizures (MS), severe malarial anemia (SMA) or prostration. Children were treated according to national guidelines for severe malaria, which included parenteral artesunate as first-line anti-malarial therapy. 581 children age 6-48 months were enrolled in the study, with a mean (SD) age of 2.1 ±0.9 years. The overall mortality rate was 43/581(7.4%), and 60% of the deaths occurred within 24 hours of admission. Mortality was highest in CM (29/84, 34.5%) and RD (12/115, 10.4%), while mortality in MS, SMA and prostration was low (1/158, 0.6%; 0/150, 0%; and 1/74, 1.3%, respectively). Coma (odds ratio, 95% confidence interval, 16.0, 6.9-37.5), acidosis (base excess ≤ -8mmol/L) (6.1, 1.6-22.5), blood urea nitrogen ≥ 20mg/dL (2.6, 1.1-6.1) and respiratory distress (3.6, 1.5-9.0) were independently associated with increased mortality among all children with severe malaria. The presence of coma, acidosis and elevated blood urea nitrogen was associated with 58.8% mortality, and the addition of respiratory distress increased mortality to 71.4%. Research to identify and prevent the causes of coma, acidosis, respiratory distress and uremia in severe malaria has the potential to substantially decrease malaria-associated mortality.

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#### COVERAGE OF AND FINANCIAL RISK ASSOCIATED WITH UNCOMPLICATED MALARIA TREATMENT AMONG CHILDREN UNDER FIVE YEARS IN MALAWI: EVIDENCE FROM NATIONAL SURVEYS

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Universal health coverage (UHC) entails access to quality healthcare without incurring financial hardships from healthcare payments. Despite UHC gaining traction as a priority public health goal in the post 2015 development agenda, and tracking progress and social protection being essential to the strategy's monitoring framework, few studies have assessed UHC for priority tropical diseases. This study investigated the coverage of and financial risk associated with treatment of uncomplicated malaria in children ≤ 5 years in Malawi. Using secondary data from two sources, the 2010 Malaria Indicator Survey (MIS) and the 2010-11 Integrated Household Survey (IHS), we obtained information on episodes of fever, use of health services, time of treatment, days lost seeking and receiving care, and welfare in relation to consumption. We estimated coverage of uncomplicated malaria treatment and associated financial risk ascertained as the ratio of malaria expenditure to non-food expenditure. We present results by social economic tertiles; poor, middle poor and least poor. Of the 2115 children with complete data, 23.8% had uncomplicated malaria of which 30.1%, 27.1% and 12.3% were poor, middle poor and least poor respectively. Overall, 26.4% of the children ≤ 5 years obtained treatment within 48 hours of fever onset. The proportions were 14.2%, 35.7% and 32.7% among the poor, middle poor and least poor respectively. Overall financial risk was 0.90%; across social economic groups this was 0.92%, 0.98% and 0.65% for the poor, middle poor and least poor respectively. No household experienced catastrophic health expenditure. The economic burden of uncomplicated malaria among children ≤ 5 years in Malawi is very low; thus household costs on uncomplicated malaria are not catastrophic. This indicates that the health system, with regards to uncomplicated malaria adequately protects households from financial risk. However, the results showed relative disparities across social economic groups in both malaria and financial burden, implying that Malawi's health policy needs to be adjusted to address these inequalities.

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#### CONGENITAL MALARIA AND ANEMIA IN NEONATES FROM LIBREVILLE, GABON

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In Gabon, a rebound of malaria morbidity is observed since 2011 and more than 79% of febrile children are anemic. The frequency of congenital malaria and its relationship with anemia was estimated among newborns hospitalized for neonatal infections in GABON. This was a prospective, cross-sectional study conducted between June and November 2015 in a sentinel site for malaria survey. All newborns hospitalized for a suspected neonatal infection were screened for malaria by microscopy and for anemia using an ABX system. A total of 100 newborns were included, the majority (77%) was less than 8 days old, 23% were very preterm and half the population had a low birth weight. Malaria was the most common mother infectious disease presented during pregnancy. The frequency of congenital malaria was 21%, with 13 children having both malaria and bacterial infection. The median parasitemia was 1500[750-4000]p/μL. The median Hb level was 15.4[11.4-17.1]g/dL and the frequency of anemia was 52%; almost all (89%) the 8 to 15-day-old babies were anemic and 66.6% of them had moderate to severe anemia. Among newborns with malaria, 62% were anemic (*versus* 38% of malaria negative ones). IPTp-SP, number of ANC visits, HbSS, were not associated with anemia in newborns. In conclusion, congenital malaria and neonatal anemia burden seems to be high among neonates and malaria is strongly associated with neonatal anemia.

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#### IMPACT OF AGE ON DIHYDROARTEMISININ-PIPERAQUINE EXPOSURE IN YOUNG UGANDAN CHILDREN

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Artemisinin-based combination therapies (ACTs) are standard for the treatment of malaria in children in Africa. Dihydroartemisinin-piperazine (DHA-PQ), an ACT with excellent antimalarial efficacy in Africa, is being evaluated for chemoprevention in children. However, our understanding of the impact of age on DHA-PQ pharmacokinetics (PK) is limited. We compared the PK of PQ in Ugandan children enrolled in a DHA-PQ chemoprevention trial at 32 and 104 weeks of age using an intensive PK design. PQ levels were collected after the 3rd dose of a standard weight-based regimen of DHA-PQ (daily dose x 3 days). Venous blood samples were taken at 0, 0.5, 1, 2, 3, 4, 6, 8, and 24 hours after the dose. Capillary blood samples were also collected 24 and 48 hours after the third dose, and on days 7, 14, and 21. To determine the impact of maturation on PQ disposition, PK parameters were compared between the same 31 children at 32 and 104 weeks of age, and between children at 104 weeks of age (n=31) and non-pregnant adult women (n=24) to compare PK in children and adults. PQ levels were measured by LC-MS/MS and the PQ area under the concentration-time curve (AUC) was measured over 21 days. Preliminary data indicate reduced PQ exposure in children aged 104 versus 32 weeks of age, as measured by AUC (6.60 vs 9.54 hr.ug/mL, p<0.01) and terminal PQ concentrations on days 7, 14, and 21 (6.19, 3.46, 2.14 ng/mL vs 12.6, 6.20, 3.55 ng/mL; all p values <0.03). Comparing children at 104 weeks of age to adults, we found decreases in PQ AUC (6.60 hr.ug/mL vs 17.6 hr.ug/mL, p<0.0001) and terminal levels on days 7, 14 and 21 (6.19, 3.46, 2.14 ng/mL vs 30.9, 17.7, 11.2 ng/mL, p<0.0001, respectively). PQ exposure was significantly lower at 104 weeks compared to 32 weeks of age. Children at 104 weeks of age exhibited significantly

lower exposure than adults. Children at both ages exhibited terminal PQ concentrations (7, 14, and 21 days) <15 ng/mL. The clinical relevance of these findings requires further study.

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### QUALITY OF CARE DETERMINANTS OF COMMUNITY CASE MANAGEMENT OF UNCOMPLICATED MALARIA IN WESTERN KENYA

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Quality of service is important in management of malaria. Prevention and control of malaria will not be achieved without timely diagnosis, treatment and monitoring. Kenya, a country with high poverty rate, has adopted World Health Organization's recommendation of community case management of malaria (CCMM). This concept has now been implemented for over four years and little or no information is available on the quality of services provided by community health volunteers (CHVs). Amref Health Africa with Global Fund has supported this in western Kenya. This study established determinants of quality of service of CCMM by CHVs. A cross sectional survey was conducted and the study lasted four weeks. The CHVs were observed as they carried out their duties and various aspects were noted and thereafter administered a questionnaire. Data was analysed using SPSS. Majority (62%) of the CHVs were found to offer quality services based on 75% cut off score considering their low literacy levels. It was realised that there was relationship at  $P < 0.05$  among stock outs of Artemether lumefantrine ( $P=0.037$ ), rapid diagnostic test kits ( $P=0.001$ ) and support supervision ( $P=0.007$ ) to quality of services offered by CHVs. Other factors including sociodemographics (such as marital status, gender, religion, and education level), referrals, other commodities except ALS and RDTs had no association with quality of services. CHVs who had ever had support supervision were 4 times likely to perform better than those not support supervised. CHVs who did not experience Artemether lumefantrine and rapid diagnostic test kits stock outs were most likely to perform three times better than those who experienced stock outs (AUOR 3.2 and 3.3 respectively at 95% CI). A good percentage of CHVs offered quality services in CCMM. Steady and continuous supply of RDTs, ALS and provision of support supervision are essential in the performance of CHVs in management of uncomplicated malaria at community level.

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### TOWARD IMPROVED HEALTH SYSTEMS RESPONSIVENESS: A CROSS-SECTIONAL STUDY OF MALARIA ENDEMICITY AND READINESS TO DELIVER SERVICES IN KENYA, NAMIBIA AND SENEGAL

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New, straightforward approaches for measuring health system responsiveness to disease are needed toward continued health systems strengthening. Despite good progress toward elimination, malaria continues to contribute substantially to the sub-Saharan African disease burden. Sustaining previous gains requires continued readiness to deliver health services in response to actual disease burden. In this study, as a measure of health system responsiveness, we examined whether malaria prevalence is a driver of health facility readiness to deliver services. Using a cross-sectional design, we examined the relationship between malaria prevalence, or endemicity, and facility readiness to deliver services

using facility survey data for Kenya, Namibia, and Senegal geo-linked to endemicity data. We tested the validity and reliability of the malaria service readiness index, the primary study outcome, and mapped service readiness components in a geographic information system. We conducted a weighted multivariable linear regression analysis of the relationship between endemicity and malaria service readiness, stratified for urban or rural facility location. As endemicity increased in rural areas, there was a concurrent, modest increase in service readiness at the facility level [ $\beta$ : 0.028; (95% CI: 0.008, 0.047)], whereas no relationship existed in urban settings. Private-for-profit facilities were generally less prepared than public [ $\beta$ : -0.102; (95% CI: -0.154, -0.050)]. Most facilities had the necessary supplies to diagnose malaria, yet availability of malaria guidelines and adequately trained staff as well as medicines and commodities varied. Findings require cautious interpretation outside the study sample, which was a more limited subset of the original surveys' sampling schemes. Our approach and findings may aid national malaria programs in identifying variations in facility performance for targeted service delivery interventions.

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### ANTIPLASMODIAL ACTIVITY IN *COCOS NUCIFERA* LEAVES FROM THE NATURAL RESERVE OF PUNTA PATIÑO, DARIÉN

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For the past few decades the search for biologically active compounds from nature has been intensified, in response to the failure of commercially available drugs due to malaria parasite resistance and costs. *Cocos nucifera*, the coconut tree, has been traditionally used to fight a number of human diseases and symptoms like diarrhea and fever, including malaria. Previous studies have associated its therapeutic activity with the presence of phenolic metabolites in the plants' husk, inflorescence, fruit and water. To date, only the husk has been reported of containing compounds against *Plasmodium falciparum*, despite other plant parts being traditionally used to treat patients. In this study we analyzed the leaves of *C. nucifera* in order to test its activity against *P. falciparum*. We sampled the leaves from *C. nucifera* plants collected from a private natural reserve in Punta Patiño, Darién. The aqueous extract of a 15 min decoction of the leaves was used against *P. falciparum* *in vitro*. Positive results with the extract (up to 70% growth inhibition at 10% v/v) led to a solid phase extraction (SPE) of the crude extract which produced fractions for further testing. The active fraction and the decoction were used in ultra performance liquid chromatography (UPLC) coupled to a mass spectrometer (LC – MS/MS) for the identification of the chemical compounds in the extracts. A series of polyphenols were found, most of them belonging to the flavan-3-ol, with epicatechin being one of them, and some flavones such as isoorientin (luteolin-6-C-glucoside). The aqueous decoction of leaves as well as the active fraction obtained by SPE were tested for cytotoxicity in Vero cells at the same antiplasmodial concentrations and no effect was observed. This suggests that the activity against *P. falciparum* from the leaves of *C. nucifera* might be caused by phenolic compounds, and that this finding merits further research to isolate and identify the possible active molecules.

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### USING GENOMIC DATA FOR OPERATIONAL DECISION MAKING IN MALARIA ELIMINATION IN SENEGAL

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Progress toward malaria elimination requires new tools and strategies to guide decision-making at a programmatic level. Molecular genetic signatures including parasite relatedness can help track drug resistance, transmission dynamics, and sources of new infections. Combining molecular genetic signatures with clinical, epidemiological, and serological indicators can identify parasite population structure and connectivity between different transmission foci. Integration of these data with health management information systems like DHIS2 can better inform decision-making. We applied molecular epidemiological tools to estimate transmission trends in Senegal based on metrics including complexity of infection, parasite relatedness, and clonality. Epidemiological modeling of genetic data detected both declines and rebounds in malaria transmission (R0) over the past decade in Thies, Senegal. In the high transmission area of Kedougou, Senegal, increased intervention deployment over the past 4 years resulted in comparable genetic signals of increasing parasite relatedness and greater clonality. Between 2013 and 2015 we observed increased monogenomic infections (from 44% to 72%), and 8% of monogenomic infections exhibit shared genomic signatures in 2015 versus none in 2013. Hence simple genomic tools, such as a molecular barcode to identify parasite lineages, rapidly reveal trends of parasite relatedness and clonality related to transmission dynamics across Senegal. Drug resistance marker surveillance tracks the consequences of increasing drug pressure on these populations. These results exemplify the utility of barcoding relatively small numbers of individual samples to provide real-time, informative data with operational implications for national and regional policies and practices to eliminate malaria. We present development of a dashboard that integrates molecular epidemiology with DHIS2 monitoring of health interventions toward malaria elimination.

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### MANIFESTATION OF MALARIA IN MANGALORE, SOUTHERN INDIA

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Severe *Plasmodium vivax* malaria including cerebral cases and fatalities has been reported from India. In Mangalore, Karnataka, southern India, malaria is focused in urban areas and potentially associated with importation by migrant workers from endemic regions of the country. In the largest governmental hospital, we aimed at assessing the clinical, parasitological, and biochemical characteristics of malaria patients and at determining respective differences between vivax and falciparum malaria. 960 patients with PCR-confirmed *Plasmodium* infection were included. Patients were predominately male (92%), migrants from other parts of India (77%), and of overall low socio-economic status. The mean age was 30 years (range, 1-82). *P. vivax* mono-infection (69.4%) predominated

over *P. falciparum* mono-infection (9.0%) and mixed *P. vivax-P. falciparum* infection (21.4%). The geometric mean parasite density was 3776/mcl (95%CI, 3403-4189). 5.3% of patients were hospitalized, with a slightly increased proportion among *P. falciparum* infected patients (7.2%). As compared to vivax malaria patients, those infected with *P. falciparum* had significantly higher parasite density, higher axillary temperature, and lower hemoglobin levels. Moreover, patients with *P. falciparum* more frequently showed vomiting, splenomegaly, an impaired general condition, and a reduced level of consciousness. One patient each had prostration, cerebral malaria, and hyperparasitemia, two patients had severe anemia (Hb<5 g/dL), and 3.1% had increased creatinine (>1.4 mg/dL). No fatality occurred. In this study from southern India, uncomplicated cases of malaria largely predominated with *P. falciparum* causing slightly more intense manifestation. Severe malaria was infrequent, and no fatal cases occurred. This contrasts with the reported pattern of manifestation in other parts of India, which requires the analysis of underlying causes.

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### RAPID ACQUISITION OF ANTIBODIES TO BOTH PREERYTHROCYTIC AND ERYTHROCYTIC ANTIGENS FOLLOWING CONTROLLED HUMAN MALARIA INFECTION WITH PLASMODIUM VIVAX AND P. FALCIPARUM

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Seroepidemiological studies on the prevalence of antibodies to malaria antigens are primarily conducted on individuals from endemic regions. It is, therefore, difficult to accurately correlate the antibody responses to the timing, number and species of prior malaria infections. This study was undertaken to assess the evolution of antibodies to the dominant surface antigens of *Plasmodium vivax* and *P. falciparum* following primary malaria infection in naïve individuals. Serum samples from malaria-naïve adults were collected before and after controlled human malaria infection (CHMI) with either *P. vivax* or *P. falciparum*. Serum samples were tested for the presence of antibodies to the circumsporozoite protein (CSP) and the 42 kilodalton fragment of the merozoite surface protein-1 (MSP-142) of both *P. vivax* and *P. falciparum* by enzyme-linked immunosorbent assay. Approximately one month following CHMI with *P. vivax* or *P. falciparum* 72% and 67% of subjects, respectively, seroconverted to homologous CSP. A higher proportion of subjects - 78% for *P. vivax* and 100% for *P. falciparum* - seroconverted to homologous MSP-142. More than 60% of the subjects demonstrated reactivity to heterologous CSP and MSP-142 and a similar proportion of subjects remained seropositive to homologous MSP-142 at > 5 months post CHMI. This study demonstrates the immunogenic nature of sporozoites as despite the short duration for which they are present in circulation prior to their migration to the liver, they are able to induce detectable anti-CSP antibodies. As expected, a higher proportion of subjects react to the blood stage antigen MSP-1 and more than half the subjects continue to demonstrate reactivity to MSP-1 several months post exposure. Presence of long-lived and heterologous reactivity may confound the determination of time and species of exposure in field settings.

### FURTHER EVALUATION OF THE PROTOTYPE NON-WOVEN FABRIC (NWF) FILTER FOR THE PURIFICATION OF *PLASMODIUM VIVAX*-INFECTED ERYTHROCYTES UNDER FIELD CONDITIONS

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Isolation of *Plasmodium*-infected red blood cells (iRBCs) is often required for experiments such as *ex vivo* drug assays, *in vitro* invasion assays, and genome sequencing. A prototype non-woven fabric (NWF) filter was developed for the purification of iRBCs, which showed great efficiency for removing WBCs in a pilot study. With the commercialization of the filters, this study aims to evaluate the efficiency and suitability of the commercial NWF filter for the purification of *Plasmodium vivax*-infected RBCs in smaller volumes of blood in a field laboratory, and to compare its performance with that of Plasmodipur® filters. Forty three clinical *P. vivax* blood samples taken from symptomatic patients at the China-Myanmar border were processed using the NWF filters. The numbers of WBCs and iRBCs and morphology of *P. vivax* parasites in the blood samples before and after NWF filtration were compared. The viability of *P. vivax* parasites after filtration from 27 blood samples was examined by *in vitro* short-term culture. In addition, the effectiveness of the NWF filter for removing WBCs was compared with that of the Plasmodipur® filter in six *P. vivax* blood samples. Filtration of 1-2 mL of *P. vivax*-infected blood with the NWF filter removed 99.68% WBCs. The densities of total iRBCs, ring and trophozoite stages before and after filtration were not significantly different ( $P > 0.05$ ). After filtration, the *P. vivax* parasites did not show apparent morphological changes. Culture of 27 *P. vivax*-infected blood samples after filtration showed that parasites successfully matured into the schizont stage. The WBC removal rates and iRBC recovery rates were not significantly different between the NWF and Plasmodipur® filters ( $P > 0.05$ ). In conclusion, when tested with 1-2 mL of *P. vivax*-infected blood under field laboratory conditions, the NWF filter could effectively remove WBCs and the recovery rates for ring- and trophozoite-iRBCs were high. The performance of the NWF and Plasmodipur® filters for removing WBCs and recovering iRBCs was comparable.

### IS TARGETED REACTIVE VECTOR CONTROL A NON-INFERIOR SUBSTITUTE FOR GENERALIZED INDOOR RESIDUAL SPRAYING IN AREAS OF VERY LOW MALARIA TRANSMISSION - RESULTS FROM A CLUSTER RANDOMIZED TRIAL

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In areas of very low, pre-elimination malaria transmission the small number of incident cases make it difficult to sustain resources for spraying all houses annually with insecticide (generalised indoor residual spraying (GIRS)). An alternative that may be more sustainable is to target IRS (TIRS) reactively only at houses in the immediate neighbourhood of

incident cases as they arise. Of 62 clusters consisting of settlements of approximately 5000 persons each in the provinces of Mpumalanga and Limpopo in South Africa, half were randomly allocated to receive the currently practised GIRS whilst the other half received reactive TIRS in response to every passively reported local case. TIRS was carried out in the immediate neighbourhood of about 8 houses per index case. Dried blood spots were collected from neighbourhoods of index cases, and in randomly selected neighbourhoods where there were no cases. Non-inferiority of TIRS was assessed in comparison to GIRS based on passive case incidence in the two study arms. Age-standardised antibody responses, sero-prevalence and seroconversion rates to malarial antigens were compared between neighbourhoods of index cases and randomly selected comparison neighbourhoods, to determine whether new cases arise predominantly in areas of previous exposure to parasites. Results of the trial will be presented. If cases arise predominantly in areas with populations who have elevated seroprevalence, then serological surveys would be highly informative for proactive targeting of vector control interventions. If targeted IRS is non-inferior to generalised IRS, it could make vector control in very low transmission settings more sustainable and more effective.

### STUDY OF QUALITY OF ANTIMALARIAL DRUGS IN INDIA

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Substandard and counterfeit antimalarial medicines poses a serious threat to public health. These counterfeit/ substandard medicines increases the mortality by decreasing efficacy; it also increases the threat of emergence of drug resistance, adverse effect from incorrect excipients/ active ingredients which may be potentially dangerous to the patients. Owing to this, a pilot study was conducted to survey quality of drugs collected from different malaria endemic areas of India. The survey was conducted in different geographical regions on the basis of malaria endemicity i.e. Uttar Pradesh (U.P.), Mizoram, Meghalaya, Gujarat, Madhya Pradesh. Antimalarial samples of ACT (Artesunate+ Sulphadoxine-pyremethamine), (Artesunate+Lumefantrine), Chloroquine, Primaquine were collected for qualitative analysis. A mystery shopper approach was used for collection of samples. The quality of antimalarial drugs from these areas were assessed by using Global Pharma Health Fund Minilab test kit. This includes physical/visual inspection and disintegration test, thin-layer chromatography. High performance liquid chromatography was carried out for quantitative assessment of active pharmaceutical ingredient. A total of 101 brands out of which 38 were for CQ, 38 for AL, 7 for AS+ SP, 18 for primaquine were tested from different sites. In this study 97.03% of the tablets passed minilab disintegration, 2.97% consisting did not passed disintegration test. The variable disintegration and retention factor might be due to improper handling during storage, humid temperature, transportation and distribution. However, HPLC analysis confirms standard active pharmaceutical ingredient in the tablets.

### ASSOCIATION BETWEEN PLASMA AND CEREBROSPINAL FLUID BIOMARKERS AND NEUROPSYCHOLOGICAL OUTCOMES AMONG CHILDREN WITH CEREBRAL AND SEVERE MALARIA IN UGANDA

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Cognitive impairment following severe malaria can result in compromised social and behavioral performance in children. We aimed

to explore the association between immunological biomarkers (IB) and neuropsychological (NP) outcomes among Ugandan children surviving cerebral malaria (CM), severe malaria anemia (SMA) and community controls (CC). Sample included 300 children: 93 (31%) with CM, 57 (19%) with SMA, and 150 (50%) CC. Von Willebrand Factor (VWF), Granulocyte Colony Stimulating Factor (GCSF), and Tumor Necrosis Factor-alpha (TNF- $\alpha$ ) were collected in plasma for all and in CSF in children with CM. NP tests included Kaufman Assessment Battery for Children (2nd ed., KABC-II), Behavior Rating Inventory for Executive Function (BRIEF), and Achenbach Child Behavior Checklist (CBCL). For each group, general linear models were used to relate NP scores to IB. Models were adjusted for anthropometric and socioeconomic variables. 158 boys and 142 girls (p=0.01) ages 5-12.8 years were enrolled. Median time from clinical disease to NP test was 2.6 years. In plasma, higher VWF was associated with lower KABC-II scores in 3 memory subtests (p=0.01, p=0.05, p=0.02) in SMA and with 2 subtests in CC children (p=0.05, p=0.04). Higher VWF was related to lower BRIEF in CM (p=0.01) but higher scores among SMA children (p=0.02). Higher VWF was associated with more Externalizing Problems (CBCL) in SM children (p=0.03). Higher GCSF in CSF was associated with more Externalizing Problems (p=0.03) (CBCL) in CM children. In plasma, higher GCSF was associated with lower KABC-II score (p<0.01) in SM children, and with lower BRIEF scores (p=0.02), and CBCL's Total Problems (p=0.03) in CC. Differences could respond to multiple comparisons performed, increasing the chance of a random association of p<0.05. Results suggest preliminary evidence towards the role of endothelial activation and angiogenesis factors in predicting neurological damage among children surviving severe malaria. Further assessment is needed to fully characterize these pathways, specifically, longitudinal studies that will allow to assess time related association between IB and NP.

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### A PILOT STUDY TO DETERMINE THE FEASIBILITY OF FAMILY PLANNING WORKERS IN BANDARBAN, BANGLADESH DELIVERING MALARIA CASE MANAGEMENT IN THEIR COMMUNITIES

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Malaria is endemic in border districts (13/64) of Bangladesh mainly in remote areas with poor access to appropriate and timely care. Pregnant women and children are at risk. To deliver quality malaria care at community level, coordination between departments of the Ministry of Health and Family Welfare (MOHFW) and the National Malaria Control Program (NMCP) is crucial. A previous study in the Bandarban Hill district observed first-line family planning health workers (FPHW) lacked adequate training to deliver malaria care at the primary level. Results indicated community members with malaria symptoms seek care elsewhere, causing delays in treatment, increased risk of severe illness and death. This pilot study was commissioned by the NMCP and the Family Planning Department to determine the feasibility of increasing access to malaria diagnosis and treatment, especially for pregnant women and children U5, in communities where they live. A central training of the trainers (ToT) was conducted for National Trainers (n=29) from the MOHFW. Trainers learned adult learning and facilitation skills; malaria epidemiology; rapid diagnostic testing; treatment for uncomplicated malaria; referral of severe cases; use of interpersonal communication skills in treating and preventing malaria; supervision skills and use of tools to improve performance of FPHWs during routine supervision. Trainers then trained FPHWs (n=57) in Bandarban. The average improvement in trainer knowledge was 37% (pre- and post-tests scores, 51% and 88% respectively), and average trainee improvement was 36% (pre- and post-tests 49% and 85% respectively). Following the training, 3 supervision visits were conducted to observe FPHWs proficiency in malaria management competencies. Competency

scores improved from 54% during visit 1, to 57% in visits 2 and 3. This pilot study showed it was feasible and acceptable to integrate FPHWs in malaria elimination initiatives and to deliver quality community malaria case management. Additional districts could benefit from a similar training and supervision programme towards malaria elimination.

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### SOCIO-DEMOGRAPHIC FACTORS INFLUENCING INSECTICIDE TREATED NET USE AMONG NIGERIAN UNDER-FIVES: EVIDENCE FROM A NATIONAL SURVEY

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The use of Insecticide-treated nets (ITN) is the most cost-effective malaria preventive measure. However, malaria remains a big killer of children in Nigeria, accounting for 11 percent of maternal mortality, 25 percent of infant mortality and 30 percent of the under-fives (U5) deaths. Despite massive distribution campaigns nationwide, ITN use among (U5s) is low. This study assessed factors influencing ITN use among U5s across households in Nigeria. We analyzed the 2015 Nigeria Malaria Indicator Survey with specific focus on ITN use in children. A total of 7,008 under-fives all slept in the surveyed households a night before the survey. However, 5,397 (77%) under-fives (U5s) slept in households with at least one ITN the night prior to the survey were studied. Binary logistic regression analysis was conducted to identify factors influencing ITN use by U5s in (1) households that have at least one ITN and (2) households with at least one ITN for every two people (universal coverage). Data analysis was conducted using STATA (version 14.0). In households with ITN ownership, only 56.6% of U5s slept under the ITN the night before the survey. Significantly, children less than one year old, those who reside in rural areas, those from other regions of the country except the South East had higher odds of using ITN the night before the survey. Also, children from households with less than 7 members and those from the lowest and second wealth quintiles had significantly higher odds of using ITN. On the second regression model among households with universal coverage, U5s who reside in rural areas, those from other regions of the country except the South West and those from the lowest and second wealth quintiles still had significantly higher odds of sleeping under ITN the night before the survey. In conclusion, our findings have programmatic implications for malaria control among U5s and malaria elimination in Nigeria. Interventions to improve ITN use should be intensified among urban dwellers, and residents of South-Western and South-Eastern Nigeria.

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### ASSESSING THE USEFULNESS OF A THRESHOLD FOR DETECTING MALARIA EPIDEMICS IN TANZANIA: A CASE STUDY IN HAI DISTRICT (KILIMANJARO REGION) AND BUKOBA MUNICIPAL COUNCIL (KAGERA REGION)

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Health officials need a threshold to detect an emerging malaria epidemic so they can take quick action to contain the spread of infection and reduce morbidity and mortality. Most methods to define epidemic thresholds require data going back five years or more. In settings such as Tanzania, where such data are not available, precise thresholds are hard to establish. There, the World Health Organization recommends the use of a three-week rolling average to set the threshold. Our study sought to assess the usefulness of this threshold. Weekly malaria data are reported from health facilities through electronic Integrated Disease Surveillance and Response (e-IDSR) using mobile technology and can be viewed in the District Health Information System (DHIS). The system sets the threshold at a twofold increase of malaria cases in the current week compared to the average of malaria cases in the previous three weeks (3 weeks AVG\*2). An alert is supposed to be triggered if the threshold is exceeded by the number of malaria cases reported in that particular week. Because no such alert has been triggered to date, we analyzed all positive malaria cases reported from week 11 of 2014 to week 12 of 2017 in Bukoba Municipality (Kagera Region) and Hai district (Kilimanjaro region) for trends in malaria cases, to see if epidemics had been undetected. In year 2015, we found that in Hai district, the reporting rate was 53% and malaria cases reported were 1,317. In weeks 18 (102 cases reported) and 23 (144 cases reported), the set thresholds were 42 and 65 cases respectively, which should have triggered the alarm. In Bukoba Municipality, we found that, in year 2016, the reporting rate was 91% and malaria cases reported were 7,461. The highest number of malaria cases were reported in weeks 3, 4, 5 and 6 with 327, 359, 326 and 316 cases which should have been investigated but, the set epidemic threshold were 653, 695, 735 and 718 cases which did not exceed to trigger alarm. These findings suggest a need to further review available historical data in order to calibrate more accurate thresholds for the malaria surveillance system to generate automatically, maximizing the system's usefulness.

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### MALARIA CONTROL: A REALIST REVIEW

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This paper presents a qualitative systematic review on various malaria control strategies, to uncover what works or doesn't work, for whom and in what context. The paper works to inform what current malaria control strategies are most useful and what barriers exist with access and use, to discover the gaps in current knowledge and suggest possible areas requiring further research. We structured the search with the following steps: a scoping review of literature on malaria control to map the evidence base and define the scope of the review; a search for evidence of what works or doesn't work; extraction of data and synthesis of evidence; and development of a narrative which includes a hypothesis. We restricted the literature to focus on tools that were available in endemic countries. There is a wealth of evidence that speaks to why malaria interventions don't work. Experiences speaking to sustained use of interventions depends on understanding how the intervention works and seeing it work consistently over time. This improves the buy in and likelihood of an individual to comply with use and maintain use in the long term, assuming they accept the design and experience minimal barriers. Results so far suggest Behaviour Change Communication is a fruitful strategy to improve uptake and use of malaria control interventions. However, more work needs to be done on promoting maintenance of change in behaviour.

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### REDUCED MALARIA COMMODITY STOCK-OUTS AT HEALTH FACILITY LEVEL THROUGH MONTHLY SUPERVISION IN BENIN

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To reduce stock-outs of essential malaria commodities at health facilities (HFs), the President's Malaria Initiative (PMI) piloted a monthly supervision approach in two purposively selected health districts in Benin, Parakou-N'Dali (PKN) and Come-Bopa Houeyogbo-Grand Popo (CBGH). These districts were selected based on having average (PKN) and low (CBGH) Logistics Management Information System (LMIS) performance scores, which was comprised of completeness, timeliness and accuracy of the LMIS reports and data. Quality Improvement Teams (QITs) made monthly visits to all HFs in PKN (20) and CBGH (48) for a nine-month period in 2015. During the monthly visits, the QITs collected information on malaria commodity availability, prescription data, and pharmacy records. They also assessed compliance with the LMIS parameters, completeness and timeliness of LMIS reports, as well as consistency, accuracy and quality of the LMIS data. During the intervention period, the proportion of HFs with health workers able to correctly fill out a LMIS report increased from 21% to 84% in CBGH and from 16% to 90% in PKN. During the same time period, the degree of discordance between the quantities of Artemisinin Combination Therapy (ACT) prescribed (as recorded in the treatment registers), and the quantity dispersed (as documented in the monthly pharmacy logs), reduced from 93% to 19% in CBGH, and from 73% to 18% in PKN. Stock-outs of Artesunate/Lumefantrine (as a proxy for all ACT presentations) also declined in both districts. Close follow-up and coaching by the QITs contributed to more reliable logistics data and improved tracking and availability of malaria commodities at the point of service delivery. The team based approach fostered effective problem solving and local resource mobilization to address identified needs by the District Management Teams. Chief Medical Doctors are able to conduct quality control by linking prescription and dispensation data. This capacity building approach is recommended in health districts with poor supply chain management at the HF level. Further assessment is needed to determine the sustainability of the approach.

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### MALARIA PROGRAMS IMPLEMENTATION IN EBONYI STATE, NIGERIA: WHERE ARE WE?

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Despite important strides in recent years, Nigeria has yet to achieve global targets of universal coverage for malaria case management nor 80% coverage for malaria in pregnancy. While available malaria interventions are effective, critical health system challenges undermine implementation. Jhpiego has developed a health systems framework and planning tool to assist malaria control programs identify and respond to these challenges. The tool was recently used with the Ebonyi State Malaria Control Program (MCP) with Jhpiego's guidance. An initial situational analysis used the 2015 Malaria Information Survey to highlight that 89% of state households had long lasting insecticide treated bed-nets but only 50% of children under five used them. Likewise, intermittent preventive treatment during pregnancy (IPTp) was only 44% for two IPTp doses and 41% for three. Use of parasitological diagnosis for malaria was low and

unacceptable. A subsequent meeting among MCP and Jhpiego staff was held to review nine health systems areas to determine reasons for the low performance on malaria indicators. The group reviewed strategies and annual workplans and then ranked each health system area on a scale from 1 (low) to 4 (high) to reflect level of progress, and then the average score computed. The highest scoring components were human resource capacity (3) and integration and coordination (3), based on findings such as integrated supportive supervision and the holding of monthly coordination and review meetings among partners at the state and local level. Community Involvement (1.9) and finance (1.8) scored lowest, based on lack of community outreach and engagement, in control efforts, and late/ sporadic release of funds for program implementation, respectively. In response, the group drew up action plans to address identified weaknesses and used monthly partners meetings for advocacy and learning. In conclusion Nigerian health workers can use health systems analysis and planning tools to identify best practices, address challenges, and create an action plan to help advance their state (and country) along the pathway to malaria elimination.

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### REGULATORY T CELL-MEDIATED SUPPRESSION OF HUMAN IMMUNE RESPONSES TO INVESTIGATIONAL MALARIA VACCINES

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Natural malaria and helminth infections induce upregulation of Regulatory T cell (Treg) responses in humans. Increased Treg frequencies may play a role in causing the immunosuppression known to be associated with malaria infection and may contribute to the reduced immunogenicity observed for some experimental malaria vaccines when trialled in malaria-endemic sites in Africa. Increased total CD4+ CD25+ FoxP3+ CD127lo Treg frequencies at baseline, as measured by flow cytometry, negatively correlate with peak antigen-specific CD4+ T cell and antibody titres in the investigational vaccine regime ChAd63 ME-TRAP / MVA ME-TRAP, but only with antigen-specific CD4+ T cell responses to RTS,S in malaria-naïve European volunteers. Both vaccine regimes themselves induced antigen-specific Treg responses, however these do not correlate with decreased antibody or effector CD4+ T cell titres. Instead, high baseline Treg frequencies appeared to be suppressive of antigen-specific Treg induction as well, demonstrating that pre-existing Tregs might be most important in suppression of vaccine-induced immunity. Therefore, in a setting where populations have naturally higher circulating Treg frequencies due to parasite infections, responses to vaccines could be suppressed. Immunogenicity and Treg frequency data from a Kenyan cohort vaccinated with ChAd63 ME-TRAP / MVA ME-TRAP will be presented. Functional data from Treg suppression assays will also be presented to understand the mechanistic basis of the heterologous effects of Tregs on different experimental malaria vaccines.

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### SAFETY AND IMMUNOGENICITY OF THE MALARIA VACCINE CANDIDATE R21 ADJUVANTED WITH MATRIX-M1 IN WEST AFRICAN ADULT VOLUNTEERS, BURKINA FASO

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A highly effective malaria vaccine would help reduce disease burden in malaria endemic countries. We have assessed the safety and immunogenicity of the malaria vaccine candidate R21, a virus-like particle comprising a fusion protein of part of the *P. falciparum* circumsporozoite protein with the hepatitis B surface antigen. A phase Ib randomised, controlled, single-blind study was conducted. Volunteers were randomly assigned in a 2:1 ratio to receive 3 doses of the malaria vaccine or the control (normal saline) intramuscularly 1 month apart. Participants were actively followed up at home daily during the 7 days following each vaccination to collect local and systemic solicited adverse events. Serious adverse events were recorded throughout the study duration. Venous blood samples were obtained for clinical safety and immunological evaluations. The study duration was 140 days for each participant. A total of 8 and 5 participants have received respectively 10µg of the malaria vaccine or a saline control immunisation. The most reported local solicited symptoms post vaccination were mild to moderate pain (25%, 2/8) and swelling (12.5%; 1/8) at injection site following each vaccination; all reported in the R21 vaccine group. Solicited systemic symptoms include moderate joint pain and headache. Neither severe adverse event nor serious adverse event was recorded. There was significant increase of anti-NANP IgG following vaccination in the R21 group compared to the controls. This was the first administration of R21 malaria vaccine candidate in Africa. The vaccine was immunogenic in semi immune adults with a good safety profile. These data are supportive for further development of the R21 malaria vaccine candidate.

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### PROTEOMIC CHARACTERIZATION OF ERYTHROCYTE DERIVED MICROVESICLES FROM MALARIA INFECTED CHILDREN

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Red blood cells infected with *Plasmodium* parasites produce microvesicles containing parasite derived proteins and mRNA. Previous studies have shown that the production of red blood cell-derived microvesicles (RMVs) is increased during malaria infection, with a correlation between elevated RMV concentration and increased severity of disease. These microvesicles have been shown to have numerous effects, including induction of transmission, upregulation of inflammation and inter-parasite communication. Purified RMVs from infected individuals have previously been seen to elicit an acquired immune response and diminish lethality from *Plasmodium* infections in mice. The goal of this study is to explore the role of RMVs from infected erythrocytes in the development of humoral immune response to malaria. We hypothesize that RMVs play a role in displaying *Plasmodium* proteins to the host immune system, leading to the production of protective antibodies. To achieve this, RMVs from malaria-infected children's plasma were isolated and analyzed using electron microscopy and proteomic approaches. Proteomic analysis indicates that several *Plasmodium* proteins are enriched in the microvesicles. Conserved proteins indicated to be consistently present in RMVs will be recombinantly expressed for characterization and use in immunosurveillance studies. The presence of naturally acquired antibodies against putative *Plasmodium* antigens in RMVs is to be cross-referenced with reduction in parasite burden and protection against disease. Results from this immunosurveillance will indicate the viability of using antigens found to be enriched in RMVs as malaria vaccine targets.

### LYMPH NODE TARGETING NANOPARTICLE BASED PLATFORM FOR MALARIA VACCINE DELIVERY

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Transmission-blocking vaccines that disrupt malaria transmission between the human and the mosquito host are considered one of the key interventions in the global effort to eradicate malaria. A highly conserved *Anopheles* mosquito midgut surface glycoprotein, Alanyl aminopeptidase N (AnAPN1), is a promising malaria transmission-blocking vaccine candidate. The AnAPN1 antibodies have been shown to completely block the development of naturally circulating isolates of *Plasmodium falciparum* and *P. vivax*. The AnAPN1-based vaccine is safe and highly immunogenic, but lacks natural boosting because the target antigen is not naturally presented to the human immune system. To circumvent this problem and improve vaccine efficacy, we have developed a polymeric biodegradable nanoparticle-based vaccine delivery platform. These biodegradable nanoparticles target the draining lymph nodes to allow effective presentation of the antigen, AnAPN1, to generate a robust priming immune response. A high-energy, multi-inlet vortex mixer based flash nanoprecipitation method was developed to generate a series of tightly size regulated virus-like biodegradable nanoparticles loaded with near infrared and Alexa488 dyes. Several biodegradable nanoparticles with different hydrodynamic diameters (30, 50, 70, and >100 nm) were tested for safety, inflammation, and mistargeting in mice. *In vivo* mice imaging studies suggest that biodegradable nanoparticle size is a key determinant of trafficking and retention in the draining lymph nodes. Our studies show that the smaller biodegradable nanoparticles (30 and 50 nm) drained more efficiently than their larger counterparts (70 and >100 nm). The co-localization of smaller biodegradable nanoparticles with macrophages and dendritic cells suggest cellular uptake by lymph node resident antigen presenting cells. We will leverage the lymph node targeting biodegradable nanoparticles (30 nm) as part of a bimodal, nano-/microparticle vaccine delivery system to induce a long-lasting AnAPN1-specific immune response in vaccinated individuals.

### A NOVEL VIRUS-LIKE PARTICLE-BASED PLATFORM FOR MULTI-ANTIGEN MALARIA VACCINES

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Malaria remains a global health priority despite numerous interventions and is worsened by the emergence and spread of drug resistant *P. falciparum*. Currently, the most advanced vaccine, known as RTS,S, has only shown modest efficacy in clinical trials and new more efficacious vaccines are needed. The RTS,S vaccine is based on the presentation of a fragment of the sporozoite antigen CSP on the surface of virus-like particles (VLPs) based on human hepatitis B virus (HBV). However, a limitation of the HBV platform is that only small antigens or antigen fragments can be incorporated into VLPs, and not larger proteins that are typical of malaria vaccine candidates. We have developed and evaluated a novel VLP platform based on duck HBV (known as Metavax) for malaria vaccine development. This platform can incorporate large and complex proteins into VLPs and is produced in a *Hansenula* cell line compatible with cGMP vaccine production. We have established the expression of several leading *P. falciparum* malaria vaccine candidates as chimeric VLPs. This includes Pfs25 and Pfs230, which are candidate transmission-

blocking vaccine antigens, the sporozoite antigen CSP, and a candidate vaccine antigens expressed during blood-stage infection. VLP formation and display of vaccine antigens was confirmed using super resolution microscopy with antigen-specific antibodies, and electron microscopy. Correct folding and antigen presentation was evaluated by western blotting and ELISA using defined antibodies. Initial studies demonstrated that the VLPs effectively induce antibodies to malaria vaccine candidates with minimal induction of antibodies to the duck HBV scaffold antigen, and that VLPs are more immunogenic than standard recombinant antigen. These results establish the utility of this VLP platform for malaria vaccines. The expression of multiple vaccine antigens in a single platform will facilitate the development of multi-stage multi-component vaccines to achieve high vaccine efficacy and transmission-blocking immunity.

### TRUNCATION OF PFRIPR REVEALS REGION THAT INDUCES ANTIBODY WITH THE MOST POTENT GROWTH INHIBITORY ACTIVITY AGAINST PLASMODIUM FALCIPARUM

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Blood-stage malaria vaccine candidates of high efficacy against *Plasmodium falciparum* remain elusive, mainly because of antigen polymorphisms and resultant allele-specific immunity in natural parasite populations. We recently reported *P. falciparum* reticulocyte binding protein homologue 5-interacting protein (PFRipr) as a promising blood-stage vaccine candidate, and highly conserved among Ugandan isolates. PFRipr is known to interact with previously reported conserved merozoite proteins, reticulocyte binding protein homologue-5 (PFRH5) and Cysteine rich protective antigen (PfCyRPA) in an invasion complex. PFRH5 binds to basigin receptor on the surface of red blood cells and is currently in clinical development. Antibodies against wheat germ cell-free system (WGCFs) expressed recombinant PFRipr based on Pf 3D7 sequences demonstrated potent growth inhibition assay (GIA) activity of both 3D7 and heterologous FVO. However, because of difficulties associated with synthesizing a large PFRipr immunogen that consists of 1086 amino acids (aa) and 83 cysteine residues, we attempted to identify the protein region(s) with functional epitope(s). The WGCFs successfully expressed 11 PFRipr antigen truncates, each containing approximately 200 aa. Rabbit antibodies against each truncate were generated, and performed GIA. We demonstrated that antibody against PFRipr region containing 215 aa had the highest GIA activity, and a promising blood stage vaccine candidate.

### ASSESSMENT OF PLASMODIUM FALCIPARUM PFS47 AS A TRANSMISSION BLOCKING TARGET FOR MALARIA

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Malaria transmission-blocking vaccines rely on functional antibodies that interact with proteins present on the surface of sexual/sporogonic stages of *Plasmodium* or in the mosquito midgut, and disrupt vector-parasite interactions critical for malaria transmission. We recently identified the female gametocyte surface protein *Pfs47*, a three domain 6-cys protein, as a key determinant of parasite immune evasion in the mosquito vector. Polymorphisms in the domain 2 of *Pfs47* were shown critical to determine immune evasion by the parasite. We investigated whether monoclonal antibodies (mAb) to *Pfs47* have transmission blocking activity by decreasing infection of mosquitoes in a standard membrane feeding assay. Thirteen mAbs generated against the full length *Pfs47* showed modest and variable transmission-blocking activity but, through domain mapping, we observed that none of these mAbs reacted against the domain 2 of

*Pfs47*. A modified version of *Pfs47* domain 2 was expressed in *E. coli* for polyclonal and monoclonal antibody production. Purified IgG from the sera of domain 2 immunized mice showed a robust and reproducible transmission-reducing activity when compared to naïve purified mouse IgG. Two independent spleen fusions were carried out using the immunized mice for monoclonal antibody production. Monoclonal antibodies against *Pfs47* domain 2 showed transmission blocking activity. Ongoing work is directed to narrow down the specific epitopes of *Pfs47* domain 2 to target. Overall, we show that *Pfs47* domain 2 can be a target for transmission blocking strategies.

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### PBG37 IS A POTENTIAL NOVEL MALARIA TRANSMISSION BLOCKING VACCINE CANDIDATE

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Transmission-blocking vaccines (TBVs) that interrupt parasite transmission are potentially important tools for malaria eradication. However, only a handful of TBV candidates have been characterized, highlighting the urgent demand to explore novel antigens for TBVs. Here, we identified a putative *Plasmodium berghei* gametocyte-stage protein, Pbg37, and evaluated its potential for TBV. A partial fragment of predicted protein was expressed in bacteria and purified recombinant protein was used to immunize mice. Antisera against the recombinant protein elicited high titers of total IgG in mice. Western blot analysis demonstrated that Pbg37 was expressed on the gametocytes, zygotes and ookinetes. IFA showed that protein localized the outer membrane of gametocytes, gametes, zygotes, retorts and ookinetes. To elucidate the function of Pbg37, the gene was knocked out in *P. berghei* ( $\Delta$ *pbg37*). The  $\Delta$ *pbg37* line exhibited considerable defects during sexual development, including a significant reduction in gametocyte number (~60%), an altered female/male ratio (~6:1) and mature male gametocytes, and a decrease in exflagellation (~36%) and ookinete numbers (~75%). Direct mosquito feeding assay showed a reduction in oocyst numbers per midgut (~90%) and infection prevalence (~24%) of the  $\Delta$ *pbg37* line. Antisera from active immunization against Pfg37 displayed significant TB ability, shown as a significant reduction in exflagellation and in ookinete number *in vitro*, and a decrease in oocyst intensity and infection prevalence after mosquito feeding. These data, together with the conservation of the gene in *Plasmodium*, suggested that Pbg37 could be a new TBV target candidate worth of future investigations.

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### ANTI-CELTOS TRANSMISSION BLOCKING ACTIVITY IN VIVO AND IN VITRO AGAINST PLASMODIUM FALCIPARUM BY EPIOTOPE-SPECIFIC MONOCLONAL ANTIBODIES

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*Plasmodium falciparum* is responsible for most of the morbidity and mortality associated with malaria. Continued success in the global malaria elimination campaign will require development effective vaccines to prevent malaria transmission. The cell-traversal protein for ookinetes and sporozoites (CeITOS) has emerged as a leading transmission blocking vaccine (TBV) candidate. We investigated the ability of epitope-specific CeITOS monoclonal antibodies (mAbs) to block transmission of *P. falciparum* sexual blood stages to mosquito stage oocyst using both *in vitro* with a standard membrane-feeding assays (SMFA) and *in vivo* with a *P. falciparum*-humanized mouse model. Standard *in vitro* culture methods

were used to produce mature gametocytes for the SMFA. The *in vivo* studies relied on an NF54 line carrying a luciferase-expressing cassette integrated in the genome. NSG mice treated with clodronate liposome supported high-level engraftment of huRBC and can be infected by this *P. falciparum* luciferase reporter line, including development of mature gametocytes infectious for mosquitoes and leading to salivary gland sporozoites. This *in vivo* laboratory model permitted a highly sensitive *in vivo* transmission blocking assay to reliably quantify early oocyst development on day 22 post infection when stage V gametocytemia reached 0.07-0.18%. Mice were randomly divided into 4 groups and 60 min. before direct mosquito feed, each mouse in the antibody-treatment group received 16 mg/kg mAb in 200µl RPMI by *iv* injection; a blank control group received of equal volume RPMI. For the *in vitro* SMFA, 400 µg/ml of CeITOS mAb was added to gametocyte culture 60 min. prior to the mosquito feed. The presence of CeITOS mAb significantly inhibited oocyst development in mosquitoes in both *in vivo* and *in vitro* assays. Importantly, the experimental results with an innovative *in vivo* humanized mouse model confirmed that circulating anti-CeITOS antibody effectively inhibits *P. falciparum* ookinete development to oocyst in mosquitoes. These results support the development of CeITOS as a transmission blocking vaccine.

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### BRIDGING HISTORICAL LUMINEX® 200™ DATA WITH LUMINEX® FLEXMAP 3D™ DATA

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Malaria, a disease caused by *Plasmodium* parasites, influences the tropical and subtropical region of the world: Sub-Saharan Africa, South-East Asia, Latin America and Middle East with ~92% death rate among children below the age of five; creating an urgent need for a vaccine. The Walter Reed Army Institute of Research (WRAIR) International Reference Laboratory for Malaria Serology (MSL) characterizes, and quantifies serum antibody responses in malaria vaccine clinical trials. The methods commonly used include enzyme-linked immunosorbent assay (ELISA), indirect fluorescent antibody test (IFAT) and, recently, multiplex assays such as xMAP technology (Luminex Corporation, Austin, TX, USA). Previously, the Luminex® 200™ instrument was utilized for acquisition of multiplex data; with obtaining more advanced instrument, the Luminex® FLEXMAP 3D™, there was a need to compare data from Luminex 200 to the data of Luminex® FLEXMAP 3D™. The advancement of technology and the need to rapidly acquire data has driven science towards multiplexing strategies. When acquiring new instrumentation comparisons of sensitivity, resolution, and accuracy from one instrument to another is key. Therefore, comparative and quantitative testing is necessary in technology transfers such as this. In this report, several assays of *Plasmodium falciparum* immune serum samples including controls from previous clinical trials were reacted with *P. falciparum* antigen-coupled magnetic beads and analyzed simultaneously on Luminex® 200™ and Luminex® FLEXMAP 3D™ instruments. Our results shows a wider linear range and a higher MFI values on the Luminex® FLEXMAP 3D™ instrument compared with Luminex® 200™ instrument. It became evident that, the instrument used in data acquisition is as equally important as the study itself.

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**PRESERVING THE INTEGRITY OF EXPERIMENTAL HUMAN INFECTION MODELS BY ANALYTICAL TESTING**

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Clinical trials rely on the voluntary participation of subjects. Lack of subject integrity can lead to results that may jeopardize the analysis of a study. We report the case of a volunteer who was one of six control subjects undergoing controlled human malaria infection (CHMI) by the bites of malaria-infected mosquitoes. This model uses drug-susceptible 3D7 *P. falciparum* parasites. Subjects were instructed to avoid anti-malarials or drugs that might inhibit parasite development or prevent infection. It was expected that they would become parasitemic between days 9-15 post-challenge. Five of six subjects were diagnosed with malaria on days 12-14 and successfully treated with chloroquine. The last subject remained negative by blood smear and PCR through day 28. The subject reported taking an over-the-counter cold medicine on the day of CHMI but denied the use of other drugs. To explore the cause of this challenge failure, blood specimens from this subject were analysed by ultra performance liquid chromatography-mass spectrometry to look for components of the cold medicine and anti-malarial drugs that could have prevented infection. Samples from 2 days pre- through 16 days post-CHMI were available. Blood levels of the components of the cold medicine, doxycycline, primaquine, quinine, and chloroquine were measured. Chloroquine was found in all serum samples beginning day 7 after mosquito bite challenge through day 16. Serum levels of chloroquine ranged from 6.5-12.6 nM corresponding with concentrations that induce 50% growth inhibition in this strain *in vitro*. No other anti-malarials were detected. While components of the cold medicine purportedly have anti-malarial activity, none was found in the subject's blood in significant concentrations. The presence of chloroquine in the blood may explain why they did not become parasitemic. Undisclosed self-administration of anti-malarials by even one control subject disrupts the integrity of the CHMI model. Investigators designing trials incorporating experimental human infection should consider analytical testing for compounds that could adversely impact the integrity of the model.

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**COMPARISON OF A MULTIPLEX AVIDITY ASSAY FOR PLASMODIUM FALCIPARUM MALARIA ANTIBODIES AT ROOM TEMPERATURE VERSUS PHYSIOLOGICAL BODY TEMPERATURE**

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Enzyme-linked immunosorbent assay (ELISA) is the dominant method of testing the avidity of antigen-antibody bonds using chaotropic agents such as urea, sodium thiocyanate, and potassium thiocyanate. Chaotropic agents work to disrupt the antigen-antibody binding by breaking hydrogen bonds of tertiary proteins which allow us to measure the avidity of specific antigen-antibody bonds. Avidity encompasses traits such as binding affinity, antibody valency, epitope density, and antibody reactivity which collectively define the strength of multiple binding interactions between an antigen and antibody. With the introduction of Luminex Corporation's xMAP technology, multiplex assays are on the rise to becoming a more rapid and efficient way of testing for multiple analytes. Multiplex avidity assays offer the ability to simultaneously detect, quantify, and categorize the avidity of antibodies across multiple antigens per microwell. We have developed the multiplex avidity assay for determining IgG subclasses

against *Plasmodium falciparum* circumsporozoite protein, a dominant target for malaria vaccine research along with its immunodominant NANP repeat region peptide, and Pf16 C-term peptide. While many commercially available multiplex kits are sold with recommended assay temperature set at room temperature (25°C), we highlight the importance of comparing our multiplex avidity assay run at the human physiological temperature (37°C). Our new multiplex avidity assay enables determination of human serum total IgG levels against *P. falciparum* antigens and has been developed and tested at room temperature in comparison to the human physiological temperature offering a more time efficient and inexpensive alternative to the traditional ELISA.

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**SAFETY, IMMUNOGENICITY AND DURABILITY OF A NOVEL MALARIA VACCINE CANDIDATE, R21 ADJUVANTED WITH AS01<sub>B</sub>**

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Falciparum malaria remains one of the leading infectious causes of morbidity and mortality worldwide. Though the search for an effective vaccine has seen unprecedented advances in recent years and one of the leading vaccine candidates, RTS,S/AS01 is going to enter pilot deployments in Africa in 2018, there has been no vaccine that has demonstrated durable high level efficacy. R21 has been developed at the Jenner Institute, University of Oxford. This is an RTS,S like construct, an antigen derived from the pre-erythrocytic circumsporozoite protein, which is an abundant coat protein that is pivotal in sporozoite development and hepatocyte invasion. Similar to RTS,S, it comprises recombinant particles expressing the central repeat and the C-terminus of the circumsporozoite protein (CSP) fused to Hepatitis B surface antigen (HBsAg). It does not include the unfused HBsAg protein found in RTS,S, which was required to allow it to form a particle. CSP comprises less than 20% of the total protein content of RTS,S and a large proportion of the antibody response induced by RTS,S is towards the HBsAg. In contrast, R21 comprises only fusion protein moieties, i.e. as 100% of its molecules, which increases the density of CSP antigen on the VLP surface. This was made possible by expressing R21 in the better expressing yeast *Pichia pastoris*, rather than in *Saccharomyces cerevisiae* and it spontaneously forms a particle just like RTS,S. In pre-clinical studies, R21 induces predominantly malaria rather than hepatitis antibodies and it has been shown to be safe, non-toxic and highly immunogenic with comparable immunogenicity and similar high level efficacy to RTS,S. We undertook a Phase I, open-label clinical trial to assess the safety and immunogenicity of R21 administered at 10µg (n=10) and 50µg (n=10) with the liposome-based adjuvant, AS01B. Volunteers in both groups received three vaccinations, 4 weeks apart. Our data show that the vaccine was well tolerated and antibody responses were durable with titres at 6 months comparable to those reported for RTS,S/AS01, at both the doses tested.

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### MALARIA PREVENTIVE PRACTICES AND ACCEPTABILITY OF SEASONAL MALARIA CHEMOPREVENTION AMONG CAREGIVERS OF UNDER FIVE CHILDREN IN RURAL AND URBAN COMMUNITIES OF KANO, NIGERIA, 2017

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Worldwide, malaria kills more than one million people yearly and remains a leading cause of morbidity and mortality in Africa of which majority are young children. Malaria control strategies now targets specific populations for maximal effectiveness. The WHO now recommends Seasonal Malaria Chemoprevention for the prevention of malaria among children under 5 in areas with high malaria transmission. This study assessed and compared malaria preventive practices and acceptability of seasonal malaria chemoprevention including factors associated. A comparative cross-sectional study was conducted in Gwale and Gezawa districts of Kano state using mixed methods. The multi-stage sampling method and structured questionnaires were used to collect quantitative data from 325 households in each LGA while qualitative data were collected through 6 Focus Group discussions and In-depth Interviews. Quantitative data were analysed using SPSS version 21 while qualitative data were analysed using a thematic framework. Majority (72%) of the rural respondents use LLINs for malaria prevention compared to less than 5% of the urban respondents ( $p < 0.05$ ). Non-treated mosquito net was commonly (82%) used in the urban areas while mosquito coil was mainly used in the rural areas (23.3%) followed by window/door netting (5.2%) and burning of mosquito paper (4.2%). The least preventive practices in both communities were mosquito repellent cream, aerosol insecticide spray and interior wall spray as they were used by less than 2% of respondents in both communities. A higher proportion of urban residents (85.7%) were more willing to accept SMC. Age (AOR 7.6, 95% CI 1.1-54.7) and awareness of SMC (AOR 6.6, 95% CI 1.9-23.5) significantly predicted SMC acceptability in the urban community while literacy status (AOR 0.3, 95% CI 0.09-0.79) significantly predicted SMC acceptability in the rural community. Distribution of SMC drugs free, use of incentives and involvement of traditional/religious leaders would enhance SMC acceptability in both communities. The government should undertake awareness campaign on the use of LLINs in the urban communities.

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### ESTABLISHMENT OF THE FIRST EXPERIMENTAL FIELD SITE IN MADAGASCAR FOR STUDYING ANOPHELES COMPETENCY TO TRANSMIT PLASMODIUM FALCIPARUM AND P. VIVAX

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Malaria is still a major health concern in Madagascar. *Plasmodium falciparum* and *Plasmodium vivax* are the major parasite species. *Anopheles gambiae* s.l. and *Anopheles funestus* are the main vectors. However, *Anopheles mascarensis*, a Malagasy endemic species, and *Anopheles merus* can also be vectors of malaria parasites locally. Additionally, *Anopheles coustani* has recently been detected carrying *Plasmodium* sporozoites. Although *Anopheles* surveillance including *Plasmodium* carriage determination is well developed in Madagascar for malaria transmission control, no vector competence studies have been carried out in this country yet. Such studies are important for a better understanding of the effective contribution of each potential vector to transmit *P. falciparum* and/or *P. vivax* and to contribute to the development of alternative strategies to interrupt malaria transmission. Towards these

aims, we have set up an experimental platform within a rural health center in a district where both *P. falciparum* and *P. vivax* are prevalent. There *Anopheles* mosquitoes are locally produced as F0 from larval catches or as F1 from gravid female catches. Female mosquitoes are fed using a membrane feeding system on the blood of *P. falciparum* gametocyte carriers and any *P. vivax* carriers who are selected among children (5-15 years) attending local schools by thick blood smears. In addition, in the same locality we performed a survey of the local mosquito population to evaluate the rate of *Plasmodium* carriage in these mosquitoes. During the 2016 and 2017 campaigns, among roughly 1000 children screened each year, 10% were detected carrying *Plasmodium* parasites with *P. falciparum* representing the major species (8-9%). Up to now we have performed 15 successful experimental infections of *An. gambiae* s.l. leading to a higher rate of infection of this mosquito species with *P. falciparum* than with *P. vivax* (55% prevalence compared to 34% - microscopic oocyst counts at D7). Our full set of data will be presented. Our data clearly show that we have now at hand the capacity to study the competence of *Anopheles* mosquitoes of Madagascar to transmit *P. falciparum* and *P. vivax*.

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### CHARACTERIZATION OF PYRETHROID RESISTANCE IN POPULATIONS OF ANOPHELES GAMBIAE S. L FROM ETHIOPIA AND EVALUATION OF LONG-LASTING INSECTICIDAL NETS

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In Ethiopia, long-lasting insecticidal nets together with indoor residual spraying are key vector control interventions. PermaNet 2.0 and MAGNet nets are widely in use and the role of PBO nets in areas with confirmed pyrethroid resistance is implicated in the recently approved insecticide resistance monitoring and management strategic document. The resistance status of *Anopheles gambiae* s.l. to deltamethrin, permethrin and alphacypermethrin, the enzymatic mechanism of resistance indirectly by synergist test and the bioefficacy of LLINs including PermaNet 3.0 was assessed in five districts using the standard WHO tube test and the WHO cone bioassay tests. Pyrethroid resistance was confirmed in populations of *An. gambiae* s.l. (mortality 30-65%) and the presence of metabolic resistance was observed as the synergist test restored susceptibility (mortality 95% to 100%) to alphacypermethrin and deltamethrin. In the case of permethrin, mortality ranged from 70% -93% after PBO test showing the involvement of two or more resistance mechanisms. Of all the four LLINs, PermaNet 3.0 was more efficacious with 90-100% knockdown and 67-100% mortality. The roof that contains PBO killed 100% of test mosquitoes. The pyrethroid-only nets produced 1-48% knockdown and 1-69% mortality, less than WHO cut-offs of 95% knockdown or 80% mortality.

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### CROSS SECTIONAL SURVEY OF LONG LASTING INSECTICIDETREATED BEDNET DURABILITY AND USE AMONG FISHING AND PASTORALIST COMMUNITIES, KENYA, 2015

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Malaria accounts for ~15% of outpatient visits in Kenya. Mass distribution of long-lasting insecticide-treated bednets (LLINs) every 3 years is a key malaria control strategy. We compared LLIN attrition, damage and net use between two communities in Kenya. Cross-sectional household surveys were conducted 1 year after mass net distribution to determine net attrition, damage and use in two communities in western Kenya from December 2015–January 2016. Initially, 720 and 628 LLINs with unique identifiers were distributed in Siaya, a fishing community, and West Pokot, a pastoralist community, respectively. Standardized household questionnaires were administered to determine net use. A household net census determined attrition (net missing) and damage through visual inspection and hole-size (area) measurement. Proportionate hole index (pHI) was calculated following World Health Organization guidelines; nets were categorized as “serviceable” (pHI <643) or “damaged beyond repair” (pHI ≥643). Use was defined as sleeping under an LLIN the night before the survey. Data were analyzed using SAS 9.1 to compare community net attrition, damage and use. At 1-year post-distribution, mass campaign nets present in fishing community households were 508 (70.6%) of 720 compared to 303 (48.2%) of 628 in pastoralists. Net attrition was lower in the fishing community (16.1%) compared to pastoralists (36.3%,  $p < 0.0001$ ), net damage was higher in fishing community (25.4%) compared to pastoralists (8.3%,  $p < 0.0003$ ), and net use was also higher in fishing community (96.7%) compared to pastoralists (81.9%,  $p < 0.0001$ ). Net attrition was significantly higher in the pastoralist community while net damage and use were significantly higher in the fishing community. Additional distribution channels to replace nets should be considered by malaria-control stakeholders in both communities where over one-third of nets were missing 1-year post-distribution. Alternative net replacement strategies and net-care messaging are needed to maintain high coverage and prevent malaria in western Kenya.

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#### IMPACT OF SEASONAL MALARIA CHEMOPREVENTION AFTER 3 YEARS AT SCALE IN SOUTHERN SENEGAL

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In Senegal, SMC is provided children up to the age of 10 years. The objective of this project was to evaluate the delivery and impact of SMC by measuring coverage using surveys, monitoring treatment efficacy using case control studies and surveys of the prevalence of molecular markers of resistance, monitoring safety through enhanced spontaneous reporting and active follow-up, and assessment of impact using sentinel surveillance. Records on outpatients and inpatients with a diagnosis of malaria were collected from 2012 to 2016 from registers in all hospitals and health centres, and from a probability sample of health posts. After a pilot implementation in 2013, over 5 million treatments were administered from 2014-2016. 92% of children received at least three monthly treatments in 2014, 90% in 2015 and 83% in 2016. A total of seven serious adverse events have been reported (2 Stevens Johnson syndrome, 1 Lyell syndrome, 1 extra-pyramidal syndrome, and 3 allergic reactions). To estimate the impact of SMC we fitted a Poisson regression model to the data on the number of cases, with covariates for the season, year, age group (3-59 months; 5-9 years; 10-19 years; and 20 years and above), and with an indicator variable for SMC set to 1 for ages 3 month to 9 years during the months of September to December from 2014-2016, and set to zero otherwise, with a random effect for the health facility. SMC was associated with a 44% reduction in outpatient cases

of malaria, a 39% reduction in malaria inpatient admissions, and a 43% reduction in hospital deaths associated with malaria. It has been assumed that SMC would prevent malaria deaths, but this is the first evidence to show that SMC programmes can reduce mortality. The SMC programme in Senegal is highly effective. Continued monitoring will be necessary to ensure the quality of delivery is maintained. In many other countries with SMC programmes there is a similarly high burden of malaria in older children. Extending the age range to include these children can be done cost efficiently and would substantially increase the impact of those programmes.

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#### EXPERIENCES AND PERCEPTIONS ABOUT INSECTICIDES TREATED WALL LINERS AS A SUPPLEMENT TO BED NETS IN A HIGH MALARIA TRANSMISSION AREA IN TANZANIA, A MIXED METHODS STUDY

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Ambivalent reception to new health interventions is not uncommon in Sub-Saharan Africa and feedback is essential to improve participant receptivity. A cluster-randomized trial of the effectiveness of non-pyrethroid insecticide-treated durable wall lining (ITWL) to control malaria in combination with long-lasting insecticidal nets (LLINs), offered an opportunity to assess levels of ITWL acceptability among communities in rural Tanzania. Focus group discussions (n=14) and quantitative surveys (n=268) were conducted 6 and 12 months after ITWL installation, respectively. In general, ITWL was well received throughout the study and judged an important contribution to the fight against malaria. Many of the positive comments related to how ITWL beautified houses, especially those build from mud and sticks and with cracked walls (87.7%; 235/268). While most participants were satisfied with ITWL installation (86.9%; 233/268), often a space was left between the wall and the material, which became a hideout for pests, including rats and snakes, leading some to perceive ITWL as a rodent attractant. An additional negative behavior was participants reporting not using their LLIN, undermining the objective of the study, where participants considered ITWL an improved replacement to LLINs. After 12 months, the majority of ITWL installations were still intact. However, most householders felt they did not have the equipment or expertise to repair damaged or fallen ITWL (89.2%; 239/268), raising issues of long-term sustainability without external maintenance. In cases where ITWL had been prematurely removed, it was re-purposed to cover outdoor pit latrines, external house walls and to protect brick stacks from rain. Importantly, at the end of the study, most respondents wished to keep their ITWL and have it repeated in future (60.5%; 162/268). ITWL would be widely accepted at the community level, if complemented with reinforced concomitant use of LLINs and with periodic maintenance and modifications to the installation design to better-fit house walls.

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### NUMBER OF PERSONS SHARING A BED NET IS POSITIVELY ASSOCIATED WITH THE RISK OF *PLASMODIUM* INFECTION IN WESTERN KENYA: IMPLICATIONS FOR MALARIA PREVENTION

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Long-lasting insecticidal bed nets (LLINs) are a core tool for preventing malaria. While the World Health Organization (WHO) recommends one LLIN for every two persons in a household, a LLIN is often shared with three or more people. However, little is known about the relationship between number of persons per net and malaria risk. We assessed net sharing and the risk of *Plasmodium falciparum* infection among children in Gembe East, western Kenya. Infection was determined for children under 10 years of age with a rapid diagnostic test (RDT), and caretakers were interviewed about net use and sharing. Of 275 children studied, 233 (84.7%) reportedly slept under LLINs the previous night. The RDT-positive prevalence was 70.8% for net users and 81.0% for non-net users. The number of persons sleeping under a LLIN ranged from 1 to 6 (median=3), with infection prevalence increasing as more people slept (53.8% for one person, 71.0% for two persons, 71.4% for three persons and 74.4% for four or more persons). Overall, infection among children who slept alone under a net was significantly lower than those of children sharing a net with two or more other persons (OR=3.92, 95%CI: 1.05-14.22 against two other person; OR=4.40, 95%CI: 1.02-19.97 against three or more other persons) while it was not significantly lower than that of children sharing a net with one other person (OR=2.22, 95%CI: 0.59-8.12). When the number of person per a net increased by one, the risk of infection increased 49% (95%CI: 1.02-2.27). These results suggest that the number of persons sleeping in a net should be fewer than three, and sleeping alone may further reduce the risk.

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### ASSOCIATION BETWEEN INDOOR RESIDUAL SPRAYING OF INSECTICIDE AND IMPROVED BIRTH OUTCOMES AMONG HIV-INFECTED PREGNANT WOMEN IN UGANDA

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Despite effective interventions to prevent malaria during pregnancy, the risk of adverse birth outcomes among HIV-infected pregnant women living in sub-Saharan Africa remains high. A recent study demonstrated a reduction in adverse birth outcomes among HIV-uninfected pregnant women protected by indoor residual spraying of insecticide (IRS). Evidence

regarding its impact on HIV-infected pregnant women is lacking. To assess the association between IRS and preterm birth (gestational period < 37 weeks), low birthweight (< 2500 grams), and fetal/neonatal death, data was pooled from two clinical trials conducted before and after a large-scale IRS campaign in Tororo District, Uganda. Participants were HIV-infected pregnant women who received insecticide treated bed nets, daily trimethoprim-sulfamethoxazole, and combination antiretroviral therapy at study enrollment. Exposure was measured as the percentage of IRS protection experienced by the mother during her gestational period. Multivariate Poisson regression with robust standard errors was used to estimate adjusted risk ratios (aRR). Of the 565 women included in our analysis, 380 (68%), 88 (16%), and 97 (17%) women were protected by IRS for 0%, > 0-90%, and > 90% of their pregnancy, respectively. Compared to women with no IRS protection during pregnancy, IRS protection for > 90% of pregnancy was associated with a significantly lower risk of preterm birth (17.1% vs. 6.2%; aRR = 0.37; 95% CI: 0.16-0.84). Preterm birth risk decreased with increasing IRS protection, indicating a dose-dependent effect (p = 0.03). IRS protection for > 90% of pregnancy was also associated with a lower risk of low birthweight (18.5% vs. 9.3%; RR = 0.58; 95% CI: 0.25-1.32) and fetal/neonatal death (5.3% vs. 2.1%; RR = 0.25; 95% CI: 0.05-1.37), although these associations did not reach statistical significance. IRS may further reduce the risk of adverse birth outcomes among HIV-infected pregnant women who are already using currently available interventions to prevent malaria during pregnancy.

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### EFFECTIVENESS OF VECTORS CONTROL INTERVENTION ON MALARIA INFECTION AND CLINICAL CASES, BENIN, WEST AFRICA

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The most commonly used methods to prevent mosquito bites are sleeping under the Long lasting insecticide treated net (LLIN) and spraying the inside walls of a house with an insecticide called indoor residual spraying (IRS). The present study aimed to evaluate the effectiveness of post deployment LLIN and IRS on *P. falciparum* infection and clinical cases. A case-control study was carried out among all age groups in the north-west Benin in 2014. Nine villages were included in the commune of Djougou (urban area) and in the commune of Coby (rural area) respectively. A case of *P. falciparum* infection was defined as positive rapid diagnosis test. A total of 594 cases of infection and 1495 controls were recruited in urban area compared to 1030 cases and 1135 controls in rural area. The malaria clinical case was defined as positive thick blood films plus fever. A total of 112 cases and 1362 controls were recruited in urban compared 90 cases and 1445 controls in rural. The prevalence rate of *P. falciparum* infection was 36.0% and 70.0% among children aged two to nine years old in urban and rural area respectively. The use of LLIN was 38.9% in urban area and 50.9% in rural. The coverage of IRS was 95.8%. In urban area, the LLINs induced 50% significant reduction of infection in only one neighbourhood but any reduction in malaria clinical cases. In the rural area, a significant protection (49%) was obtained among all population only if the LLINs were associated to IRS. Malaria remains a major public health problem in Benin. It is an urgent need to build a strong surveillance system of malaria and evaluated as soon as possible what are working and which intervention need better management and a large behavior change communication.

## QUANTIFYING SEASON PATTERNS OF ITN USE ACROSS CLIMATIC ZONES IN SUB-SAHARAN AFRICA

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Insecticide-treated nets (ITNs) are the primary tool for malaria prevention in sub-Saharan Africa, and while population access to ITN is often below the RBM target of 80%, the ITN use:access ratio, the primary indicator for ITN use among those with access, is regularly above the target of 0.80 in a majority of countries. The main barriers to ITN use are known to be low perceived mosquito abundance and feelings of discomfort, primarily related to heat, but seasonal determinants of ITN use have never been quantified. We examined subnational patterns of seasonal ITN use and access in three countries to assess relationship with seasonal rainfall and for mosquito density (rainfall lagged by 1 month), controlling for zone and urban/rural residence. Cluster means for ITN use:access ratio were generated from cluster means for ITN use and ITN access derived for each survey. Clusters were categorized as dry, early rainy, or late rainy season based on month of survey fieldwork and monthly average rainfall patterns (1990-2012 averages) for three zones per country. Cluster use:access ratios were linearly correlated with rainy season in Senegal 2014 cDHS and 2015 cDHS (coef 0.151 and 0.192,  $p < 0.05$  respectively), but lagged mosquito abundance was a stronger predictor than rainy season (coef 0.170 and 0.226,  $p < 0.05$  respectively), controlling for zone and urban/rural residence. In Tanzania, cluster use:access ratios were also linearly correlated with rainy season in 2015-16 (coef 0.045,  $p < 0.05$ ) and to a slightly higher degree with lagged mosquito abundance (coef 0.069,  $p < 0.05$ ). In Ghana, no seasonal correlation was observed with three seasonal categories, but binary categorization of rainfall showed a 0.05 point increase in use:access ratio from dry to rainy season ( $p < 0.05$ ). Urban residence was associated with decreased use:access ratio in Senegal and Tanzania, but with increased ratio in Ghana. These preliminary results demonstrate that mosquito abundance drives use of ITNs among those with access to a greater degree than rainfall itself, boosting use:access ratios by 6-22 points. Additional surveys will be analyzed and comprehensive results presented.

## WHO BUYS NETS? FACTORS ASSOCIATED WITH MOSQUITO NET PURCHASE IN SUB-SAHARAN AFRICA

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Bednets, particularly long-lasting insecticidal nets are the primary tool for preventing malaria infection and reducing associated morbidity and mortality. While bednets are typically obtained free from public sources, nets are often purchased from private retail channels. Using data from the 2015/16 Tanzania DHS data, we explore household level factors associated with the purchase of nets in Tanzania. Nets were defined as purchased if obtained from private health facilities, shops, markets or accredited drug dispensing outlets. Both rural and urban households across all wealth quintiles in all regions of Tanzania owned purchased nets. The following household factors were included in a logistic regression model: sex and age of the head of household, presence of a child under five or currently pregnant woman; household size; indoor residual spraying, distance to market, wealth quintile; urban/rural residence; regional zone and altitude. Presence of an under five or pregnant woman (adjusted odds ratio: 1.26 and urban residence (aOR: 5.32) were significantly associated ( $< 0.05$ ) with increased odds of owning a purchased net. Odds of owning a purchased net also significantly increased with wealth (aOR: 2.08, 2.91, 4.95, 8.19 among poorer, middle, richer and richest quintiles respectively). However, female heads of household, altitudes above than 1000m and household sizes of more than 3 people were significantly associated with lower odds of owning purchased nets (aOR: 0.80, 0.44 and 0.80 respectively).

Compared to Western zone, residence in Eastern zone was significantly associated with higher odds (aOR: 2.04) while residence in Southern Highlands or Zanzibar was significantly associated with reduced odds of purchasing nets (aOR: 0.65 and 0.40 respectively). Findings can help donors and the National Malaria Control Program gain insight on the contribution of net sales on their overall ITN strategy as well as inform the design and implementation of market shaping strategies to shift consumers to purchase ITNs. Additional findings on ITN purchase behavior will be presented along with data from other countries in sub-Saharan Africa.

## HUMAN, PARASITE AND ENVIRONMENTAL FACTORS ASSOCIATED WITH ANOPHELES MOSQUITO HETEROGENEITY AMONG HOUSEHOLDS OF SOUTHERN MALAWI

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Simulation models show that transmission heterogeneity enables pathogen persistence even when population averages are theoretically too low to maintain endemicity. For malaria, heterogeneous exposure to *Anopheles* mosquitoes creates both differential infection risks and potential transmission contributions. Further, asymptomatic human reservoirs of *Plasmodium falciparum* infection are common. We investigated factors that influence spatial distribution of competent vectors to understand heterogeneity in human population transmission potential, hypothesizing that humans with gametocytes are exposed to more *Anopheles* mosquitoes than those without. We surveyed ~80 households (HH) in each of 4 endemic villages during the rainy and dry seasons of 2015. HH locations and land use/cover within 50m were observed. Consenting HH members were asked about malaria symptoms, treatments and prevention. Blood was collected to test for *P. falciparum* parasites by PCR and gametocytes by qRT-PCR for *Pfs25*. Mosquitoes were sampled by CDC light traps in most houses (rainy,  $n=247$ ; dry,  $n=303$ ) and identified to species by microscopy and PCR. Female *Anopheles* were found in 26% of houses during the dry season (median where present: 1; range: 1-15) and 71% during the rainy season (median: 7, range: 1-215). Better housing materials and closed eaves were associated with lower *Anopheles* abundance in both seasons; nearby grazing animals were associated with lower abundance only in the dry season. The HH-level association between *Anopheles* presence and at least one HH member with gametocytes was strongly modified by a village interaction (interaction  $p=0.01$ ), being positive in three but significantly negative in one. These results suggest that despite relative proximity between villages (20 – 65 km), risk factors for household exposure to *Anopheles* are significantly modified by season and village-level factors. Further analyses of the factors underlying geographic differences in relationships between human parasite reservoirs and vector heterogeneity should reveal predictors of transmission 'hot spots' and enhance future targeting of interventions.

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### EVALUATION OF FOUR ROUNDS OF LONG LASTING INSECTICIDAL NET DISTRIBUTION THROUGH SCHOOLS IN SOUTHERN TANZANIA

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In order to explore and evaluate innovative strategies for increasing use and ownership of ITNs, National Malaria Control Programme (NMCP) and partners are piloting the distribution of long lasting insecticidal nets (LLINs) through schools in Southern zone of Tanzania as a Keep-Up-Strategy to maintain universal coverage following a mass distribution. The pilot is funded through the United States Agency for International Development, the President's Malaria Initiative (USAID/PMI) and implemented by a consortium of partners (including: RTI International, Tanzania Red Cross Society, PSI, JHU/COMMIT) led by the NMCP with support from the Swiss Tropical and Public Health Institute. The evaluation consists of annual cross-sectional surveys in both implementing and non-implementing areas to compare changes in net coverage and use over time. Across the first three evaluation rounds, data was collected on 2828 and 1869 implementation area and non-implementation area households, respectively. Results from the second round evaluation showed that population access to LLINs in implementation areas was maintained, increasing from 63% in 2013 to 68% in 2015 ( $p$  for change=0.683) while access in the non-implementing areas dropped significantly from 51% to 39% during the same time period ( $p$  for change=0.002). Results from the third round evaluation in 2016 are currently still under review, however population-level access to LLINs in implementation areas declined significantly to 55% ( $p=0.013$ ). Data from the fourth round evaluation will be analyzed using repeated-measures logistic regression methods to model change in access over time, allowing adjustment for potential confounders such as socioeconomic status and school attendance. The potential for school-based distribution of LLINs as an effective Keep-Up-Strategy may be complex and dependent on a variety of factors. The results of this research provide new information on this method of LLIN distribution and the factors associated with its performance.

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### A SYSTEMATIC REVIEW OF INDOOR RESIDUAL SPRAYING TO INVESTIGATE THE IMPACT OF PYRETHROID RESISTANCE ON MALARIA TRANSMISSION

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Vector control, delivered through the mass distribution of long-lasting insecticide treated bednets (LLIN) and indoor residual spraying (IRS) of insecticide, is one of the most effective tools for preventing malaria. There are growing concerns that recent gains in malaria control are under threat due to the emergence of mosquitoes that are resistant to pyrethroid insecticides. IRS offer a greater range of insecticide options for protecting indoor areas whilst pyrethroids are the only class of insecticide currently used in LLINs. Long-lasting IRS formulations with novel active ingredients are now available. In this study, a meta-analysis of experimental hut trial data is used to characterise the entomological impact of pyrethroid resistance on IRS in Africa. The epidemiological consequences are assessed using a dynamic transmission model of malaria which is used to predict the public health significance in areas with differing epidemiology and history of malaria control. The benefit of IRS campaigns using these new products is investigated throughout sub-Saharan Africa considering different levels

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of pyrethroid resistance, seasonality in vector abundance and existing use of LLINs. Results indicate that the effectiveness of IRS campaigns can be substantially improved when switching from pyrethroids to new insecticide compounds but that IRS coverage and impact vary substantially between locations.

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### CONSIDERATIONS FOR FORECASTING IRS INSECTICIDES

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Indoor residual spraying (RS) is a powerful tool in the fight against malaria. The success of an IRS program depends on several requirements, including adequate and sustainable logistics resources. For IRS to be effective there must be high spray coverage of all structures in a defined area. In order to obtain high coverage, sufficient quantities of insecticide and related materials must be available. A deliberate, data-informed quantification is a critical step in planning for a successful spray campaign. Forecasting considerations for IRS insecticides, include the following: -Spray campaigns are short in duration and the timeframe for conducting them is fixed. Delays in product arrival can be detrimental to an IRS program. -For 3GIRS, demand is uncertain, and there are a limited number of manufacturers. 3GIRS manufacturers must cover the risk in the uncertain demand, which has contributed to higher prices compared to other insecticides. -Given the duration of spray season and lead times, national malaria control programs (NMCPs) may need to place an order for the following year's spray season, before they have the most recent spray season data available, and well before any post-spray entomological monitoring data are available. When conducting a forecast, the total cost of managing the insecticide must be considered. Different insecticides have different supply chain considerations, i.e. a capsule suspension will have significantly higher freight and storage costs compared to a wettable powder. -Unlike other health commodities, where maximum and minimum stock levels are utilized, consumption of IRS insecticides is concentrated in a limited time, so products do not need to continually be managed between max and min. Managers should strive for accurate forecasts and include minimal buffer stock. Other factors may influence the confidence that managers have in an IRS forecast: whether new areas are being sprayed, whether the spray season is shifting, whether an enumeration activity has been conducted, whether funding is firm, and whether stock on hand from the previous campaign is known.

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### EVALUATING THE EPIDEMIOLOGICAL IMPACT OF SHIFTING IRS PRODUCTS FROM 2011-2014 IN NORTHERN GHANA

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The indoor residual spraying (IRS) of insecticides has contributed substantially to recent successes in malaria control, positioning IRS as one of the key components of the global malaria control strategy. In Ghana, insecticide resistance concerns prompted a switch from pyrethroids to a microencapsulated formulation of pirimiphos-methyl (PM CS) starting in 2012. In conjunction with this switch, Africa Indoor Residual Spraying (AIRS) entomological surveillance has tracked the resistance status of local vector populations and the effect of IRS on key vector indicators through sentinel site monitoring. Previous analysis of data from Bunkpurugu-Yunyoo, Savelugu Nanton, Tolon/Kumbungu, and Tamale districts found that compared to IRS with a pyrethroid in 2012, IRS with PM CS in 2013 had a much more robust impact on reducing the indoor resting density of *An. gambiae*. In addition, from 2010 to 2014, the US President's Malaria

Initiative (PMI) supported enhanced anemia and parasitemia monitoring via longitudinal cross sectional surveys in Bunkpurugu-Yunyoo. This monitoring was previously reported to show a small 5% decline in parasite prevalence from 2011 (52.4%) to 2012 (47.7%), when a pyrethroid insecticide was used for IRS. In 2013 the insecticide was switched to an organophosphate and a much larger 57% decline in parasite prevalence, from 47.7% to 20.6%, was observed, suggesting a positive impact on transmission reduction resulting from a change in the class of insecticide. To supplement these analyses, we conducted a retrospective, observational analysis of the epidemiological impact of IRS with the varying insecticide classes, using routine health facility data to compare malaria incidence rates in the aforementioned districts. Changes in incidence observed in these districts are compared from year to year in the context of their varying IRS implementation status and products to provide an estimate of intervention impact. These analyses are triangulated with the previous entomological and parasitemia results to further our understanding of the impact of IRS with PM CS in a region with documented pyrethroid resistance.

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#### DETERMINATION OF ESBL PREVALENCE AND COMMON MECHANISMS IN ENTEROTOXIGENIC *ESCHERICHIA ISOLATED FROM DIARRHEA SAMPLES COLLECTED IN NEPAL DURING 2001-2016*

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Multidrug resistant (MDR) bacteria have rapidly spread globally and are now endemic in many areas of South Asia. However, there are a lack of comprehensive data from many developing countries in the region to determine the extent of MDR prevalence and common antimicrobial resistance mechanisms. In Nepal, Enterotoxigenic *Escherichia coli* (ETEC) is one of the leading causes of both acute infant and traveler's diarrhea. The MDR prevalence and resistance mechanisms of ETEC isolates responsible for enteric infections are unknown. From 2001-2016, 273 ETEC isolates were collected from acute diarrheal samples from traveler's clinics and hospitals in Nepal. The isolates were first characterized using multiplex PCR assays to determine toxin genes and colonization factors (CFs). Isolates were then screened for Extended Spectrum Beta-lactamase (ESBL) production via the Microscan Automated Microbiology System. ESBL-positive isolates were further screened for genetic components potentially responsible for the observed resistance, and conjugation studies were performed to characterize plasmid-mediated components. ETEC toxins were confirmed in the isolates (97%) and the CFs CS2 (12.8%), CS3 (22.7%), CS6 (25.6%), and CS21 (59.7%) were shown to be the most prevalent. 40/273 isolates were identified as ESBL positive. Isolates from 2008, 2013, 2014, and 2016 had ESBL prevalence rates of 1.5%, 34.8%, 31%, and 35% respectively. CTX-M-15 was identified as the most dominant ESBL gene (77.5%) followed by TEM (40.0%), SHV (20.0%), CTX-M-14 (2.5%), and CIT (2.5%). 42.5% of the isolates carried multiple ESBL genes. The ESBL-positive ETEC isolates showed a higher association with the CFs CS2 (35%), CS3 (32.5%), CS6 (45%), and CS21 (70%) compared to the whole population. A significant number of post-2013 ETEC isolates demonstrated ESBL resistance, and this prevalence will most likely increase over time. Persistent surveillance and characterization of enteric ETEC isolates are vital to track the community presence of MDR bacterial species in order to recommend treatment strategies and mitigate the spread of resistant pathogens.

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#### SPECIATION, SEROTYPING AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF *SHIGELLA* ISOLATES IN NORTHERN SRI LANKA

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Shigellosis causes high disease burden, especially in children below 5 years. *Shigella flexneri* (SF) is the predominant cause of morbidity and mortality. With socioeconomic improvement the circulating species changes from *S flexneri* to *S sonnei* (SS). In Sri Lanka the highest number of cases is reported from Jaffna district of Northern Province, mostly based on clinical diagnosis. Microbiological information is lacking from this region for three decades due to an ethnic war which ended in 2009. *S sonnei* is now the commonest cause of shigellosis reported in Sri Lanka. This study aimed to determine the *Shigella* species, antimicrobial susceptibilities, age and outcome related to shigellosis in northern Sri Lanka. A prospective descriptive study was done of stool cultures received from June 2014 to December 2016. Culture processing, organism identification and antimicrobial susceptibility testing were performed according to standard laboratory methods. Serotyping was done at Medical Research Institute, Colombo. 45/1126 (4%) stool cultures yielded *Shigella* species in 41 children and 4 adults; all residents of Northern Province. 92.7% (38/44) children were ≤ 5 years and predominantly male [57.9% (22/38)]. No deaths reported. 64.4% (29/45) were SF and 35.6% (16/45) were SS. At time of writing, 10/29 SF II and 5/29 SF VI serotypes and 2/16 SS phase I and 12/16 SS phase II were identified. All SF and SS demonstrated sensitivity to mecillinam and ceftriaxone. 96.6% SF and 93.8% SS were ciprofloxacin sensitive. Almost all SF (93.1%) and SS (93.8%) were resistant to co-trimoxazole and all isolates showed either complete/intermediate resistance to furazolidone. All SF were resistant to nalidixic acid but all SS were sensitive. Ampicillin sensitivity in SS was 100% but 58.2% (17/29) SF were resistant. Of the serotyped SF, all SF II were resistant to ampicillin and all SF VI sensitive. *Shigella flexneri* is the predominant species in northern Sri Lanka. All SF and SS were non-susceptible to furazolidone, a commonly used antibiotic in other regions. Long term analysis will determine if *Shigella* species will change with socioeconomic improvements in this region.

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#### EARLY CHILDHOOD STUNTING AMONG HIV-EXPOSED, UNINFECTED INFANTS IN KENYA; THE IMPACT OF MATERNAL AND INFANT DIARRHEA

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The growing population of HIV exposed uninfected (HEU) children in sub-Saharan Africa is at increased risk of stunting (length-for-age z score [LAZ] ≤ -2) as compared to HIV unexposed children. Risk factors for stunting in HEU children may differ from other populations due to differences in acquired and passive immunity from HIV exposure, breastfeeding practices, and/or socioeconomic vulnerability. Associations between maternal factors (age at birth, education, income, malnutrition, HIV viral load, CD4 percent, diarrhea) and infant factors (low birth weight, preterm birth, diarrhea, breastfeeding) and infant stunting were assessed using data from a pre-antiretroviral therapy era cohort (1999-2002) of HIV-infected Kenyan mothers and their uninfected infants. Mothers were enrolled

during pregnancy and mother-infant pairs were followed for 12 months postpartum. Surviving infants with 12-month LAZ data were included in the analysis. Using log binomial regression models, stunting prevalence at 12 months was compared between infants with and without cofactors of interest during the first 10-months postpartum (assuming 2-month length effect lag). Among 273 HEU infants who completed follow-up, 18% (48) were stunted at 12 months. During the first 10 months of life, mothers reported that 71% (193) of infants had any diarrhea, 25% (68) had diarrhea  $\geq 3$  times, 14% (40) had at least one episode of prolonged diarrhea ( $> 7$  days), and 22% (61) reported that they themselves had experienced at least one episode of diarrhea. Any maternal diarrhea (prevalence ratio [PR]=1.74; 95% confidence interval [CI]=1.02, 2.94;  $p=0.04$ ) and  $\geq 3$  infant diarrhea episodes (PR=1.88; 95% CI=1.08, 3.03;  $p=0.02$ ) during the first 10 months postpartum were associated with infant stunting. Statistical tests for other maternal and infant risk factors did not reach significance. Prevention and expedited management of diarrhea in HIV-infected mothers and infants may reduce the burden of chronic malnutrition in HEU children, a population at particularly high risk of mortality and developmental shortfalls.

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### QUANTITATIVE ANALYSIS OF DIARRHEA ETIOLOGY IN TRAVELERS' DIARRHEA IN NEPAL

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Traveler's diarrhea (TD) is one of the most common illnesses experienced by western travelers traveling to developing countries. Nepal is one of the major destinations for trekking and its undisturbed natural beauty. The risk of developing TD is high due to the developing tourism infrastructure in Nepal. Studies describing TD diarrhea etiology in Nepal are limited with the most recent reporting of samples collected in 2001-2003. More recently, a clinic-based surveillance study was conducted in Nepal collecting TD samples from 2012-2014. A wide range of conventional diagnostic assays were performed to determine diarrhea etiology which were time and labor intensive. Previously reported at ASTMH 2016, partial TD samples from Nepal and Thailand were combined for quantitative analyses to further demonstrate pathogen-disease association using the enteropathogenic panel TaqMan® Array Card (TAC), which was developed and evaluated by the University of Virginia. This current study focused on 631 TD samples from Nepal composed of 425 cases and 206 asymptomatic controls. Analyses of these pathogens have shown that there is a significant risk of developing TD by *Campylobacter* spp., enterotoxigenic *Escherichia coli* (ETEC), norovirus, rotavirus, *Shigella* spp., and *Cryptosporidium*. Comparison of the diarrheagenic *E. coli* (enteroaggregative *E. coli*, enteropathogenic *E. coli*, and ETEC) detected by TAC and conventional methods showed that TAC detected diarrheagenic *E. coli* at a significantly higher percentage and EAEC was significantly detected more in the cases when compared to the controls. *Cyclospora*, endemic to Nepal, was detected at a lower percentage with no significant associated risk of diarrhea. However, the collection time of positive samples coincided with the seasonal infection peak in June/July. Attributable fraction of each pathogen to TD was calculated and each of these pathogens attributed over 70% of TD cases with the highest attributable fraction by ETEC and *Shigella* spp. at 88% each. Further quantitative analyses will provide a greater insight into a limited knowledge of diarrhea etiology and TD epidemiology in Nepal.

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### DETECTION *CRYPTOSPORIDIUM* AND *CYCLOSPORA* OF DIGITAL DROPLET PCR (DDPCR) FROM TRAVELERS' DIARRHEA AND NEPALESE STOOL SAMPLES

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*Cryptosporidium* (CR) and *Cyclospora* (CY) are microscopic parasites that cause diarrhea in both children and adults worldwide. Specifically, CY is endemic to Nepal with peak infection between May and October. Western travelers visiting endemic regions are at a greater risk of infection. Both CR and CY exist in the environment as oocysts before sporulation upon entering the host. Microscopy is the gold standard for parasite detection but variability in oocyst characteristics and the low infectious dose for both parasites (10-100 oocysts) can lead to misidentification. Diagnostic methods such as ELISA and real-time PCR have been developed to alleviate the sensitivity issue but have not been adopted as alternative methods of detection. Previous detection of CR and CY by real-time PCR showed an increased number of CR and CY detected but at a high threshold cycle (Ct) indicating a low copy number which suggested that CR and CY might be under-detected. Digital droplet PCR (ddPCR) is reported to have increased detection sensitivity and an assay using ddPCR was developed and evaluated to detect CR and CY from positive samples with high Cts and samples that were pathogen negative from diarrhea; stool samples collected from travelers and the indigenous population in Nepal. The assay was developed based on a real-time PCR assay and evaluated under various ddPCR conditions using both genomic and plasmid DNA containing the target gene and was further optimized for a duplex assay. Two hundred and forty six samples (66 previously CR and/or CY positive and 180 pathogen negative) were used for the study. Results generated by ddPCR were 100% concordant with the previous CY and CR positive results by real-time PCR with an additional 12 CR and/or CY positive samples detected from previously pathogen negative samples. This is a 6.7% detection rate from pathogen negative samples which can decrease the burden of pathogen negative samples. With an expanded evaluation of ddPCR on a larger number of samples, ddPCR has the potential as an alternative diagnostic platform with increased sensitivity for other relevant low infectious dose pathogens.

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### ANTIBIOTIC RESISTANCE OF *SALMONELLA ENTERICA* ISOLATES FROM PORK CARCASSES IN SLAUGHTERHOUSES IN LIMA, PERU

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The aim of this study was to detect the frequency of resistant *Salmonella* by isolation techniques in pork carcasses intended for human consumption. Three hundred carcasses from two slaughterhouses in Lima, Peru were studied. Samples were taken by swabbing the skin of the head, abdomen, back and leg. They were processed following the protocol for isolation of bacteria based on ISO 6579:2002. The isolates were identified by biochemical tests and specific antisera. In the next phase of the study, sensitivity tests were performed. The Kirby Bauer technique was used, following the CLSI recommendations against 10 antimicrobials: Amikacin, Ampicillin, Chloramphenicol, Oxytetracycline, Streptomycin, Nalidixic acid, Tobramycin, Ciprofloxacin, Neomycin and Gentamicin. Finally, the detection of extended spectrum (Cefotaxime,

Ceftazidime, Cefepime, Aztreonam with Amoxicillin with Clavulanic acid disk diffusion test) and AmpC (Cefotaxime-Ceftazidime with Cloxacillin disk diffusion test, Cefoxitin-Ceftriaxone disk diffusion test) betalactamases were performed. In 6.3% (19/300) of pork carcasses were detected *Salmonella* spp. The isolates were serotyped and identified as *Salmonella enterica* subsp. *enterica* serotype Derby. Resistance was detected mainly to Chloramphenicol 100% (19/19), Oxytetracycline 100% (19/19), Tobramycin 47.4% (9/19), Streptomycin 36.8% (7/19), Ampicillin 5.3% (1/19) and Gentamicin 5.3% (1/19). All isolates were negative for extended spectrum and Ampc betalactamases. The detection of resistant *Salmonella* strains could lead to problems in the treatment of the disease, because the antimicrobials no longer work or no desired results are obtained. The results confirm the need to implement control measures and detection of the bacteria to reduce the frequency of contaminated pork that reaches the consumer.

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### ENTEROPATHOGEN PROFILE IN HUMANS AND DOMESTIC ANIMALS IN COASTAL DISTRICT OF ODISHA, INDIA: POSSIBLE ZONOTIC TRANSMISSION AND CONCERNS

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Infectious diarrhea is a major cause of morbidity and mortality particularly among children of <5 years of age. The source of diarrheal pathogens can be varied that depends largely on an interplay between human, environment, animals and food/water. In this study we investigated presence of major diarrheal pathogens in human and animal fecal samples to assess epidemiology and possible transmission routes responsible for disease burden. We examined human fecal samples (n=310) and animal fecal samples (n=150) during May 2016 to January 2017. PCR amplifying targeted pathogen specific genes was employed to diagnose different strains of diarrhoeogenic *Escherichia coli* or DEC, *Shigella* species, *Salmonella* sp., *Vibrio cholera*, *Cryptosporidium* sp., and *Giardia* sp. PCR positive samples were subjected to sequence analysis for further confirmation. We observed DEC was the most frequently detected pathogen in both humans (29.03%) and animals (38.66%). Other enteropathogens viz., *Shigella* (13.87%), *Cryptosporidium* (4.1%), Adenovirus (3.87%) and *Giardia* (0.64%) were also detected in humans. *Cryptosporidium* sp. was predominately detected among goats (17.77%) followed by cow (11.11%). The detection rates were remarkable for Shiga toxin-producing *E. coli* or STEC (10.32% in humans and 21.33% in domestic animals) and enteropathogenic *E. coli* or EPEC (15.80% in humans and 14.66% in domestic animals). Detection rate of *Cryptosporidium* sp in humans was significantly higher in rural area in comparison to urban counterpart (p=<0.0001). Co-infection with two or more enteric pathogens were also found in humans as well as animals (14.19% and 19.33% respectively). Sequencing results showed STEC, EPEC and *Cryptosporidium parvum* infection were the predominant infections both in humans and common domesticated animals in the studied region. Genetic similarity study suggests a great potential of transmission of infections from domestic animals to the inhabitants in the resource poor communities. Further studies are warranted to understand more on persistence, transmission dynamics, and associated consequences of these enteric pathogens in this region.

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### EVALUATION OF THE MULTIPLEX LUMINEX GASTROINTESTINAL PATHOGEN PANEL (XTAG GPP) TESTING IN THE DIARRHEIC MILITARY DEPLOYED TO COBRA GOLD AND BALIKATAN EXERCISES

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Traveler's diarrhea is a major infectious disease threat to US military forces deployed to Southeast Asia and can greatly hinder operations. Eighty-four stool samples collected from 2012-2016 during the US military exercises, Cobra Gold (Thailand) and Balikatan (Philippines), were evaluated for enteric pathogens using routine microscopy, ELISA and real-time RT-PCR and results were compared with the FDA approved Gastrointestinal Pathogen Panel (GPP) (Luminex). The GPP assay is able to detect 15 pathogens as compared to conventional diagnostic tests which can be time and labor intensive. By conventional methodology, *Campylobacter* was the most prevalent pathogen at 70.6% (12/17) for Balikatan and 41.8% (28/67) for Cobra Gold. Other pathogens detected in the Balikatan samples included enterotoxigenic *Escherichia coli* (ETEC) (11.8%) and *Salmonella* (5.9%). Additional pathogens detected in the Cobra Gold samples were *Salmonella* (16.4%), Norovirus (11.9%) and ETEC (4.5%). No pathogen was detected in 5.9% and 19.4% of the Balikatan and Cobra Gold samples respectively. A total of 15 samples were reported as negative by all assays, however, an additional 5 samples were negative by GPP, these 5 additional samples were positive for Rotavirus, Adenovirus, *Campylobacter* and *Vibrio cholera* by conventional methods. Two negative samples by routine methods were identified as *Salmonella* positive by GPP. The GPP assay showed increased sensitivity towards *Campylobacter*, *Salmonella*, Norovirus GII, ETEC and Shiga-toxin producing *E. coli* (STEC) with sensitivities ranging from 97.6 - 100% and specificities ranging from 94.0 - 100%. Multiple pathogens were identified by the GPP assay in 9/84 (10.7%); these were 66.7% *E. coli* O157, 44.4% *Campylobacter*, 33.3% Norovirus and 22.2% for both ETEC and *Salmonella*. GPP was shown to be convenient, rapid and sensitive assay that allowed for faster turnaround time and for identification of co-infections in a single assay. Although GPP may replace conventional methods in certain areas, there is still a need for culture to perform antibiotic susceptibility tests and for certain organism confirmation testing.

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### ANTIMICROBIAL RESISTANCE IN *ESCHERICHIA COLI* AND *SALMONELLA* SPP. ISOLATES FROM FRESH PRODUCE IN THE PHILIPPINES AND THE IMPACT TO CONSUMERS' HEALTH AND SAFETY

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Foodborne diseases associated with fresh produce consumption have escalated worldwide, making the microbial safety of produce of critical importance. Bacteria that have increasingly been detected in fresh produce are *Escherichia coli* and *Salmonella* spp., both of which have been shown to progressively display antimicrobial resistance. The study focused on the assessment of bacterial antimicrobial resistance from different kinds of fresh produce from various open air markets and supermarkets in the Philippines. Using the disk diffusion assay on a total of 50 bacterial isolates obtained from 410 fresh produce surveyed, monoresistance to tetracycline was observed to be the most prevalent (38%), followed by multidrug resistance to tetracycline, chloramphenicol, ciprofloxacin, and nalidixic acid (4%), and lastly by dual resistance to tetracycline and chloramphenicol (2%). Using multiplex and simplex polymerase chain reaction (PCR) assays, *tetA* (75%) and *tetB* (9%) were found in tetracycline

resistant isolates, whereas *catI* (67%) and *catIII* (33%) were detected in chloramphenicol resistant isolates. Sequence analysis of *gyr* and *par* genes from the ciprofloxacin and nalidixic acid resistant isolates revealed different mutations. Based on the results, fresh produce act as a reservoir of these antibiotic resistant bacteria which may pose health threats to consumers.

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### PROJECTING THE POTENTIAL IMPACT AND COST-EFFECTIVENESS OF A DIAGNOSTIC FOR LONG-TERM CARRIAGE IN TYPHOID FEVER

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Typhoid fever remains a major public health problem globally, causing an estimated 12 million illnesses and 130,000 deaths annually. As new public health strategies against typhoid fever are considered, there are key questions on the importance of addressing the reservoir of long-term carriage, which may represent a barrier to control and elimination in many settings. While new serologic diagnostic markers hold promise for identifying long-term carriers, the potential population health impact and cost-effectiveness remains unclear. We modeled typhoid transmission dynamics across a range of settings, varying the contribution of carriers to endemic transmission, and simulated the changes in typhoid incidence and cost-effectiveness of a hypothetical carriage diagnostic for typhoid fever. We developed a dynamic, age-structured transmission and cost-effectiveness model that simulated a hypothetical carriage diagnostic under two potential strategies: 1) population-wide household screening; and 2) continual screening through the healthcare system. We simulated a 10-year vaccination program, and sourced data on costs, disability, and typhoid natural history and biology from literature. We estimated a test cost of US\$4.00 with US\$1.00 delivery cost (for population-wide screen), and 1% case fatality rate from typhoid fever. We performed sensitivity analyses on key model parameters. The incremental cost-effectiveness ratio (ICER) was calculated in 2016 US\$ per disability-adjusted life year (DALY) averted. We defined strategies as highly cost-effective if the ICER was less than the GDP per capita of a low-income country (US\$1,035). We found that aggressive strategies to diagnose and treat carriers provided substantial reductions in incidence across a range of settings, although more realistic uses of the diagnostic test yielded only modest reductions in incidence. Our results indicate that population-wide screening for carriage would be unlikely to be cost-effective in resource-constrained settings, although targeted usage to high-risk subpopulations could potentially be impactful and cost-effective.

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### ASSOCIATION BETWEEN ORAL REHYDRATION SALTS DURING HOME TREATMENT, AND DEHYDRATION AND EXTENDED CASE FATALITY FOLLOWING A MODERATE-TO-SEVERE DIARRHEAL EPISODE IN LOW AND MIDDLE INCOME COUNTRIES

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Diarrhea causes a high burden of disease globally, with most deaths occurring in low- and middle-income countries. Despite extensive promotion of oral rehydration salts (ORS), continued feeding, and zinc supplementation for diarrhea treatment at home, multiple surveys have reported inadequate uptake of these interventions. The cross-sectional design and lack of clinical outcome data in most studies limit understanding of effectiveness of home treatment, across developing countries. We hypothesized that home treatment with ORS and zinc will reduce dehydration and extended case-fatality risk. Using data from the 7 sites of the Global Enteric Multicenter Study of diarrhea in sub-Saharan Africa and South Asia, we conducted secondary analyses to estimate prevalence of ORS and zinc administration during home treatment of moderate-to-severe diarrhea (MSD). We estimated risk ratios (RR) and 95% confidence intervals (CI) for dehydration (requiring IV rehydration) and death at 50-90 days follow-up visit, in relation to ORS administration

at home before seeking care. Zinc administration was low across countries, during home treatment (3.9%) and at health facilities (10.0%), more so in the African sites (0.0 – 9.4%) than in the Asian sites (7.8 – 37.9%). In multivariable pooled analyses adjusted for age, sex and socioeconomic status, home treatment with ORS was associated with lower incidence of dehydration (RR 0.90, 95% CI 0.83-0.97). Home treatment with ORS was also associated with lower case-fatality at 50-90 days following an MSD episode, but the association was not statistically significant (0.87, 0.60-1.26). The role of other factors, such as nutritional status, socioeconomic parameters, and concomitant therapy, in this association still needs to be examined. Our analysis suggests that home treatment with ORS protects against dehydration, and might protect against short-to-intermediate term extended case-fatality among children with MSD in low-income settings. Further studies should address a broader range of potential confounders and evaluate interventions to facilitate uptake and effective use of home treatment.

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### VIABILITY OF *VIBRIO CHOLERAE* ISOLATED DURING THE CHOLERA EPIDEMIC IN PERU IN 1991

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*Vibrio cholerae*, is the etiological agent of cholera and caused the great epidemic that affected Peru in 1991 and lately an epidemic in Haiti. There are few reports of its viability in routine culture media. The objective of the study was to observe the viability of *V. cholerae* isolated at the Microbiology Service of the National Institute of Child Health, Lima, Peru, during the 1991 epidemic. Fifty strains of *Vibrio cholerae* O1, Inaba and Ogawa serotypes, were processed and that were stored in 4mL glass vials, hermetically sealed with rubber caps, using the following medium: LIA, SIM, Simmons Citrate, Peptonade Broth with 0% ClNa and a semisolid preservation medium with peptone, ClNa and agar. The cultures were kept at room temperature. Cultures were realized to verify viability after one, 5, 10, 20 and 25 years; 45 of 50 strains were found viable with phenotypic characteristics in all of the mentioned media, while 5 strains were discarded (10%), due to contamination or dryness. Conclusion: 90% of *V. cholerae* strains stored previously were found viable; these results suggest that the medium used is a simple alternative to keep *V. cholerae* viable for research and teaching purposes.

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### A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED TRIAL TO ASSESS THE PROTECTIVE EFFICACY OF ORALLY DELIVERED BOVINE SERUM IMMUNOGLOBULIN (BSIGG) SPECIFIC FOR THE COLONIZATION FACTOR CS6 FOLLOWING CHALLENGE WITH THE CS6-EXPRESSING ENTEROTOXIGENIC *E. COLI* (ETEC) STRAIN B7A

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Enterotoxigenic *Escherichia coli* (ETEC) is a major cause of diarrhea in children and travelers. The pathogenesis of ETEC diarrhea involves colonization (via colonization factors (CFs)) which are surface proteins mediating intestinal adhesion. The role of CFs as protective antigens has been substantiated by a number of population-based studies. There is

a dearth of evidence supporting CS6 in diarrheal pathogenesis, nor is a correlation in the host immune response to CS6 and protection. The development of CF-based vaccines against a majority of circulating ETEC strains is dependent on the protective capability of included antigens. We designed a passive immunoprophylaxis trial to estimate the protective efficacy of serum-derived hyperimmune bovine immunoglobulins (BSIgG) against CS6 following challenge with CS6-expressing ETEC (strain B7A). Up to 60 volunteers were enrolled in this double-blind study and randomized 1:1:1 to receive (1) BSIgG generated against whole cell, killed ETEC strain B7A (O148:H28 CS6+ LT+ST+), (2) BSIgG raised against purified CS6, or (3) non-hyperimmune BSIgG. All groups received 1.0 g of their assigned product orally three times daily for 7 days. On the third day of administration, all volunteers were challenged with 1x10<sup>10</sup> colony forming units of B7A. The primary endpoint for this study was post-challenge development of moderate to severe diarrhea (MSD) as determined by an independent adjudication board. Fifty-nine subjects were recruited, enrolled and have completed the challenge phase of this study. BSIgG was well tolerated without incurring serious adverse-events. MSD was induced in a subset of volunteers, however the study remains blinded to the investigative team. Data are being processed and data entry and cleaning are underway. The primary endpoint data will be adjudicated in June 2017 after which the study will be unblinded. The results from this study may provide insight into the potential of CS6 as a protective antigen (critical to ETEC vaccine development), and the efficacy of BSIgG (fostering studies investigating the protective efficacy of a multivalent anti-ETEC BSIgG product).

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### CASES OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS: ASSESSING ITS RISE IN HOSPITAL AND COMMUNITY-ASSOCIATED CASES

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Methicillin-resistant *Staphylococcus aureus* (MRSA) has since become a major cause of illness and death in our healthcare setting. Risk factors for HA-MRSA include hospitalization, older age, invasive devices, and residence in long-term care facility, including exposure to antimicrobial agents. HA-MRSA isolates are often resistant to several antimicrobial drug classes in addition to beta-lactams. The CA-MRSA infections usually affects young, healthy persons and associated with sharing towels or athletic equipment, participating in contact sports, living in unsanitary and crowded areas, using illegal intravenous drugs. Directions were given out for clinical microbiology laboratories to submit invasive isolates of MRSA to our unit, where we perform antimicrobial drug susceptibility tests on all isolates and characterize all isolates that were resistant to <3 non-beta-lactam antimicrobial drug classes. Most isolates were obtained from blood cultures. The full model for predicting invasive infection with CA-MRSA compared with HA-MRSA included age, seasonality, and hospital exposure, plus specimen type. The only significant predictors of CA-MRSA infection compared with HA-MRSA were age <69 years, which was associated with increased risk ([OR] 5.1, 95% [CI] 2.06-12.64), and hospital exposure (OR 0.07, 95% CI 0.01-0.51), which was associated with decreased risk. Most patients were hospitalized for their infections and the proportion of patients admitted to intensive care units did not vary by strain. Patients infected by MRSA were younger than those infected by other strains. The number of invasive MRSA infections reported and the number of invasive infections caused by CA-MRSA is on the increase. The increase of CA-MRSA poses a unique public health threat. It is now clear that CA-MRSA no longer causes only SSTIs but now causes an increased proportion of invasive infections in a rural state.

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### HIGH PREVALENCE OF SUSPECTED NOSOCOMIAL COLONIZATION WITH METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* AT A TERTIARY CARE HOSPITAL IN SOUTHERN SRI LANKA

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Methicillin-resistant *Staphylococcus aureus* (MRSA) causes a high burden of community-acquired and nosocomial infection. Prior colonization with MRSA is a recognized risk factor for MRSA infection. In resource-limited settings, few studies have explored MRSA colonization prevalence. The purpose of this study was to describe prevalence, risk factors, and frequency of in-hospital acquisition of MRSA colonization in Sri Lanka. From Sep 2016- Mar 2017, consecutive patients admitted to orthopedic wards and every fifth patient admitted to medical and general surgical wards were enrolled at a 1,500-bed tertiary care hospital in Sri Lanka. A nasal swab was collected from the anterior nares within 24 hrs of admission and in the 48 hrs prior to discharge. Clinical and demographic data were collected. Standard microbiologic procedures were performed to identify MRSA. A total of 502 patients were enrolled, including 152 medical, 201 general surgical, and 150 orthopedic patients. Median (IQR) age was 45 (28- 61) years and 57% were male. Median (IQR) hospitalization duration was 3 (2- 5) days. At admission, 31 (6.2%) were colonized with MRSA; colonization was higher in orthopedic (12.0%) compared to medical (4.0%) and surgical (3.5%) patients,  $p=0.002$ . Patients colonized with MRSA were younger (median 38 vs 46 years,  $p=0.03$ ) and more likely male (74.2% vs 56.3%,  $p=0.05$ ). At discharge, 24 (6.7%) patients were colonized with MRSA (12.8% orthopedic, 8.4% medical, and 1.3% surgical patients,  $p=0.001$ ). Of the 24 patients, almost half (10, 41.7%) were not colonized with MRSA at admission. MRSA isolates showed low susceptibility to oral antibiotics (tetracycline 64.5%, clindamycin 19.4%, trimethoprim/ sulfamethoxazole 0%). MRSA colonization was much higher in orthopedic than in surgical or medical patients at this Sri Lankan hospital. Almost half of patients with MRSA at discharge were not colonized at admission, raising concern for nosocomial MRSA acquisition. MRSA isolates showed low susceptibility to traditional oral antibiotics. Improved infection control and targeted decolonization may decrease MRSA colonization and infection in this setting.

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### WHAT IS THE MAJOR ISSUE TO TACKLE NEONATAL INFECTIONS IN LOW INCOME COUNTRIES? EVIDENCE FROM A COMMUNITY-BASED COHORT STUDY IN MADAGASCAR

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Tackling neonatal sepsis is extremely challenging in low-income countries. Critical data on the burden of severe bacterial infections in neonates is scarce because neonatal deaths may occur rapidly when access to care may be limited. There is a particular lack of data regarding infections occurring in the community, which may differ from cases admitted to the hospital. Also, the role of the different factors involved in the transmission of multiresistant bacteria remains unclear, particularly mother-to-child.

Data are needed for these countries to prioritize interventions to decrease neonatal infections. We conducted a prospective cohort of 981 newborns in Madagascar between September 2012 and October 2014. Exhaustive identification of pregnant women on a geographic basis allowed us to enroll newborns at birth. Children were followed-up using active (home-visits) and passive monitoring. Data on clinical symptoms developed by the children and all results of biological and bacteriological samples taken were collected. The prevalence of extended-spectrum beta-lactamase producing Enterobacteriaceae and *agalactiae streptococcus* carriage among pregnant women was 18% and 13%, respectively. The incidence of community-acquired neonatal infections was 35.8 cases per 1,000 live births [95% CI, 25.4-50.8], with a great majority during the first week of life (85%). The incidence rate for multiresistant neonatal infection was 5.5 cases per 1,000 live births [2.2-13.2]. Almost two-thirds of the pathogens isolated were resistant to current WHO-recommended treatment for neonatal sepsis. In Madagascar, the incidence of bacterial neonatal infections is alarmingly high in the community. No evidence of high rates of multiresistant infections was found. The role of the mother as a potential reservoir of transmission of multiresistant neonatal infection has been highlighted. Public health measures should prioritize interventions to improve the prevention, early diagnosis, and case management of neonatal infections to decrease neonatal mortality due to severe bacterial infection, rather than bacterial resistance.

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### TYPHOID FEVER OUTBREAK IN HARARE, ZIMBABWE, 2016-2017

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Typhoid fever is a bacterial infection caused by *Salmonella* Typhi and spread through fecal-oral transmission. It causes an estimated 20 million illnesses and 200,000 deaths worldwide annually. Zimbabwe has experienced repeated outbreaks of typhoid fever over the last decade. On October 13, 2016, an outbreak was detected in the Mbare suburb of Harare. The City of Harare City Health Department (CHCHD) led an investigation in collaboration with the Ministry of Health and Child Care, WHO and CDC to characterize and identify opportunities to control the outbreak. Suspect cases were defined as fever  $\geq 38.0^{\circ}\text{C}$  and presence of at least one of the following: chills, malaise, headache, sore throat, cough, constipation or diarrhea. Investigators generated a line list cases from 36 clinics and three hospitals. Cases were confirmed by isolation of *S. Typhi* from stool and blood samples at the CHCHD laboratory, and antibiotic susceptibility testing was done using the disk diffusion technique. Investigators conducted an environmental assessment to test and map water sources. From October 13, 2016 to March 8, 2017, 867 suspected and 77 confirmed cases, with 4 deaths (case fatality rate 0.5%) were reported. Suspected cases occurred in persons aged <1 to 78 years (median 18); 48% were female. Eighteen (23%) of 77 *S. Typhi* isolates were resistant to ciprofloxacin and 10 (13%) showed decreased ciprofloxacin susceptibility. All but one of the 18 ciprofloxacin-resistant isolates were from patients who presented after Dec 31, 2016, representing 39% of the 44 isolates from this time period. Sewage contaminating underground water, which was the main water source in a suburb with erratic piped water supplies, likely contributed to this outbreak. Of 30 boreholes tested, 15 (50%) were contaminated with fecal coliforms; mapping indicated that cases were clustered around contaminated boreholes. Response efforts included: repairing sewer lines and boreholes, providing point-of-use water

treatment products, educating affected communities, training in rapid response, correct case management, improved surveillance and continued monitoring of antibiotic resistance.

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### IMPACT OF NUTRITIONAL STATUS ON PEDIATRIC PATIENTS AGED FROM 6 TO 59 MONTH HOSPITALIZED FOR SUSPECTED INVASIVE BACTERIAL INFECTIONS AT CHU-GT, BAMAKO, MALI

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Malnutrition and invasive bacterial infections are major causes of morbidity and mortality in the world. In Mali, under five mortality rate is one of the highest in the world and one half of under-five death is somehow linked to malnutrition. Few studies assess the impact of nutritional status on patients with suspected invasive bacterial infection in Mali. We retrospectively paired malnourished and well-nourished children aged from 6 to 59 month enrolled in the hospital based surveillance of invasive bacterial infection from January 2013 to December 2015. Cases and controls were paired by age group and date of enrollment  $\pm$  15 days. Case was defined as children 6-59 month malnourished (Z-score  $\leq$  -2) admitted with suspicion of invasive bacterial infection (SIBI). Blood culture or culture of any normally sterile body fluid was performed to identify potential pathogens. Controls were well-nourished children with Z-score  $>$  -2 and hospitalized for SIBI. 172 patients were enrolled (86 cases and 86 control). The mean weight of malnutrition cases was 6.59 Kg (Min 3; Max 13) while it was 9.94 Kg (Min 5; Max 19) for Non-malnourished. 10 Blood culture (4.65%) were positive (8 cases and 2 control). Cases were 4 time at risk of having pathogen in blood culture (95%CI 0.87 - 18.29) compared to control. Streptococcus pneumoniae is the predominant pathogen in cases (5/8, 62.5%). 12 deaths (9 cases and 3 control) all with no pathogen isolated in blood culture (OR= 2.13, 95%CI 0.79-5.74). Malnutrition is an important risk factor of death in patient with or without confirmed bacterial infection. Nutritional support should be implemented to support patients.

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### USE OF C-REACTIVE PROTEIN AND PROCALCITONIN TO TARGET ANTIBIOTIC PRESCRIPTION IN CHILDREN UNDER-FIVE WITH UNDIFFERENTIATED FEVER: RESULTS FROM A CLINICAL TRIAL IN TANZANIA

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At the outpatient level about 1/4 of febrile children have no localizing symptoms, and test negative for malaria. Given the lack of diagnostic tools, antibiotic overuse is a major challenge. We assessed the use of point-of-care inflammatory marker results (C-reactive protein [CRP] and procalcitonin [PCT]; intervention arm) versus disease-specific criteria (urine dipstick, typhoid rapid test, or ear discharge; control arm) in deciding on antibiotic prescription for undifferentiated fever among pediatric

outpatients (sub-cohort of a larger trial, e-POCT, that included consecutive febrile patients). Antibiotics were prescribed only to children with CRP  $\geq$ 80mg/L or PCT  $\geq$ 4 $\mu$ g/L (intervention), and to patients with positive urine dipstick, typhoid test or acute ear discharge (control). 13% (52/401) met criteria for antibiotic prescription in the intervention, versus 16% (67/413) in the control arm ( $p=0.19$ ). 12% (47/402) had PCT  $\geq$ 4 $\mu$ g/L, 2% (8/402) CRP  $\geq$ 80mg/L and 1% (3/402) both. There were 3 clinical failures (1 death) and 6 clinical failures (3 deaths) by day 7 in the intervention and control arms ( $p=0.33$ ). 1.2% (10/814) and 2.7% (22/814) had a positive blood or urine culture, respectively (result available late in the episode, not used for clinical management). In the intervention arm, 3/4 and 9/13 of children with positive blood /urine culture, respectively, had been identified by CRP/ PCT and thus treated with antibiotics. In the control arm, 2/6 of those with positive blood culture (1 positive urine dipstick, 1 ear discharge) were treated. All patients with positive blood/urine culture but not treated with antibiotics were cured at day 7. In the Dar es Salaam outpatient population, using a simplified approach of combined CRP and PCT cutoffs to decide on antibiotic prescription among children with undifferentiated fever was comparable in terms of antibiotic prescription and clinical outcome to a disease-specific diagnostic strategy. The limited relationship between the presence of bacterial disease and clinical outcome speaks in favor of using host biomarkers to identify serious infection, whatever type of pathogen is causing it.

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### BACK-CALCULATION OF THE INCIDENCE OF LEPROSY-RELATED IMPAIRMENT - GLOBAL PATTERNS AND FORECASTS

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The primary approach to reducing the public health burden of leprosy is early diagnosis of symptomatic cases thereby reducing both transmission and the incidence of leprosy-related impairment (commonly reported as cases with grade 2 disability; G2D). The 2020 target for leprosy is less than 1 new case with G2D per 1,000,000 of the global population; using the incidence of new cases with G2D both as an aim and as a proxy for reduced detection delays and transmission. Annual national-level data published by the World Health Organisation in the Weekly Epidemiological Record were analysed, each country being analysed separately. A back-calculation method was used in a fully Bayesian framework to make probabilistic inferences about the incidence of leprosy-related impairment in 2020 and beyond, and in which years the number of new leprosy cases with G2D are expected to be less than 1 per 1,000,000 of the population nationally and internationally.

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### DESCRIPTION OF THE LOCAL EPIDEMIOLOGY OF BACTERIAL ETIOLOGY IN CLINICAL NEONATAL SEPSIS IN RURAL SOUTHEASTERN CAMBODIA

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Neonatal deaths are the largest contributor to under-5 mortality worldwide, and sepsis is the second most prevalent diagnosis among neonatal deaths in low and middle income countries (LMIC). Most data and evidence informing guidelines for the treatment of neonatal sepsis in LMICs comes from the US and western Europe, which a growing body of evidence is showing to have a very different epidemiology when compared to LMICs. We performed an 18 month prospective study in a rural provincial hospital in southeastern Cambodia to describe the local

epidemiology of bacterial causes of neonatal sepsis. Neonatal nurses completed a 1-page data sheet in Khmer at time of admission. One-half to 1ml blood was collected by aseptic technique and inoculated into locally produced pediatric blood culture bottles, then incubated for 7 days and checked once daily based on the microbiology laboratory's protocol. Organisms were identified by Gram stain and basic biochemical testing. A total of 128 blood cultures were obtained from patients between April 2015 and November 2016. Three isolates identified were presumed pathogens, and all were gram negative rods (1 *Escherichia coli*, 1 *Klebsiella pneumoniae*, and 1 *Acinetobacter* spp.). Eleven were coagulase negative *Staphylococcus* and 2 were other skin contaminants, resulting in 2.3% true positives and 10.2% contaminants. High rates of contaminants pose a challenge to obtaining and interpreting blood culture results in neonates with clinical sepsis in a rural region of Cambodia. The positive rate is approximately 10 times that seen in the United States and western Europe, and the presence of gram negative rods rather than group B *Streptococcus* as the primary pathogens could help influence local treatment guidelines.

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### EXPERIMENTAL CONFIRMATION OF UNIQUE, FUNCTIONAL RICIN-B LIKE LECTIN DOMAINS IN PATHOGENIC *LEPTOSPIRA*

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Comparative genomic analysis of the genus *Leptospira* predicts that a large family (2-15) paralogous members) of secreted proteins—so-called Virulence Modifying [VM] proteins—are involved in leptospirosis pathogenesis. *In silico* analysis predicts that VM proteins contain amino-terminal ricin B-like lectin (RBLL) domains, which are found only as large gene families in pathogenic *Leptospira* (and, notably, Andean *Bartonella* spp), but no other known pathogens. RBLL domains are predicted to bind terminal galactosyl residues on glycoproteins, based on known properties of the B chain of the potent toxin, ricin. This study aimed to understand potential functional roles of leptospiral RBLL-domain-containing VM proteins. Soluble recombinant RBLL-domains as mCherry fusion proteins with an HA epitope tag and His6 affinity purification tag from two VM proteins (LA-3, LA-6) were produced in *E. coli* and purified using nickel affinity chromatography. Recombinant protein solubility, hence presumably proper folding of the cysteine-rich proteins, could be easily seen by pink color seen in the supernatant of the cell pellet lysate, allowing for rapid tracking in purification. Pulldown assays using the terminal galactosyl exposed model protein, asialofetuin, plus recombinant VM RBLL domains in the presence of 1% Tween-20, demonstrated that both VM RBLL domains interacted with asialofetuin, as detected using Western immunoblot and SDS-PAGE silver staining. IFA using specific mouse polyclonal antisera did not yield signal with *in vitro*-grown *L. interrogans*. These data demonstrate that soluble, recombinant, cysteine-rich VM proteins are functional, and supports the hypothesis that RBLL domains in pathogenic *Leptospira* bind to host glycoproteins, supporting their potential role in host-pathogen interactions. Ongoing work to identify potential host cell binding targets for VM proteins, cell binding *in vitro* and *in vivo* using fluorescence tracking, and investigation of *in vivo* VM protein expression will shed light into mechanistic functional roles of the VM protein family in leptospirosis pathogenesis.

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### SALMONELLA SEROGROUP C1 SEROVARS ISOLATED FROM BLOOD OF INFANTS IN BAMAKO, MALI, FROM 2002 TO 2014

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Non-typhoidal *Salmonella* (NTS) are the leading cause of foodborne infections worldwide, and a major cause of bloodstream infections in infants and HIV-infected adults in sub-Saharan Africa (SSA). *Salmonella*

Typhimurium (serogroup B) and *Salmonella* Enteritidis (serogroup D) are the most common serovars that cause invasive NTS disease in this region. However, data describing rarer invasive NTS serovars, particularly those belonging to serogroups C1 and C2, circulating in SSA are lacking. We previously conducted systematic blood culture surveillance on pediatric patients in Bamako, Mali from 2002 to 2014. Of 687 NTS isolates, 87% belonged to four serovars: *Salmonella* Enteritidis (36%), *Salmonella* Typhimurium (32%), *Salmonella* I 4,[5],12:i:- (6%) and *Salmonella* Dublin (13%). Here, we present data on 27 *Salmonella* serogroup C1 strains that were isolated during this previous study. No serogroup C2 strains were identified. The 27 serogroup C1 strains were typed by serum agglutination and molecular methods including Multi-Locus Sequence Typing (MLST). Of these, sixteen strains were *Salmonella* Paratyphi C, four were *Salmonella* Colindale and two were *Salmonella* Virchow. Interestingly, five strains were identified as the very rare *Salmonella* Brazzaville using a combination of O- and H-agglutination and flagellin gene typing. Phenotypic characterization showed that *Salmonella* Brazzaville produced biofilm, as measured by crystal violet binding, and exhibited catalase activity, which were not statistically different to the gastroenteritis-associated *Salmonella* Typhimurium sequence type (ST) 19. All tested *Salmonella* Paratyphi C strains were poor biofilm producers and showed significantly less catalase activity than *Salmonella* Typhimurium ST19. Overall, our study provides insight into the *Salmonella* serogroup C1 serovars that cause invasive disease in infants in Mali. Additionally, we show that MLST and flagellin gene sequencing, in association with traditional serum agglutination, are invaluable tools to help identify rare *Salmonella* serovars.

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### THE DEVELOPMENT OF A DUAL-TARGET REAL-TIME PCR ASSAY FOR THE DETECTION OF *BRUCELLA* SPECIES

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Brucellosis is one of the most widespread zoonotic diseases and is endemic in many countries across the globe. To advance brucellosis surveillance and control, it is important to continue to develop methods for rapid detection of *Brucella* species. Though many PCR assays have been developed for the detection of *Brucella* species, currently there is only one commercial kit available for PCR-based detection of *Brucella* species. During previous *Brucella* surveillance efforts it was found that a commercial kit provided false positive results because it had only a single target that shared homology with *Ochrobactrum* species. *Ochrobactrum* is close to the genus *Brucella*, is commonly found in soil and can cause opportunistic infections. To develop an improved assay for PCR detection of *Brucella* species, two genes (*bcs31* and *IS711*) were selected as targets through literature search and bioinformatics tools. The *bcs31* target has a single copy in the *Brucella* genome and encodes a 31 kDa immunogenic protein, while *IS711* is an insertion sequence specific to the genus with variable copy number in different *Brucella* species. We developed and optimized a dual-target TaqMan real-time PCR assay for detection of *Brucella* species. The target genes had no significant DNA sequence homology with other bacteria in the NCBI database. We tested *Brucella* species [*B. abortus* (11), *B. melitensis* (2), *B. suis* (4), *B. ovis* (4), *B. neotomae* (4), *B. canis* (2), *B. ceti* (3) and *B. pinnipedialis* (1)], commonly found bacteria [*B. anthracis* (2), *Y. pestis* (2), *E. coli* (2), *F. tularensis* (2), *Staphylococcus aureus* (2), *P. aeruginosa* (2), *K. pneumoniae* (2), *E. gergoviae* (2), *E. cloacae* (2), *E. aerogenes* (2), *S. sakazaki* (2), *Shigella* (2) and *Proteus* (2)] and *Ochrobactrum* species (2) for specificity. The specificity was 100% for both targets. To estimate limits of detection, *Brucella abortus* strain S19 DNA was used. For *bcs31* target, the LOD was 100 fg of DNA, while for *IS711* it was 10 fg of DNA. Because of its specificity and sensitivity this dual-target TaqMan real-time PCR assay provides an advanced tool for the detection of *Brucella* species for future use in surveillance.

## SPECTRUM OF MULTI-DRUG RESISTANT GRAM NEGATIVE BACTERIA ISOLATED FROM HOSPITALIZED CHILDREN WITH FEBRILE ILLNESS IN THREE REGIONAL REFERENCE HOSPITALS IN UGANDA

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Antimicrobial resistance is a global public health problem contributing to childhood morbidity and mortality. In resource-constrained countries identification of bacterial isolates and antimicrobial susceptibility testing (AST) is a challenge due to limited laboratory capacity. As part of an ongoing acute febrile illness (AFI) sentinel surveillance program, we report the prevalence of multidrug-resistant (MDR) gram-negative bacteria (GNB) among children at three regional reference hospitals in Jinja (eastern), Mubende (central), and Arua (northwestern), Uganda. All hospitalized children  $\leq 14$  years old with a history of fever or documented temperature  $\geq 37.5^\circ\text{C}$  were tested for malaria by microscopy or rapid diagnostic test; those with a negative malaria test result qualified for blood culture. A single blood culture bottle per patient was collected and incubated in BACTEC machines; species identification was performed at a Makerere University medical microbiology laboratory. AST was done using Kirby-Bauer disk diffusion method. MDR was defined as resistance to  $\geq 3$  of the following antimicrobials: ampicillin-sulbactam, ceftazidime, ciprofloxacin, gentamicin, trimethoprim-sulfamethoxazole, and meropenem. Among 7,360 children hospitalized during July 2016-Feb 2017, blood cultures were performed on 1,351 (18.4%), with 1,246 (92.2%) yielding no growth; 38 (2.8%) yielding a likely contaminant, and 67 (5.0%) yielding a likely pathogen. Of the pathogens isolated, 43 (64.2%) were GNB of which 10 (14.9%) were MDR, including *Klebsiella pneumoniae* (3), *Citrobacter freundii* (2), *Escherichia coli* (2), *Enterobacter species* (2), and *Klebsiella oxytoca* (1). Of the 10 MDR GNB, 6 were isolated from patients in Jinja, 5 from patients in Arua, and 5 produced extended spectrum  $\beta$ -lactamase (ESBL). MDR-GNB are isolated frequently from pediatric inpatients and pose a treatment dilemma for health workers. The ongoing surveillance project will continue to yield critical information on bacterial pathogens and their antimicrobial susceptibility patterns to inform patient care and antimicrobial stewardship guidelines.

## A NOVEL DIAGNOSTIC KEY FOR LEPROSY BASED ON ARTIFICIAL INTELLIGENCE

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Leprosy is an infectious disease caused by *Mycobacterium leprae* and approximately 200,000 new cases are registered each year. Brazil is one of the countries most affected by the disease and Governador Valadares, city of Minas Gerais state, has presented high incidence along the years, making it an ideal location to study epidemiologic interactions and to determine clinical patterns for this disease diagnosis. This diagnosis remains based on the appearance of clinically relevant manifestations what give margin to misunderstandings. This work propose a fast, accurate and friendly method of clinical classification of leprosy in order to optimize the public health service. Using the Brazilian National Notifiable Disease Surveillance System (SINAM), data were collected on cases of leprosy between January 5, 2001 and January 24, 2017, for the microregion surrounding Governador Valadares, totaling 3,368 patients in the analysis of pattern recognizing. The database include the follow variables: age, gender, number of lesions, entry mode, detection mode, bacilloscopy, number of affected nerves, clinical form and operational classification of leprosy. The median age at notification was 39 years (range 1 to 97), 55.5% were female and 39.5% were Tuberculoid cases. The data set of these variables was analyzed using a type of artificial intelligence called Random Forest (RF) to predict the clinical form of leprosy of new suspect patients. The RF proved to be quite robust for the classification of leprosy even with an error about 30% in the clinical classification (Indeterminate, Tuberculoid, Dimorph, Virchowian). On the other hand, in the operational classification (Paucibacillary and Multibacillary) this error decreased to 9%. The main source of error comes from Indeterminate cases which are mostly classified as Tuberculoid by RF, emphasizing the importance of more tests to make an accurate diagnosis. Once this situation is minimized or solved, could be created a clinical classification key for leprosy which public health professionals can use with great precision. Financial support: FAPEMIG

## UNRECOGNIZED BURDEN OF LEPTOSPIROSIS IN RURAL NEPAL: EVIDENCE FROM A SERO-EPIDEMIOLOGIC SURVEY

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Leptospirosis is an important cause of morbidity and mortality globally, but there is significant geographical heterogeneity in the burden of disease. In Nepal, there are limited data on the burden of leptospirosis, particularly outside of the Kathmandu Valley. Furthermore, most published data are based upon IgM ELISAs, which have poor specificity. To investigate the burden of leptospirosis in peri-urban and rural areas and identify the predominant serogroups circulating within Nepal, we conducted a sero-epidemiologic survey. We sampled an age and sex-stratified population of patients seeking care with mild acute febrile illness lasting  $\geq 3$  days at a tertiary referral hospital serving a peri-urban and rural population. We performed microscopic agglutination tests (MAT) using a panel of 29 reference strains representing globally important serogroups. We assumed positive titers  $>1:100$  to represent prior exposure. We used generalized linear and additive models to investigate trends in seropositivity according to age, sex and population density of residence. Among 210 individuals, 35 (16.7%) had a positive titer for at least one serovar. The most common serogroups detected were Icterohaemorrhagiae (29), Australis/Bratislava (20), and Cynopteri (13). We found no difference in seropositivity between men and women (13.8% vs 18.7%;  $p=0.45$ ). Seropositivity increased with age from 0% (0/15) among children  $<5$  years; 14% among children 5-9 years (3/22), 20% among 10-19 year olds (12/61), and 18% among those  $>20$  years of age (20/112). We found a trend towards higher seropositivity in lower population density areas ( $p=0.16$ ). There is a substantial burden

of exposure to leptospires among both men and women in peri-urban and rural areas in Nepal, which may be underappreciated as a cause of illness. Improved awareness of leptospirosis and access to accurate diagnostics are needed in clinical settings to improve management of patients with acute febrile illnesses.

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### PATHOGENS AND THEIR SUSCEPTIBILITY TO ANTIMICROBIALS USED FOR EMPIRIC TREATMENT OF INFECTIONS

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Antimicrobial resistance is a global health challenge. Significant gaps in surveillance remains, especially in Africa where blood cultures are not done routinely in public hospitals and treatment of suspected infections is mainly empirical. We here report data obtained from hospitalized children in a pilot study and the phase III RTS,S/AS01 malaria vaccine trial. This cross-sectional study was conducted at Kombewa County Hospital in the periods September 2008 to March 2009, when all admitted acutely ill children aged 2 months to 4 years were eligible for inclusion, and January 2010 to October 2013 when all admitted children in the RTS,S vaccine trial were eligible. Using a protocol defined algorithm, patients were clinically evaluated and routinely had blood cultures by the BACTEC® system and antibiotic susceptibility assays done. In 2008/2009, of the 75 positive cultures, 26 (34.67%) were positive for *Salmonella paratyphi* type B (*S. paratyphi* B). While all were susceptible to ceftriaxone, > 95% were resistant to amoxicillin/clavulanate (amoxiclav), chloramphenicol and cotrimoxazole (CTX). Of 4 (5.3%) *Streptococcus pneumoniae* (*S. pneumoniae*) isolates, 75% and 100% were susceptible to penicillin and chloramphenicol respectively but all were resistant to CTX. The 16 (17%) *Staphylococcus aureus* isolates were susceptible to Penicillin but resistant to CTX. Between 2010 and 2013, of 56 positive cultures, 24 (42.9%) were *S. paratyphi* B isolates with susceptibility to ciprofloxacin at 100% and to ceftriaxone at 70.8%. Resistance to chloramphenicol was 81.8%, to amoxiclav and CTX 100%. There were 4 (5.3%) *S. pneumoniae* isolates; 100% were susceptible to penicillin, chloramphenicol and ceftriaxone but all were resistant to amoxiclav and CTX. In conclusion, *S. paratyphi* B remained the commonest organism isolated within the time periods. There was increased chloramphenicol resistance in *S. paratyphi* B isolates. *S. pneumoniae* susceptibility to penicillin remained high. Resistance to CTX and amoxiclav was high.

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### THE BURDEN AND DISTRIBUTION OF TYPHOID FEVER IN AFRICA

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The existing estimates of the global burden of typhoid fever (ranging between 10 and 20 million infections in 2010) are based on rather simplistic extrapolation of the incidence observed during some 20 longitudinal studies. We revisited these estimates for Africa using more statistically sound methods backed by more informative explanatory covariates. We collated historic typhoid occurrence data from peer-reviewed literature, ProMED online articles, and databases of country's health administrations. After locating typhoid occurrences onto 5x5 km square grids of a map of Africa, we explored the impact of the covariates (e.g., access to improved water and sanitation and environmental covariates such as precipitation and temperature) on typhoid occurrence

using a boosted regression tree method. This led to a 5x5 km square grid map of probability of typhoid occurrence in Africa. We then examined the association between estimated probability of typhoid occurrence and observed typhoid annual incidence again on 5x5 km square grids, creating a map of predicted typhoid incidence at the (sub) national and continental level. The occurrence probability and incidence rate of typhoid fever appear to be influenced more by socioeconomic factors such as access to clean water or population density while environmental factors such as precipitation or altitude still have an impact. The estimated annual incidence of typhoid fever in Africa is around one million and thus is lower than the previous estimates (e.g., ~3 million) although its 95% credible interval includes them. Recent typhoid transmissions have been more frequent in Middle and Eastern Africa, potentially influenced by El Niño-Southern Oscillation. The updated disease burden shows significant spatiotemporal heterogeneity of typhoid fever transmission in Africa, implying that simplistic extrapolation of observed transmission events may not be adequate for estimating its continent-wide burden and we need more sophisticated tools to account for various factors influencing the transmission of typhoid fever.

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### CLINICAL CHARACTERIZATION OF LEPROSY IN A TERTIARY CENTER IN PERU

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Leprosy diagnosis requires a high-index of suspicion, recognition of clinical signs and symptoms and, if available, laboratory confirmation. In low-resource settings, the limited access to specialized diagnostic tools makes clinical diagnosis important, and early clinical recognition has implications for appropriate management and prevention of disability. We describe the experience in clinical diagnosis of leprosy in a tertiary health facility in Lima, Peru. We reviewed the data collection forms of the patients diagnosed with leprosy in the Cayetano Heredia Hospital in Lima, Peru between January 2001 and December 2016. We registered the patients' sex, age at onset, duration of disease, clinical diagnosis using the Ridley-Jopling clinical classification, and slit-skin smears for identifying acid-fast bacilli. A total of 26 patients were clinically diagnosed with leprosy, 19 (73.1%) were males. The mean age at disease onset was 43.5 ± 15.3 years (19 - 69) and the median of disease duration was 18 months (3 - 120). Out of the total number of patients, 3 (11.5%) had been previously treated and 9 (37.5%) had a known leprosy contact. Clinically, 11/26 (42.3%) patients were classified as lepromatous leprosy, 7/26 (26.9%) as mid-borderline leprosy, 4/26 (15.4%) as borderline lepromatous leprosy, and 4/26 (15.4%) as borderline tuberculoid leprosy. Acid-fast bacilli were found in 23/26 (88.5%) patients. Disability grade I (anesthesia) was found in 5/21 (23.8%) patients. Clinical diagnostic approach to leprosy using the Ridley-Jopling classification represents a valuable tool in resource-constrained settings where no laboratory confirmation is available. Further evaluation of this diagnostic approach in leprosy treatment outcomes is needed.

### RICKETTSIAL INFECTION: AN UNEXPECTED CAUSE OF FEVER IN PATIENTS HOSPITALIZED WITH ACUTE FEBRILE ILLNESS IN INDONESIA

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*Rickettsia typhi*, the etiological agent of murine typhus, is a flea-borne Gram-negative rod found in tropical and sub-tropical areas worldwide. The global burden of human infection is unknown, primarily due to the similarities in clinical presentation with other infectious diseases, particularly typhoid fever. While *R. typhi* infection is generally mild, presenting as an acute fever and rash, it can become severe and even fatal if not accurately diagnosed and treated. Unfortunately, developing countries often lack the diagnostic capacity to accurately distinguish infections of *R. typhi* from other tropical diseases. To understand the etiological profile of acute febrile illness in Indonesia, an observational study was conducted at 8 top-referral hospitals across the country from 2013-2016. 1,486 patients were enrolled and monitored at three time points: at enrollment, once between days 14-28, and at 3 months after enrollment. Demographic and clinical data were collected, and blood samples were obtained at each study visit for standard-of-care diagnostics and study-specific serological and molecular tests. 102/1486 (7%) patients were found to be infected with *R. typhi* and 1 patient was found to be infected with *R. felis* by PCR. In all cases, patients were misdiagnosed, most frequently with typhoid fever. *Rickettsial* infections were observed at all study sites, the highest prevalence being in Surabaya, East Java (12.5% of cases). Of the 89 patients who died during the study, *R. typhi* was identified as the etiological agent in 6 (6.7%/7%) cases. The detection of *Rickettsia spp.* highlights both the importance of including these pathogens in the differential diagnosis of acute febrile illness and the need for enhanced diagnostic capacity in Indonesia to improve clinical case management and decrease morbidity.

### CLINICAL, SEROLOGICAL AND MOLECULAR DIAGNOSIS OF TYPHOID FEVER, A SIGNIFICANT CAUSE OF ACUTE FEBRILE ILLNESS AMONG HOSPITALIZED PATIENTS IN INDONESIA FROM 2013-2016

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Typhoid fever remains a significant public health problem, with an estimated 21 million infections and 222,000 deaths occurring annually worldwide, primarily in developing countries. In Indonesia, the burden of disease is unknown, largely due to both challenges in clinical differentiation and reduced laboratory diagnostic capacity at health centers nationwide. To better understand how hospitalized patients presenting with an acute febrile illness are diagnosed and managed in Indonesia, an observational study was conducted at 8 top-referral hospitals across the country from 2013-2016. 1,486 patients were enrolled and monitored during enrollment, once during the 14-28 days following enrollment,

and at 3 months after enrollment. Demographic and clinical data were collected at enrollment, and blood samples were collected at each study visit for use in standard-of-care diagnostics and advanced, study-specific testing. Typhoid fever was clinically diagnosed in 248 cases, of which 43 were confirmed by blood culture and 167 by rapid diagnostic test. Only 34 of the 167 cases could be verified by ELISA and/or PCR, leading to additional testing that identified the misdiagnosed etiological agents *Rickettsia spp.* (30), dengue virus (19), chikungunya virus (7), influenza A (3), *Leptospira spp.* (2), other pathogens (6), and unknown (66). This discordance between standard-of-care and study-specific diagnoses revealed that the TUBEX TF rapid diagnostic test frequently generated false-positive results, particularly for patients infected with *Rickettsia spp.* A clinical diagnosis of typhoid fever was missed in 15 cases where *Salmonella Typhi* was identified by molecular testing. The results from this observational study suggest that typhoid fever remains a significant cause of hospitalization for patients with acute febrile illness in Indonesia. This outcome, along with our findings regarding laboratory diagnostics, will strengthen and improve the successful diagnosis of typhoid fever in Indonesia and other developing countries.

### HUMAN ECHINOCOCCOSIS: EVALUATION OF DISEASE ACTIVITY BY SEROLOGY

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Burden of human echinococcosis has changed worldwide with varied clinical profile and wider therapeutic options. A retrospective survey hydatid disease cases between 2006 and 2016 was conducted at the Hospital for Tropical Diseases, London. Clinical outcome, serological response and factors associated with adverse outcomes were assessed. Cyst-specific total IgG antibody and IgG2 subclass antibody response were serially measured by enzyme-linked immunosorbent assay (ELISA) as correlates of disease activity. 160 cases were diagnosed during the study period. Cystic echinococcosis (CE) accounted for 97% (n=155) of cases while 5 (3.1%) cases had alveolar echinococcosis. Pre-and post-treatment total IgG and IgG2 among WHO cyst types showed the mean ODs of total IgG to be significantly higher across all cyst types than those of IgG2. Post-treatment change in mean ODs was similar for both total IgG and IgG2 for all cyst types. Drug therapy was received by 127 (79.4%) cases, of which 99 (78%) received combined albendazole and praziquantel while 28 (22%) received albendazole alone. The median duration of albendazole therapy was 12 months. Radical surgery was performed in 70 (71.4%) cases while 28 (28.6%) had conservative surgery. Significant change in total IgG and IgG2 was demonstrated following surgical intervention. The median OD remained the same for total IgG after radical or conservative surgery while the greatest change in IgG2 was in the conservative surgery group. Both total IgG and IgG2 antibody levels remained elevated in active persistent disease as compared to inactive disease. IgG2 antibody response had better correlation with disease activity during post-treatment follow-up. Serologic follow-up over the years showed steady decline in total IgG and IgG2 following treatment and concurrent rise with disease reactivation. There was no disease recurrence. Serology is a useful tool for monitoring of disease activity and evaluation of response to treatment of human echinococcosis. Prospective trials are required to establish the optimal IgG2 antibody response during treatment using standardized serologic methods.

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### LUNG AND LIVER CYSTIC ECHINOCOCCOSIS - FACTORS ASSOCIATED WITH HEALTH-RELATED QUALITY OF LIFE AFTER SURGICAL TREATMENT

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The burden of Cystic Echinococcosis (CE) considers the impact of the disease and its treatment on the health of subjects which is measured by the reduction in health related quality of life (HRQoL). Current estimates of CE disease burden did not include important variables such as involved organ, type of surgical treatment, time since surgery, and disease relapse. To obtain a more detailed assessment of the impact of the disease and its treatment, we evaluated the HRQoL in a historical cohort of surgically treated individuals. The Short Form 36 questionnaire (SF-36) was applied to study population. Health domains' scores obtained by the SF-36 were standardized using means and standard deviations from the general non-institutionalized Peruvian population, and the probability to have a score below the population mean was estimated. A total of 163 subjects were evaluated. There were CE-attributable long-term effects in all health domains, except for the general health domain. The domains of vitality, role limitations due to physical problems and due to emotional problems are the most affected. Among the characteristics associated with the probability to have a score below population mean, females showed an association with vitality and mental health domain; living in endemic areas with, social functioning, and role limitations due to emotional and physical problems; and disease relapse with the general health domain. Our study demonstrated a long-term effect in HRQoL among surgically treated CE patients, predominantly in the domains of the mental health component. Among the factors related to the disease, development of a new cystic lesion was the only one that showed an impact in the general health domain.

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### NEUROINFLAMMATION IN NEUROCYSTICERCOSIS USING RAT ORAL INFECTION VERSUS INTRACRANIAL INFECTION

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Neurocysticercosis (NCC), a disease caused by *Taenia solium* larval stage (cysticerci) in the Central Nervous System, is the most common neurological disease of parasite etiology. NCC is a leading cause of seizures and epilepsy in the developing world. Research into the pathophysiology of the disease and appropriate treatment is hindered by lack of cost-effective and physiologically similar animal models. To address that, a novel rat NCC model using oral infection with activated *T. solium* oncospheres was developed, by the natural route of infection. Holtzman rats were infected in two separate groups: one group was inoculated by oral route and the second group by intracranial route. Histological examination of the tissue surrounding cysticerci was performed and compared by the two route of infection. Results show that using oral route, the infected rats developed cysticerci in the brain tissue after three months and the cysticerci can be located in the parenchymal, ventricle, or submeningeal brain tissue. When compare the two route of infection we found a statistically significant difference in the proportion of rats that develop cysticerci. Using oral infection the proportion of infected rats was less than

intracranial infection. Conversely, the infection dose in oral infection was higher (more than 5000 activated oncosphere) than intracranial infection (less than 500 activated oncospheres). However, the histopathology and neuroinflammation was not different. We observed, a layer of collagen tissue, inflammatory infiltrate cells, perivascular infiltrate, angiogenesis, spongy change, neuronal damage, and mass effect in the tissue surrounding the cysticerci. This study presents a suitable animal model for the study the pathogenesis and epileptogenesis of human NCC.

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### NEUROINFLAMMATION AT DIFFERENT TIMES AFTER INFECTION WITH *TAENIA SOLIUM* LARVA STAGE USING RAT MODEL

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The neurocysticercosis (NCC) is a parasitic infection caused by the establishment of *Taenia solium* cysts in the human brain. This infectious disease is a major cause of adult acquired epilepsy. It is reported that the parasite cause inflammation in the tissue surrounded the cyst. Therefore, some studies mentioned that the epileptogenesis could be associated with neuroinflammation and alteration of the blood-brain barrier integrity. One of the limitations to study the brain tissue neuroinflammation in patients with NCC is the difficulty in accessing to brain tissue, so it is necessary to use an animal model. Our group developed rat NCC animal model, that we used in the present study to evaluate the brain immune histopathology. The aim of this study was to evaluate neuroinflammation at different times after infection. We infected 30 Holtzman rats by intracranial route, with *T. solium* post oncospheres, and six rats none infected as control group. After infection, they were euthanized at different times (15, 30, 60, 90 days after infection). The rat brains tissue were processed to detect neuroinflammation using GFAP and Iba-1, to measure activation of astrocytes and microglia respectively, and CD68 to identify macrophages. We evaluated BBB disruption by detected IgG extravasation in the parenchymal brain tissue. We compared the proportion of immunoreactivity between the groups. The results show that reactivity to GFAP was lower at 15 days compared to 30, 60, and 90 days after infection ( $p < 0.05$ ). But the reactivity between 30, 60 and 90 days was not statistically different ( $p > 0.05$ ). The reactivity to Iba-1 and CD68 had the same pattern at the time, to 15 and 60 days both showed greater reactivity than to 30 and 90 days. Also, we observed, that immunoreactivity to IgG in the tissue surrounded the cyst at different times after infection (15, 30 and 60 days) were not statistically different ( $p > 0.05$ ), but the reactivity increased to 90 days. In conclusion, the development of the parasite in rat brain tissue caused neuroinflammation, have shown astrogliosis and to Iba-1 and CD68 decreased the reactivity with the time after infection.

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### CLONING AND EXPRESSION OF THE TRYPSIN-LIKE PROTEIN TSAG5 IN AN INSECT'S CELLS EXPRESSION SYSTEM FOR ITS POTENTIAL USE IN THE IMMUNODIAGNOSTICS OF SINGLE CYST NEUROCYSTICERCOSIS

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Neurocysticercosis is a disease caused by the accidental ingestion of *Taenia solium* eggs and it is the major cause of acquired epilepsy in Peru. The diagnosis is based on the observation of the cysts using imaging tests, such as magnetic resonance imaging (MRI) and computer tomography (CT) scan. The diagnosis can be complemented using serological tests. TsAg5 is a protein with trypsin-like activity, which has been detected in cyst's fluid and in excretion/secretion antigens of *T.solium*. In this study, we express the trypsin-like domain of TsAg5 using baculovirus as a vector to transfect Sf9 insect's cells. The expression of the domain was checked using a Western Blot assay. Then, we purified the protein using Affinity chromatography and Ion exchange chromatography. A sensitivity of 39.62% has been detected to diagnose single cyst Neurocysticercosis using TsAg5 expressed in prokaryotic vectors (Rueda *et al*, 2011). It is thought that TsAg5 expressed in a eukaryotic system would be more sensitive to diagnose single cyst Neurocysticercosis due to the post-translational modifications the system provide. This would be confirm using serological tests such as ELISA and EITB, in blood serums of patients positive to single cyst Neurocysticercosis.

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### PRODUCTION OF KIT INHOUSE IMMUNOELECTROTRANSFER BLOT TEST WITH PURIFIED CYSTICERCUS VESICULAR FLUID ANTIGEN OF TAENIA SOLIUM MIX-NATIVE FOR DIAGNOSIS OF HUMAN CYSTICERCOSIS

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Peru does not produce a kit in-house with purified antigen for diagnosis of human cysticercosis. The objectives are to purify Ag.by four methods.- Evaluate EITB test for serological diagnosis of Cysticercosis using purified Ag. Were selected pigs parasitized with cysticercosis from endemic areas of Peru.-Cysticerci were collected to obtain Ag.and quantifi the proteic concentration. Protein profile was performed by elctrophoresis. The purification of the Ags.were performed by four methods: Ammonium sulfate, Chromatography of molecular exclusion, Electroelution, and Affinity Chromatography with Lentil-Lectin. The affinity chromatography method was evaluated and selected; the sensitivity of the purified Ag.was determined with EITB and were evaluated with 50 cysticercosis-positive sera, Specificity with 50 cysticercosis-negative. -The study determined that the Ags.totals had a higher proteic concentration(3.6-2.7µg/µL), and purified Ag.had 0.82µg/mm. -Affinity Chromatography by electrophoresis identified 8 specific antigenic glycoproteins between 13-35KDa. The other methods were not evaluated by EITB because they did not correspond to the specific antigenic proteins between 13-35KDa. Using EITB test with purified Ag. we showed 100% sensitivity and specificity. -Electrophoresis with Coomassie Blue and silver nitrate, allowed to visualize purified antigenic proteins. -We conclude that of the four purification methods, affinity chromatography is of choice and recommended for purifying glycoproteins from Ag.LVC.*T.sol*. Eight specific diagnostic glycoproteins were identified(13-35KDa).-The purified Ag. improved the diagnostic efficacy of the EITB test. -Purification of glycoproteins to produce kit in-house for diagnostic NCC reliable, affordable and quality, could benefit and solve this public health problem in developing countries

of South America(Peru) and could be applied in Asia, Africa and other countries, after standardization, field validation to transfer and implement laboratories in endemic areas. The kit can be patented.

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### BANDING PATTERNS OF THE ENZYME-LINKED IMMUNOELECTROTRANSFER BLOT (EITB) CORRELATE WITH THE INFECTION STATUS IN PORCINE CYSTICERCOSIS

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The enzyme-linked immunoelectrotransfer blot (EITB) detects antibody bands against seven antigens of the *Taenia solium* larvae and is the serological reference test for porcine cysticercosis. *T. solium* antigens comprise three protein families (GP50, T24-42, and 8kDa) with different structure and function, and the heterogeneity of the antibody responses (banding patterns) against these protein families seems to be related with the characteristics of infection in humans. Nonetheless, the immune response in pig infections may differ from that observed in humans for many reasons including a heavier infective dose, different age at infection, maternal immunity, concomitant larval cestode infections, life span of infection among others. We analyzed the data on EITB banding patterns and *T. solium* infection status (not infected and infected with degenerated or viable cysts) of 491 pigs from two cross-sectional studies conducted in two endemic areas of Tumbes and Piura, in northern Peru. EITB banding patterns were grouped in homogenous classes using Latent Class Analysis (LCA), and its association with infection status, age and sex of pigs was assessed. A four-class model was specified by LCA. Infection status of pigs was strongly associated with classes ( $p < 0.001$ ), being the absence of cysts more frequent in classes 1 (negative or positive to GP50 protein family, 91.9%) and 2 (positive to T24-42 protein family, 69.9%), and viable cysts more frequent in classes 3 (positive to the 8kDa protein family, 55.6%) and 4 (strongly positive to the 8kDa protein family, 100%). Degenerated cysts were not found in the class 4, and viable cyst burden was higher in class 4 (median 1775.5, range 14-4492) and statistically significant ( $p = 0.001$ ). Pigs >8 months were more frequent in classes 3 and 4 (75% and 90%) and pigs ≤8 months were more frequent in class 1 (57%). Sex distribution was similar in all classes. EITB banding patterns correlated with the infection status of cysticercosis in pigs, so their interpretation improves the diagnosis of cysticercosis in porcine populations.

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### SOCIALIZING EVIDENCE TO TRANSFORM COMMUNITY BARRIERS IN CYSTICERCOSIS PREVENTION AND SURVEILLANCE IN NORTHERN PERU

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*Taenia solium* is a common cause of preventable epilepsy in developing nations worldwide. Ring control strategy, which involves targeted screening and treatment for taeniasis in households in proximity to heavily infected pigs, was shown to be effective when surveillance is carried out by research teams. However, for ring strategy to be sustainable, surveillance and reporting would ideally be implemented and maintained by community members. Community based participatory research (CBPR) theory suggests education alone is not sufficient to promote nor sustain this type of community-level behavior change. This was demonstrated in a recent education pilot study in which scarce adoption of community reporting led to inadequate parasite control in community-led ring strategy. By comparison, a process called SEPA has been shown to be effective and sustainable for dengue prevention in Nicaragua and Mexico. The process employs multiple modalities to socialize physical, economic, and epidemiologic evidence of disease in communities, and invests in developing community members' capacity to facilitate community-level behavioral change. In a mixed-methods pilot study involving 7 villages in Peru (pop. 1,673), we are applying a community-engagement intervention based on SEPA principles in an effort to improve adoption of community surveillance and reporting in ring-strategy. The main outcome measure is seroincidence of porcine cysticercosis in pigs born during the 12-month study period. Preliminary qualitative results are promising, including improved knowledge and retention of *Taenia* lifecycle, stronger statements of intention to change, and community generation of prevention and surveillance alternatives. Furthermore, increased willingness to publicly share experiences with *Taenia* (porcine cysticercosis, human taeniasis and neurocysticercosis) suggest this community-based process intervention also may diminish disease stigma, a previously stated barrier to behavior change and reporting. We will present methodologic insights and interim results of this on-going pilot study.

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### A NOVEL MAGNETIC PARTICLE-BASED APPROACH FOR THE PURIFICATION AND CONCENTRATION OF MONOCLONAL ANTIBODIES FROM CELL CULTURE SUPERNATANT

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Affinity chromatography is the most common method of antibody purification. This method requires samples (serum or ascitic fluid) with high antibody concentrations. However, in other samples such as large volume cell culture supernatants (CCS), this method requires one or more pre-concentration steps, complicating the experimental procedure as well as potentially damaging the antibody's native structure due to precipitation. We used a magnetic-particle based platform to evaluate the performance of the Protein L which is a multi-domain bacterial surface protein that interacts with the variable region of the kappa light chain of many immunoglobulins without interfering with the antigen binding site. First, IgM antibodies were captured by a protein L monolayer covalently coupled to the surface of iron oxide magnetic particles (mean diameter 1µm); then these particles were separated by using a magnetic field within the first 2 minutes without the need for centrifugation, an ELISA assay was performed to evaluate the performance of the purification. Then, purified antibodies were eluted as usual using regular buffers utilized in

affinity chromatography (0.1M glycine pH2). Finally, the purified antibodies were concentrated by ultracentrifugation. The loading efficiency of this magnetic platform was 2000 IgM antibodies per mg of particles, in other words, in 100 ml of CCS, using 50 mg of particles, we obtained from 0.7 up to 1 mg of IgM antibodies; these particles can be re-used up to 15 times without affecting their purification performance (OD first use 2.41; last use 2.56). Furthermore, the ELISA assay showed a clear difference in the absorbance before and after purification (OD 0.89-2.23 and 2.42-2.92, respectively). SDS-PAGE analysis showed the presence of two bands corresponding to the molecular weights of 70kDa and 25kDa, related to IgM antibodies. The use of magnetic particles combined with an ultracentrifugation method can be exploited to purify and concentrate IgM monoclonal antibodies from CCS without damaging of the structure and simplifying the procedure.

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### FIELD BASED SCREENING FOR CIRCULATING ANTIGEN IN URINE SAMPLES FOR THE DETECTION OF SEVERE FORMS OF NEUROCYSTICERCOSIS

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Neurocysticercosis (NCC), resulting from infection with *Taenia solium* larvae, exerts a heavy burden of epilepsy and other neurological disease in most developing countries. Infection is quite frequent in endemic regions: 5 to 20% of general population show antibody responses to cysticercosis and 10 to 20% of all villagers show residual brain calcifications in the very few available imaging studies performed in field conditions. In this context, while serological testing of endemic populations is easy and practical compared to imaging, the high prevalence of asymptomatic infection and the high background seroprevalence make it difficult to identify a practical outcome of serological screening initiatives. We aimed to screen a highly endemic population looking for the subgroup of individuals with elevated parasite antigens using noninvasive urine sampling, on the hypothesis that some asymptomatic villagers may harbor multicystic NCC or early subarachnoid NCC and may potentially benefit from early detection. We performed a cross sectional study in Sapillica (Piura, Peru). Initially, adult villagers were invited to provide a 5-ml urine sample that was processed in a sandwich ELISA using monoclonal antibody B158/B60. Those with high antigen levels were offered non-contrast magnetic resonance imaging of the brain machine including FLAIR and FIESTA sequences using a 1.5 T machine in a nearby city. Urine samples were collected from 429 out of 601 eligible participants. There were 25 participants with urine ELISA ratios above 2.5 (maximum 36.4). From these, 17 accepted to have a MRI performed. Three people had subarachnoid NCC diagnosed by MRI. In this population, a minimum of 0.7% of all sampled individuals had viable subarachnoid NCC. Urine antigen screening can identify asymptomatic individuals in early stages of subarachnoid NCC.

## ANGIOGENESIS AND BLOOD-BRAIN BARRIER DISRUPTION IN RAT MODEL FOR NEUROCYSTICERCOSIS

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The main infectious disease affecting the central nervous system (CNS) is caused by *T. solium* metacestode. This disease called Neurocysticercosis (NCC) is a major health problem in developing countries, especially in regions having poor sanitary conditions where pigs are raised. The study of molecular changes and host response in this infection have been difficult due to the organ affected. For this reason, animal models have been used in order to elucidate NCC pathology. A common feature presented in many CNS disease are vascular alteration and blood-brain-barrier (BBB) disruption, we hypothesized that angiogenesis and BBB disruption are presented in NCC as well. The following study uses a rat model for NCC to test this hypothesis exploring the expression of the vascular endothelial growth factor A (VEGF-A), the endothelial barrier antigen (EBA), and the presence of immunoglobulin G (IgG). Additionally, we used an *in vitro* experiment to test whether cysticercus antigens can induce angiogenesis. We found that VEGF-A is overexpressed in the tissue surrounding parenchymal and corticomeningeal cyst. BBB evaluation showed low expression of EBA in vessels close to the parasite and IgG was found in the tissue surrounding the cyst suggesting the compromise of the BBB. An endothelial cell tube formation assay using human umbilical vein endothelial cells (HUVECs), showed that excretory and secretory antigens of *T. solium* cysticercus can induce the formation of tubes. This data supports the idea that angiogenesis in NCC might be caused by the parasite itself and not only by the inflammatory response. Together this novel finding reports a new characteristic in NCC pathology and shows the effects of parasite antigens. These results indicate that further studies are needed to study the role the contribution of parasite antigen in BBB disruption in NCC.

## ANTIPARASITIC TREATMENT IN NOVEL RAT MODEL FOR NEUROCYSTICERCOSIS

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Neurocysticercosis is an infection caused by the larvae stage of *Taenia solium* and it is considered an eradicable disease. Among symptoms associated with it are seizures, headache, dementia, and other neurological disorders. We have not known exactly how these symptoms are started but they are correlated with the parasite's viability stage. Using a rat model of neurocysticercosis we standardize a treatment using different combinations of albendazole, oxfendazole and praziquantel. Also we use magnetic resonance imaging and serology as a complement to check the efficacy of treatment. A total of 48 infected rats were divided in 3 groups: 17 rats received 30mg/Kg albendazole with 100 mg/Kg praziquantel, 17 rats received 100mg/Kg oxfendazole with 100 mg/Kg praziquantel, and 14 rats received vehicle solution as a control. We reduced the parasite's viability in 30% using albendazole, and 40% using oxfendazole, although treatment with oxfendazole caused an increment of 14.9% of fibrosis. MRI post-treatment was used to assess cyst resolution but it was not a good estimator when there are several cysts. Which both drug interventions we obtained all stages of viability as we can find in an infected human brain, therefore, it will allow us to study the underlying mechanisms associated with parasite's viability stage and pathological host response.

## POTENTIAL CROSS REACTION OF GP50 WITH TAENIA HYDATIGENA IN SEROLOGIC DIAGNOSIS OF PORCINE CYSTICERCOSIS USING ON ENZYME-LINKED IMMUNOELECTROTRANSFER BLOT (LLGP EITB)

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The antibody detection assay, enzyme-linked immunoelectrotransfer blot (LLGP EITB), is often used as a serologic marker of *Taenia solium* porcine cysticercosis exposure given its excellent reported performance (sens. 99%, spec. 100%). However, only a limited number of parasites were originally evaluated as potential cross reactors. Other *Taenia* sp. are often co-endemic, including *Taenia hydatigena* which also infects pigs, raising the possibility of potential cross-reaction. In particular, the specificity of the LLGP EITB diagnostic band GP50 has been questioned. Antigen-detection assays, such as the ELISA B158/B60, are known to cross-react with other *Taenia* sp. In an ongoing study in Tumbes, Peru, where a successful cysticercosis elimination demonstration project had previously been conducted, we routinely purchase 6-8 week-old seronegative piglets from community corrals to use as sentinels in a transmission study. We encountered a corral in which 14/19 (73.7%) of tested piglets were seropositive on LLGP EITB, all of which were reactive only to the GP50 diagnostic band. These piglets were also strongly positive to Ag-detection ELISA (mean ODR 51.4) suggesting active infection with *Taenia* sp. We were able to purchase 7 of these GP50 positive piglets for subsequent necropsy; in 4/7 we found active *T. hydatigena* metacestode infection while none had *T. solium* metacestode infection. We returned to the corral where these pigs were raised to capture and purge resident dogs with Arecolin; 2/5 of the dogs captured excreted *T. hydatigena* adult stage tapeworms in their stool. The results of this investigation suggest that the GP50 glycoprotein band on LLGP EITB is likely to cross-react with *T. hydatigena* in pigs and should be interpreted with caution. Controlled experimental infection of pigs with *T. hydatigena* and other related cestodes is needed to confirm whether there is indeed cross-reactivity. These results should not be generalized to the use of LLGP EITB for diagnosis of neurocysticercosis, as humans are not known to be susceptible to *T. hydatigena* infection.

## COMMUNITY ENGAGEMENT AND HEALTH EDUCATION TO INCREASE KNOWLEDGE OF THE TAENIA SOLIUM LIFE CYCLE IN NORTHERN PERU: BASELINE AND PRELIMINARY RESULTS

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*Taenia solium* infection is a leading cause of acquired epilepsy in low and middle income countries. In Northern Peru, we are piloting a community-based research (CBR) intervention, inspired by a model called Socializing Evidence for Participatory Action (SEPA), which was developed in a community-level dengue prevention study. SEPA was shown to be effective in increasing community knowledge of dengue risk factors, as well as increasing community-developed prevention and control activities in Nicaragua and Mexico. The model includes sharing physical and epidemiologic evidence, while facilitating the collection of local knowledge through dialogue to plan community-led interventions. The model also emphasizes the formation of community-led work groups to provide outreach to community members and to develop locally relevant prevention and control strategies. Our pilot study, began in December 2016, includes 4 intervention and 3 comparison villages in northern Peru (pop. 1,673). Intervention villages are receiving the CBR approach described above, while comparison villages are receiving standard door-to-door education. Baseline data was collected on household-level knowledge of the *T. solium* life cycle, attitudes, social norms, intention to change behavior, and current *T. solium* prevention and control activities. Follow up household measures are being collected every 4 months. We are using qualitative methods to measure household-level knowledge, asking open-ended questions about three stages of the *T. solium* life cycle, then categorically classifying answers as correct or incorrect. Our poster will present results from baseline, 4-month, and 8-month follow up time points for the effects of our community-based, SEPA-inspired intervention on household-level knowledge of the *T. solium* life cycle, comparing proportional improvements in knowledge between the intervention and comparison groups.

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### DIAGNOSIS OF TAENIASIS USING A FIELD ASSAY FOR DETECTION OF COPROANTIGENS IN RURAL COMMUNITIES OF NORTHERN PERU

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*Taenia solium*, the etiologic agent of neurocysticercosis, is an important cause of preventable epilepsy in low and middle income nations. The gold standard diagnostic test is the coproantigen-detection ELISA (CoAg ELISA) which has high sensitivity (98%) and specificity (99%) to detect *Taenia* species. However, the assay requires advanced laboratory infrastructure and expensive reagents which limits its use and results in delays in diagnosis. An inexpensive alternative that can be employed in rural health posts in endemic areas is needed. We previously developed a modified version of the assay (field CoAg ELISA) using commercial bottled water for preparation of the buffers, powdered milk in place of fetal bovine serum as a blocking agent, and a toner-based plate to bypass the need for a spectrophotometer. Reagents are stored at -20 °C instead of -70 °C. In

this study we compared the performance of the field CoAg ELISA against the standard CoAg ELISA, as described by Allan et al, in a set of 1602 stool samples collected from 9 endemic communities in Piura, Peru. 1.5 ml aliquots of stool stored in PBS 5% formaldehyde were used for both assays. Cut-off points used in Peru for the CoAg ELISA are: Negative, percent positivity (PP)<7.5; Suspect, repeat test 7.5≤PP<14; Suspect, collect additional sample 14≤PP<4; Positive, PP≥40). A previously developed visual scale of color intensity corresponding to these 4 cut-off levels was used to classify the samples using the field CoAg ELISA. Inter-test agreement was excellent when comparing 4 categories (k=0.92). When samples were categorized dichotomously as positive (PP≥40) or negative (PP<40) on the standard CoAg ELISA, and positive at only the highest color intensity category vs negative (all others), agreement was lower (k=0.67); however, the field CoAg ELISA detected all true positives (28/28; 100% sensitivity) and correctly identified most true negatives (1547/1574; 98.3% specificity). These results demonstrate that the field CoAg ELISA is an excellent alternative to the standard CoAg ELISA for diagnosis of taeniasis in regions without advanced laboratory infrastructure.

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### SPATIAL AND TEMPORAL VARIATIONS IN TAENIA SOLIUM EXPOSURE AMONG PIGS IN RURAL PERU

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*Taenia solium*, the pork tapeworm, is a leading cause of seizure disorders in Peru. Humans carry the intestinal tapeworm and release infectious eggs into the environment during open field defecation. Past studies have shown that pigs testing positive for exposure to *T. solium* antibodies tend to cluster around the locations of human tapeworm carriers. Little is known, however, about how these spatial clusters vary over time in natural endemic settings. In this analysis, we examined the spatial-temporal patterns of seroincidence in pigs with the goal of identifying and characterizing persistent hotspots of *T. solium* transmission. This analysis was conducted on pigs from six rural villages in Peru. Serum samples were drawn from the full pig population every 4 months over a period of 16 months, and samples were analyzed with enzyme-linked immunoelectrotransfer blot (EITB) for antibodies against *T. solium* exposure. Incident seropositive pigs were those that had 1 or more reactive EITB band and were part of the at-risk cohort born into the villages during the study period. To detect spatial-temporal clusters, we used the SaTScan space-time scan statistic, which accounts for natural spatial variations in population density while scanning space-time windows for elevated disease rates. Overall, we detected 10 spatial-temporal clusters in the six study villages that had a combined rate ratio of 2.49 (95% CI: 2.29, 2.91), as compared to non-cluster pigs. The median cluster size had a radius of 170m (IQR: 115, 190) and spanned approximately 8 months. The existence of distinct spatial-temporal foci of transmission with extreme rates of disease occurrence indicates persistent (≥8 months) focal sources of *T. solium* eggs in the environment of endemic villages in rural Peru. These are likely attributable to persistent untreated tapeworm carriers residing in close proximity to cluster sites. Spatial-temporal analysis of pig infection may allow control programs to target persistent foci of transmission for anti-helminthic treatment or improved sanitation infrastructure, which may ultimately help reduce the burden of cysticercosis in these communities.

### EFFECTS OF IMMEDIATE VS. DELAYED IRON THERAPY ON NEUROBEHAVIORAL FUNCTION IN UGANDAN CHILDREN WITH SEVERE MALARIA

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Malaria-induced inflammation may limit iron availability in the immediate post-infectious period, and may act on the developing brain via pathways similar to nutritional iron deficiency (ID). We conducted a randomized clinical trial to determine the effects of 4-week delayed (D) vs. immediate (I, current standard of care) iron therapy on neurobehavioral outcomes in children with severe malaria (SM) and ID. Children <5 years of age with severe malarial anemia (SMA) (n=77) or cerebral malaria (CM) (n=79), and healthy community children (CC) (n=83) were enrolled. All children with CM or SMA and 38 CC had ID as assessed by zinc protoporphyrin (ZPP) concentration ( $\geq 80 \mu\text{mol/mol}$  heme), and were randomized to I or D iron therapy. Assessments of executive function, socioemotional function, and observer-rated behavior were performed at baseline and 6 and 12 months later. At 12 months, no differences were seen in executive function, socioemotional function or observed behavior between the I and D arms in children with SMA, CM, or the CC. Delayed iron therapy does not appear to improve neurobehavioral outcomes in children with SM, but it is also unclear how many of these children require iron, since ZPP is affected by inflammation. Further investigation on which iron measures best reflect brain iron status may help to unravel the complex relationships between malaria infection, ID, and cognitive and behavioral development.

### PARASITIC INFECTIONS DURING PREGNANCY IN GABON: BIRTH OUTCOMES AND IMMUNOLOGICAL CHANGES

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Low birthweight (LBW) remains important in sub-Saharan Africa and particularly in Gabon. In a multicentre study in Benin, Gabon, Mozambique and Tanzania on HIV-negative pregnant women and their offspring, determinants of LBW were malaria, very young maternal age, first pregnancy, poor gestational nutrition and small stature of the mother. In Gabon, the major parasitic infections including malaria, schistosomiasis and *Loa loa* are common in pregnant women. Most research groups in affluent countries have assessed the interaction between helminths and host's immune system and whether by modulation of bystander responses, helminths could influence the outcome of vaccinations or inflammatory diseases or conditions such as pregnancy, and only a few groups have taken these questions to areas where helminth infections are highly endemic. We showed that malaria and urogenital schistosomiasis during pregnancy are associated with higher proportions of LBW in Gabon. In immunological studies maternal infection with *L. loa* was associated with expansion in the neonatal cord blood of functionally active regulatory T cells (Tregs) that kept Th1 and Th17 immune responses in check, providing some insights on the impact of *in utero* exposure on the offspring's development and health. Schistosomiasis was associated with significant increases of frequencies of Tregs that play an important role in controlling Th1 and Th2. Despite expansion of Tregs during loiasis and schistosomiasis, there was no evidence these changes induce adverse pregnancy outcomes,

raising the question regarding their clinical impact. Mefloquine as an alternative preventive treatment showed more effective than sulfadoxine-pyrimethamine in preventing malaria infection and anemia, despite a poor tolerability. Interestingly, it was effective against concomitant schistosomiasis suggesting it could serve as a combined intervention for both infections during pregnancy. More intervention studies are necessary to further determine whether getting rid of parasites either by screening, prevention or treatment strategies would improve the outcome of pregnancies in endemic areas.

### PREDICTING MORTALITY FOR ADOLESCENT AND ADULT PATIENTS WITH FEVER IN RESOURCE-LIMITED SETTINGS

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Febrile illnesses are a major cause of mortality in sub-Saharan Africa. Early identification of patients at increased risk of death may expedite interventions to avert adverse outcomes. We aimed to evaluate the performance of the Integrated Management of Adolescent and Adult Illness (IMAI) criteria, quick Sequential Organ Failure Assessment (qSOFA) score, Modified Early Warning Score (MEWS), Assimwe score, Rapid Emergency Medicine Score (REMS), and Rapid Acute Physiology Score (RAPS) to predict in-hospital mortality among a prospective cohort of febrile patients in Tanzania. We evaluated 420 patients aged 10 years or old hospitalized in the period 2007-08. Of the 45 patients who died, 32 (71.1%) were HIV-infected. The respiratory and central nervous system (CNS) were the most common sites of infection among the patients who died. At univariate analysis (OR [95%CI]), in-hospital mortality was significantly associated with peripheral oxygen saturation <90% [3.7 (1.7-8.0)], Glasgow coma scale <15 [11.4 (5.1-25.3)], haemoglobin <9 g/dl [4.5 (2.4-8.4)], CD4+ <200 cells/ $\mu\text{l}$  [4.1 (1.4-12.4)], and CNS infection [3.7 (1.8-7.9)]. IMAI severe respiratory distress without shock, IMAI severe pneumonia, qSOFA score  $\geq 2$ , and REMS score  $\geq 16$  were also significantly associated with in-hospital mortality. After controlling for confounders, only IMAI severe respiratory distress without shock (OR 3.2, 95% CI 1.2-8.3) and IMAI severe pneumonia (OR 2.6, 95% CI 1.2-5.6) remained significantly associated with in-hospital mortality, but areas under the receiver operating characteristic curve (AUROC) showed poor discrimination (0.57 [95%CI 0.47-0.66] and 0.61 [95%CI 0.52-0.70], respectively). Severity scores did not perform well in predicting fatal outcome among febrile in-patients in Tanzania. Although impractical, IMAI severe respiratory distress without shock and severe pneumonia criteria were significantly associated with in-hospital mortality. Further studies are needed to develop a robust and simple tool for early identification of severely ill febrile patients in sub-Saharan Africa.

### PREDICTING MORTALITY FOR ADOLESCENT AND ADULT PATIENTS WITH FEVER IN RESOURCE-LIMITED SETTINGS

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Febrile illnesses are a major cause of mortality in sub-Saharan Africa. Early identification of patients at increased risk of death may expedite interventions to avert adverse outcomes. We aimed to evaluate the performance of the Integrated Management of Adolescent and Adult Illness (IMAI) criteria, quick Sequential Organ Failure Assessment (qSOFA)

score, Modified Early Warning Score (MEWS), Assimwe score, Rapid Emergency Medicine Score (REMS), and Rapid Acute Physiology Score (RAPS) to predict in-hospital mortality among a prospective cohort of febrile patients in Tanzania. We evaluated 420 patients aged 10 years or old hospitalized in the period 2007-08. Of the 45 patients who died, 32 (71.1%) were HIV-infected. The respiratory and central nervous system (CNS) were the most common sites of infection among the patients who died. At univariate analysis (OR [95%CI]), in-hospital mortality was significantly associated with peripheral oxygen saturation <90% [3.7 (1.7-8.0)], Glasgow coma scale <15 [11.4 (5.1-25.3)], haemoglobin <9 g/dl [4.5 (2.4-8.4)], CD4+ <200 cells/ $\mu$ l [4.1 (1.4-12.4)], and CNS infection [3.7 (1.8-7.9)]. IMAI severe respiratory distress without shock, IMAI severe pneumonia, qSOFA score  $\geq$ 2, and REMS score  $\geq$ 16 were also significantly associated with in-hospital mortality. After controlling for confounders, only IMAI severe respiratory distress without shock (OR 3.2, 95% CI 1.2-8.3) and IMAI severe pneumonia (OR 2.6, 95% CI 1.2-5.6) remained significantly associated with in-hospital mortality, but areas under the receiver operating characteristic curve (AUROC) showed poor discrimination (0.57 [95%CI 0.47-0.66] and 0.61 [95%CI 0.52-0.70], respectively). Severity scores did not perform well in predicting fatal outcome among febrile in-patients in Tanzania. Although impractical, IMAI severe respiratory distress without shock and severe pneumonia criteria were significantly associated with in-hospital mortality. Further studies are needed to develop a robust and simple tool for early identification of severely ill febrile patients in sub-Saharan Africa.

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### ASSOCIATION OF MIRNA-122 WITH LIPIDS IN A SUB-POPULATION OF HYPERTENSIVE PATIENTS IN SUB-SAHARAN AFRICA

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Dyslipidemia contributes to atherosclerosis and is a risk factor for cardiovascular disease. miRNA-122 is liver specific with suggested roles in cholesterol, fatty acid and lipid metabolism. This study was aimed at determining the association between dyslipidemia and plasma levels of miRNA-122. Triglycerides, total cholesterol, high density lipoprotein-cholesterol (HDL-c) and Low density lipoprotein-cholesterol (LDL-c) were quantified in plasma samples of hypertensive (n=200) and control (n=200) subjects. miRNA-122 was extracted and quantified with qPCR. Lyophilized qPCR miRNA-122 template standards were obtained from Origene Technologies, Rockville, USA. After lipid profile analysis, subjects were further divided into dyslipidemic and non-dyslipidemic groups. Results show that 44% (n=88) of hypertensive patients and 23.5% (n=47) of the control group had dyslipidemia. Dyslipidemic hypertensives and controls showed significantly ( $p < 0.05$ ) higher levels of miRNA-122 when compared with non-dyslipidemic subjects. LDL-c and total cholesterol showed significant positive correlation with miRNA-122 ( $r = 0.53$ ,  $r = 0.51$  respectively,  $p < 0.05$ ). Whereas correlation with triglycerides and HDL-c was weak and not significant ( $r = 0.10$ ,  $r = -0.10$  respectively,  $p > 0.05$ ). This findings support earlier studies suggesting that miRNA-122 may have a role in lipogenesis. Therapeutically targeting miRNA-122 using RNA-based gene silencing strategies will possibly ameliorate dyslipidemia, thereby reducing the risk of atherosclerosis and cardiovascular disease.

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### Q FEVER IN SOUTHERN CALIFORNIA, A CASE SERIES OF TWENTY-ONE PATIENTS

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Q fever was first described in Southern California in 1947, when the dairy industry was flourishing and geographically concentrated. It was found to be endemic and enzootic to the region, mostly associated with exposure to livestock. Since the 1950s few regional studies have been conducted. We describe 21 patients diagnosed with Q fever in Southern California with the aim of contributing toward the understanding of Q fever and its clinical presentation, geographic distribution and risk factors. A retrospective chart review was conducted on patients diagnosed with Q fever at our institution between 2000 and 2016. Cases were categorized as either acute or chronic based on available titers. Demographic information, laboratory data, imaging results, data on risk factors and treatment were reviewed and analyzed. The majority of patients presented with an acute febrile illness (85.7%), other common presenting symptoms included headache, cough, hepatomegaly, and arthralgias or myalgias. In 12 of the 21 cases, diagnostic titers were obtained greater than 15 days from symptom onset and 57.1% of cases went on to develop either chronic infection or death. Q fever was thought to be a contributing factor to death in most cases. The geographic distribution of cases appeared to favor rural and less populated areas. The delay in obtaining diagnostic titers by more than 15 days for the majority of patients was associated with increased severity and mortality seen in the cases. The majority of the chronic cases went on to develop endocarditis, which is the most common manifestation of chronic Q fever. We feel that Q fever is underdiagnosed in this region, which can be attributed to its frequent asymptomatic presentation or ability to present with nonspecific symptomatology. Cases in Southern California continue to occur intermittently over long periods of time in a long-standing endemic pattern. Although further epidemiologic studies are needed, it appears that the locality of our cases was closely linked with local dairy farms.

#### 500

### ANTI-MOSQUITO SALIVA IMMUNITY, MAST CELLS AND CLINICAL PRESENTATION OF DENGUE

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Mosquito saliva has a profound impact on transmission of vector-borne pathogens such as dengue virus (DENV) and immunity against saliva modifies the local response during pathogen transmission. To investigate the effect with of anti-saliva immunity, we measured IgG and IgE antibody levels against DENV and *Ae. aegypti* salivary proteins, in volunteers from a DENV endemic area. We also looked at the impact of antibodies on the role of mast cells by incubating pre-sensitized human mast cells (LAD2) with human serum from dengue patients. We found that histamine levels in the supernatant of pre-sensitized mast cells were significantly correlated with total anti-DENV-IgG antibodies, and that IgG antibody levels against salivary D7-long protein were significantly correlated with TNF $\alpha$  gene expression in mast cells challenged with SGE. We also found a significant association between platelet count and TNF $\alpha$  gene expression in mast cells challenged with DENV and SGE, while leucocyte count was associated with histamine concentration. DENV infection and mast cell degranulation were significantly correlated with anti-DENV-IgG4 antibodies

levels. We also found that DENV-exposed individuals presented higher hybrid IgG4 molecules when compared with European controls, although total IgG4 antibody levels were not significantly different between the groups. Our data also showed a significant positive correlation between the concentration of hybrid DENV-SGE antibodies and the immune response against salivary proteins in controls versus dengue patients. We hypothesize that the presence of hybrid DENV-SGE antibodies may influence antibody-mediated responses against dengue. Thus, we prepared antibody fractions and incubated them with U937 monocyte cell line and primary dendritic cells along with DENV. Our pilot analysis showed significant differences in virus infection in cells incubated with either specific anti-DENV, anti-SGE or hybrid antibodies. The full results will be discussed during the presentation.

## 501

### **CORD BLOOD MATERNAL MICROCHIMERISM PREDICTS DECREASED RISK OF NON-MALARIAL FEVER DURING CHILDHOOD**

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Despite large improvements in global childhood mortality in the last 20 years, an astonishing 5.9 million children died before their fifth birthday in 2015. Three of the top five causes of death are infectious, including pneumonia, diarrhea, and neonatal sepsis. Little is known about the host intrinsic factors that affect susceptibility to these illnesses, and in particular, thus far overlooked is the role of a small number of maternal cells and DNA acquired by the fetus during pregnancy, known as maternal microchimerism (MMC). We recently found that children with MMC at delivery were more likely to become infected with malaria but, surprisingly, when infected were less likely to become sick or to be hospitalized as compared to children without MMC. We hypothesized that this effect is the result of maternal regulation of pro-inflammatory fetal and infant immune responses, limiting immune-mediated pathology, and that such regulation may extend to other infections of global health importance. We additionally hypothesized protection from those infections in which pathology may be immune-mediated (e.g. pneumonia) but a neutral effect for those in which pathology is predominantly pathogen-mediated (e.g. diarrheal illness). Using samples and data from a nested birth cohort from Tanzania, we investigated the ability of MMC to predict four outcomes in longitudinal models: 1) non-malaria fever, 2) non-malaria hospitalization, 3) lower respiratory tract infection, and 4) diarrheal illness. Cord blood MMC was protective against non-malarial fever (AOR=0.42, p=0.01) but not non-malarial hospitalization. In addition, there was a suggestion of protection against lower respiratory tract infection (AOR=0.72, p=0.2) but not diarrheal illness. In conclusion, MMC may protect from immune-mediated pathology secondary to common childhood infections, which has broad implications for perinatal and postnatal infections and response to immunization. Future studies should investigate the direct effect of maternal cells on fetal and infant immune responses.

## 502

### **WHERE HAS THE BLOOD GONE IN PATIENTS WITH SEVERE ANAEMIA IN SEVERE MALARIA DUE TO PLASMODIUM FALCIPARUM INFECTION AND SEPTICEMIA**

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Malaria tops the list of sicknesses seen regularly by most general practitioners especially at the grassroots in Lagos Nigeria. Between 1991

and 2014, 10,721 patients were treated for malaria in our grassroots hospital located in Bariga a high density area of Lagos state Nigeria. All but 9 cases responded very well to treatment. The 9 cases presented with severe anaemia as a complication. The first case died, 8 survived but one was almost fatal. Trying to correct the severe anaemia with blood transfusion was counterproductive. The improvement in the haemoglobin level of the patients after the transfusion was short lived as the patient became pale again within 24 to 72 hours of the transfusion. Further transfusion was of no use as the patient reverted to square one. The liver and spleen which might not be palpable on admission gradually became so and could attain huge sizes. None of these 9 patients had haematemesis or melena. Jaundice if present was mild. Bilirubin and urobilinogen if raised, were just slightly so. In 2014, this picture which was hitherto seen in patients with severe malaria due to *Plasmodium falciparum* infection was also seen in our 9th case who presented with sepsis. In the absence of indexes of severe haemolysis, haematemesis and melena capped with the phenomenon of transient improvement in haemoglobin level after blood transfusion, a pertinent question to ask is where has the blood gone in these patients? In a country with a galloping inflation and a national minimum wage less than \$50 per month, the grassroots from where all these cases came cannot afford the investigations that will be required for more detailed studies on a large scale. We look forward to getting sponsors for the project.

## 503

### **ASSESSMENT OF ENDOTHELIAL PROGENITOR CELLS IN HYPERTENSIVE DISORDERS OF PREGNANCY**

**Dorotheah Obiri<sup>1</sup>**, Daniel Oduro<sup>2</sup>, John Tetteh<sup>3</sup>, Thomas Addison<sup>3</sup>, Amma Larbi<sup>3</sup>, Emmanuel Kakra Dickson<sup>3</sup>, Kwame Adu-Bonsaffoh<sup>4</sup>, Samuel A. Obed<sup>4</sup>, Kwadwo A. Kusi<sup>3</sup>, Michael Ofori<sup>3</sup>, Ben Gyan<sup>3</sup>

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Hypertensive disorders of pregnancy particularly preeclampsia, are a global challenge associated with maternal and foetal mortality. The resultant systemic inflammation has been linked to endothelial activation and subsequent dysfunction to the endothelium. Host immune response to this damage has been associated with the recruitment of rare endothelial progenitor cells (EPCs) to sites of damage where they repair or form new blood vessels. This study accessed the levels of endothelial progenitor cells in women having a normal delivery and those diagnosed with hypertensive disorders in pregnancy. EPCs were measured by the co-expression of surface antigens on progenitor cells originating from the bone marrow to sites of injury. EPC levels from normal pregnancy (controls) and hypertensive disorders (cases) were estimated using antibodies specific to CD309, CD34, and CD133. Blood was sampled from the peripheral, cord and placental blood. The percentage EPC levels were assessed at the different blood collection sites (periphery, cord, and placenta) in normal and hypertensive pregnancies. No difference were found in these sites between cases and controls. A higher number of EPCs were found in the placental blood than the cord blood among normal pregnancies. Placental and peripheral blood did not show any significant difference. For hypertensive pregnancies, there was a higher number of EPCs in the placenta compared to periphery but not in the cord blood. The high levels of EPCs in the placental blood for both normal and hypertensive pregnancies indicate the high vascularisation that occur within the placenta. Lower EPCs in peripheral blood in hypertensive pregnancy might indicate an impaired endothelial repair hence the pathology.

## 504

**JAPANESE ENCEPHALITIS VACCINE: IS THE BOOSTER DOSE REGULARLY ADMINISTERED?**

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Japanese encephalitis (JE) is one of serious vector-borne viral encephalitis disease found worldwide, especially in Asian, the Western Pacific countries, and in northern Australia. Although JE rarely affects travellers, its serious consequences and unpredictable epidemiology makes its prevention a very important part of the pre-travel consultation. The aim of this study was to evaluate the JE vaccination patterns of travellers into a pre-travel consultation. For this purpose, we used the electronic centralized vaccination database (ARSLVT, I.P.) to collected data of prescribed JE vaccines, from the Travelers Medical Center of ACES Almada-Seixal, Portugal, between January 2000 and December 2015. The nominal information extracted included, sex, date of birth, vaccine type, vaccine scheme and inoculation date. Data was entered into Microsoft Excel® and descriptive analyses performed. Of the 374 surveyed travellers, 228 (61%) were male, 146 (39%) were female, and the mean age was 41 years (range: 18-83 years). Regarding the JE vaccine, 240 had received the total scheme of the vaccine (25% JE-VAX conventional, 8% JE-VAX accelerated, 25% IX-conventional, 11% IX-accelerated), and 134 had been given only the first dose. Regarding the booster dose, only 5 (1%) travellers had received the vaccine, 2 had not yet received it and 367 had no administration dates. Results shows that the majority of individuals who had been previously immunized with JE vaccine before traveling, had not administrated the booster dose after arrival.

## 505

**DYING ONE WAY OR ANOTHER: AN ANALYSIS OF COMBAT AND NON-COMBAT DEATHS AMONG U.S. TROOPS IN VIETNAM, 1960-1975**

**David Adams**<sup>1</sup>, Femi Taiwo<sup>1</sup>, Joseph Miller<sup>2</sup>, Valerie Adams<sup>2</sup>, Kali Neil<sup>3</sup>

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An extensive literature exists on US casualties of the Vietnam War, 1960-1975. Much, however, has focused attention on mortality from engagements with pro-communist forces. An equally large body of literature has examined morbidity and mortality from infectious diseases, not least malaria. One largely ignored area of research, however, is mortality as the result of personal and interpersonal violence. More specifically, in-country deaths caused by "suicide"; "intentional" and "accidental" homicide; and "accidental self-destruction". This study will draw on archival data (RG 330) from the National Archives of the United States. We will compare "cases" (deaths by "accidental homicides," "intentional homicides," "accidental self-destruction," and "suicide") with a randomly selected group of "controls" who were killed during engagements with Vietnamese communist forces. Analysis of these data reveals that risk for specific types of non-combat violent death and in-country survival differentials significantly varied according to a number of factors, i.e., proximity to the Demilitarized Zone (DMZ), rank, service branch, climatological factors, and time of year—not least holidays such as Thanksgiving and Christmas.

## 506

**LIFE AND DEATH IN 17TH CENTURY JAMAICA: TROPICAL DISEASE AND BRITISH COLONIAL AMBITIONS**

**David Adams**<sup>1</sup>, Valerie Adams<sup>2</sup>, Femi Taiwo<sup>1</sup>, Kali Neil<sup>3</sup>, Joseph Miller<sup>2</sup>

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Much has been written about the rise of European colonization of the Americas in the 16th and 17th centuries. To the British went various slices of the Americas, to the French others, to the Spanish others, and to the Portuguese yet others. Therein lay the problem, however, as these nations maneuvered for control of as much territory as possible. It was the Caribbean, though, that proved an especially interesting international free-for-all. This presentation is a case-study of the impact of tropical disease on British troops in Jamaica during the 1650s. Although accounts by the ranks are few, those written by their officers offer an important window into maladies that afflicted officers and men alike. They shed significant light on infectious diseases that often killed more efficiently than a Spanish or French sword or round. Utilizing the first-hand account by General Robert Venables, this study highlights the impact of tropical diseases on British ambitions in mid-17th century Jamaica.

## 507

**TELEPHONE ADMINISTRATION OF THE PATIENT SPECIFIC FUNCTIONAL SCALE (PSFS): A VALID, RELIABLE, AND PATIENT-REPORTED OUTCOME IN GLOBAL SNAKEBITE RESEARCH**

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The lack of inexpensive, reliable, validated patient-reported outcomes (PRO) is a gap in global snakebite trials. With the widespread use of cellphones in LMIC, a telephone-administered validated PRO would be a useful tool in future trials. In a recent snakebite clinical trial the in-person administered Patient Specific Functional Scale (PSFS) performed well. Telephone administered PSFS was used between in-person assessments. The objective of this study is to determine if the administration of PSFS via telephone has similar performance to in-person administration in snakebites. We performed a secondary analysis of the "The Efficacy of Fab Antivenom versus Placebo plus Optional Rescue Therapy on Recovery from Copperhead Snake Envenomation: a Randomized, Double-Blind, Placebo-Controlled Clinical Trial." We compared telephone to in-person administration of PSFS by evaluating the following scale psychometric properties: (a) content validity (ceiling and floor effects), (b) internal structure and consistency (Cronbach's alpha coefficient), and (c) temporal and external validity using Intraclass Correlation Coefficient (ICC). In-person PSFS was performed on 3, 7, 14, 21 and 28 days post-envenomation. Telephone PSFS was performed on 10, 17, 24, and >28 days post envenomation. All seventy-four patients were included in the analysis. The mean age was 43 (SD17.6) years. Fifty-two percent were male and 89% were adults. There was a tendency towards floor effects in the total PSFS with a median score of 3 (range 0–8) and Q1 0–0, Q3 5–8, with 28% of the participants scoring 0 at baseline. At follow up, the ceiling effects were seen as the median total PSFS score was 9 (range 5–10) with Q1 5–8, Q3 10–10, and 48% of the participants scored 10. We found good to excellent internal consistency (Cronbach alpha 0.91 (95% CI 0.88, 0.95) in-person and 0.81 (0.73, 0.80) via telephone. There was good temporal stability with ICC 0.83 (0.72, 0.89) in-person and

0.80 (0.68, 0.88) via telephone. Telephone-administered PSFS is a valid and reliable PRO to assess recovery in snakebite compared to in-person administered PSFS.

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### RICKETTSIOSIS IN PEDIATRIC PATIENTS: CLINICAL SERIES IN LABORATORY CONFIRMED CASES IN SOUTHERN MEXICO

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Wild and domestic animals can be carriers of ectoparasites like ticks, fleas or dust mites, which constitute vectors of *rickettsial* infections in humans. The identification of epidemiological traits and clinical manifestations of the infection and is relevant for clinical research purposes regarding the true incidence and physiopathological processes associated with *rickettsial* infections. In the state of Yucatan, Mexico four confirmed cases of rickettsioses were reported by the Department of Epidemiology in 2016. *This study was undertaken to analyze the epidemiological and clinical characteristics of four confirmed cases. The four cases occurred in different locations, but the exposure to dog's ectoparasites was reported in all four cases. Common clinical manifestations were fever, rash, blood analysis regarding leukocytosis, neutrophilia and presence of lymphopenia with or without hepatic or splenic hypertrophy. We observed that the presence of antecedents of contact with the arthropod vector in domestic dogs were readily advised by the family members of the patients. However, we cannot assure that it was intentionally investigated during the initial anamnesis in the first medical consultation. Without a doubt, the anamnesis is an orienting process and it is relevant in the process of differential diagnosis, which is why it should not be ignored during the initial medical consultations nor in the elaboration of hospital clinical histories. Each of the four cases were poorly diagnosed. The patients' symptomatic profiles of fever and rash were misdiagnosed with viral entities transmitted by the Aedes vector. The doctors who made the diagnoses did not initial suspect the presence of a bacterial infection. Even though the emergent and reemerging illnesses transmitted by vectors of the Aedes genre have been the cause of recent epidemics in the region, it is always important to conduct a pertinent anamnesis that will allow one to distinguish between different etiologies, particularly when the existing evidence does not support a viral profile.*

## 509

### IDENTIFYING RISK FACTORS FOR PERINATAL DEATH AT TORORO DISTRICT HOSPITAL, UGANDA

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Many countries in sub Saharan Africa face a disproportionate burden of perinatal deaths, defined as stillbirths (fetal deaths before delivery, at or after 22 weeks gestation) and neonatal deaths within 7 days of birth. However, data to inform targeted interventions is often lacking. To identify risk factors for perinatal death, we conducted a retrospective case-control study using data from birth registers at Tororo District Hospital, Uganda on all cases of pregnancies with perinatal deaths from January to December 2014. Controls were derived from the admissions immediately preceding and following the cases of perinatal death. There were 185 case pregnancies with perinatal deaths, including 7 sets of twins with 2 perinatal deaths each, and 354 control pregnancies. Stillbirths accounted for 145/192 (75.5%) of the perinatal deaths, and 69/145 (47.6%) of these were "macerated," that is with findings suggestive of death prior to labor. The remaining 47/192 (24.5%) of perinatal deaths were neonatal demises. The following factors were associated with increased risk of perinatal

death: prematurity (odds ratio (OR) 19.9; 95% confidence interval (CI) 7.6 - 65.3), multiple gestation (OR 8.0; CI 2.8-27.7), breech presentation (OR 8.0, CI 1.6-77.1), low birth weight (OR 6.8; CI 4.1-11.5), antepartum hemorrhage (OR 3.6, CI 1.1-13.7). Presenting in spontaneous labor (OR 0.1; CI 0.1-0.3) and vaginal delivery (OR 0.3; CI 0.2-0.6) were associated with decreased risk of perinatal death. There were no associations between perinatal death and nulliparity, HIV infection, preeclampsia, or prior uterine scar. To assess for factors more likely associated with perinatal death during or secondary to labor, fresh stillbirths and neonatal deaths were compared to macerated stillbirths. Multiple gestation (OR 3.4; CI 0.9 - 18.9) and presentation in labor (OR 2.8; CI 1.4 - 5.6) were the only of the aforementioned factors that were associated with increased risk of perinatal death during or after labor and delivery rather than before. Our results identify potential areas for focused intervention to decrease the frequency of adverse birth outcomes in similar settings.

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### CREATIVE SOLUTIONS FOR NEONATOLOGY CARE IN A LIMITED RESOURCE SETTING IN BURUNDI

**Alyssa A. Pfister**

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Kibuye Hope Hospital (KHH) is a rural hospital that serves approximately 2 million people in the center of Burundi. To address the high rates of morbidity and mortality for premature infants in the region, a Neonatal Intensive Care Unit (NICU) was developed using creative solutions despite limited resources. The 11-bed NICU was opened in September 2016. Common complications of prematurity and challenges related to limited resources were addressed in the following innovative ways: Hypothermia: Incubators were designed and fabricated using local materials including a battery back-up system. Limited nursing staff: Hospital engineers created a unique bed design with built-in incubator to enable mothers to stay with their babies under a shared mosquito net. Mothers are educated in how to express breast milk and give it to their infants via nasogastric tube. Jaundice: The Bili-Hut™ provides portable phototherapy to treat neonatal jaundice. Respiratory complications: Prophylactic aminophylline is given to premature infants to reduce the risk of apnea of prematurity given the lack of continual monitoring. No surfactant is available to treat respiratory distress syndrome, but oxygen by nasal cannula assists many premature infants. Infections: No cultures are available, but two negative CRPs are used as a surrogate for eliminating the risk of bacterial sepsis and thus stopping unnecessary antibiotics. Risk of necrotizing enterocolitis (NEC): Given the lack of parenteral nutrition available, prevention of NEC is the primary goal through slow advancement of expressed breast milk feeds. At the first signs of feeding intolerance, feeds are stopped and D10 is given along with broad-spectrum antibiotics. In the first 6 months after the opening of the NICU, 172 patients were admitted including transfers from surrounding provinces. Babies with birth weights as low as <900 grams and gestational ages <28 weeks have survived. The overall mortality rate is 29.7%. Forty infants have been treated for neonatal jaundice. Mothers have learned how to effectively care for their infants and mother-baby bonding has been enhanced through the unique KHH bed design.

## 511

### MITIGATING IRON DEFICIENCY ANEMIA IN SCHOOL AGED CHILDREN IN MADURAI, INDIA

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Iron Deficiency Anemia (IDA) is one of the most common nutritional deficiencies in the world. The objective of our research was to demonstrate to the Balamandram school and City of Madurai that IDA can be significantly mitigated via cost effective intervention. The research was conducted in two stages. The first stage consisted of demonstrating that IDA levels can be mitigated via iron and vitamin supplements. The second part of the research consisted of developing a diet plan that

would augment iron levels by similar amounts as the supplements yet be much more cost effective and easy to implement. The goal was to convince the Balamandiram School and Corporation of Madurai to increase funds for mitigating IDA and bringing the children's IDA levels above the WHO recommended thresholds. The first part of the study involved measurement of IDA levels bi-monthly which allowed for close and consistent monitoring of the effectiveness of the newly introduced iron and vitamin supplements. Over a 15-month period, the research demonstrated a 19.3% average improvement in hemoglobin levels for the 34-student (ages 7-17) control group. While almost all students in the control group showed low (well below WHO thresholds) hemoglobin levels initially, the iron and vitamin supplements helped the 7-9 and 10-11 age groups significantly improve their hemoglobin levels and surpass the WHO thresholds. The other age groups also demonstrated significantly improved hemoglobin levels although they were still slightly under the WHO thresholds for their age brackets. Although the supplements provided to the students proved to be effective and won approval from the school, the cost of the supplements were considered too high. Therefore, the second part of the research consisted of working with the Food Technology Department at Fatima College in Madurai to develop a diet based iron augmentation that provided an equivalent amount of nutritional iron. The Corporation of Madurai approved additional funds of \$0.30 per student per day for the augmented diet, a 66.67% increase.

## 512

### INCIDENCE OF SERIOUS PATHOLOGY IN PATIENTS PRESENTING WITH CHRONIC LOW BACK PAIN IN A SUB-SAHARAN AFRICAN OUTPATIENT SETTING

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Locally applicable diagnostic guidelines for patients presenting with chronic low back pain in sub-Saharan Africa are lacking. Local practice typically includes lumbar spine x-rays and an erythrocyte sedimentation rate, but there is no evidence to justify this practice. To determine whether current developed world recommendations should be used or the current local practice continued, the incidence of serious pathology in chronic low back pain patients presenting in these settings must be more clearly understood. This study seeks to evaluate the incidence of serious pathologies in patients presenting with more than one month of low back pain to the outpatient department of a rural hospital in Kenya. IRB approval was obtained from the hospital. Patients presenting to the outpatient clinic at AIC Kijabe hospital in July 2015 endorsing low back pain lasting longer than 1 month with no previous diagnosis for their symptoms were included. Final diagnoses were evaluated for serious pathology defined as infection (bacterial, tuberculosis, HIV/AIDS related), or cancer (primary or metastatic), and the prevalence of these serious etiologies was calculated. Preliminary results of 40 patients indicate a serious pathology rate of 10%, with a possible rate of 17.5% (some diagnoses were inconclusive). Of the 4 cases with serious pathology identified, 3 were attributable to tuberculosis (7.5%) and 1 was confirmed malignancy (2.5%). This is significantly higher than reports from the developed world of 0.7% and 0.01%, respectively. This pilot study indicates significantly higher rates of serious pathology in patients presenting with low back pain in a sub-Saharan African setting than in the developed world. This is congruent with similarly conducted studies in African settings, and suggests that the local diagnostic practice may be justified. Further studies conducted with a larger sample size and more varied population are needed to assess whether these results translate to the sub-Saharan African population on a larger scale.

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### PREVALENCE OF SKIN CONDITIONS IN SCHOOLCHILDREN IN URBAN WESTERN AND NORTHERN UGANDA

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Skin conditions account for 25% of physician visits in sub-Saharan Africa, but there are few studies reporting skin disease prevalence in the community. Within the structure of a larger group A streptococcal study in Uganda, aimed at understanding drivers of rheumatic heart disease (RHD), our aims were (1) to determine prevalence of skin conditions seen in children and (2) to characterize skin bacterial infections--which some data suggest may contribute to RHD. Consented children at 3 primary public schools in Mbarara (west) and Gulu (north) were eligible for skin examination. Nurses conducted total body skin exams, except for the anogenital region and female breasts. The dermatology team then evaluated those with positive screens. Bacterial infection was defined as clinical evidence of impetigo, cellulitis, folliculitis, furuncles, pustules, and abscesses. When pus or crust was present on exam, a specimen was obtained and stored/transported in STGG (skim milk, tryptone, glucose, glycerin) media for microbiology culture and sensitivity. For cases of tinea capitis presenting with pustules, a specimen was obtained for microbiology, but these cases were not considered to be clinically suspected bacterial infection as tinea capitis can present with pustules. Of 3,261 children screened, 41% (1310/3261) had a skin condition including: tinea capitis (71%), tinea corporis (9.6%), eczematous dermatitis (4.5%), acne (4.1%), trauma (3.0%), tinea versicolor (2.3%), post-inflammatory changes (2.3%), interdigital toe web infection (2.1%), clinically suspected bacterial infection (1.8%), warts (1.3%), arthropod bites (0.8%), seborrheic dermatitis (0.8%), and scabies (0.5%). No case of scabies or eczematous dermatitis was impetiginized. Dermatophyte infections were most prevalent, consistent with other studies from this region. The significance of bacterial infection prevalence will need to be considered in the context of microbiology results and known RHD prevalence at these primary schools in order to draw conclusions on the role that group A streptococcal skin infection plays in the development of RHD in these urban areas of Uganda.

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### THE CLINICAL FEATURES, COMPLICATIONS AND TREATMENT OF NODDING SYNDROME

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Nodding syndrome (NS) is a debilitating neurologic disorder affecting children and adolescents in East Africa. We are conducting a series of studies in the most affected districts of Uganda. Our aim is to determine the aetiology and pathogenesis, provide a comprehensive description of clinical features, comorbidities and complications, develop a staging system and supportive treatments and initiate studies of specific treatments. First, we conducted a pilot study of 22 patients to describe the clinical features, determined patients' treatment needs and used this data to develop a supportive treatment program. Secondly, we conducted a detailed clinical, neurophysiologic and imaging study of 223 patients to describe the progressive development of symptoms, complications and comorbidities of NS. Third, based on preliminary aetiology studies that suggest potential cross-reacting antibodies with *Onchocerca volvulus*, we are enrolling 230 patients in phase II placebo-controlled trial of doxycycline as treatment. The studies suggest that NS is a neurologic disorder with multisystem involvement. Complications of the untreated disease develop

through five worsening clinical stages. Treatment with anticonvulsants and a program of physical, occupational, speech and language, psychological and nutritional rehabilitation therapy improves outcomes, function and wellbeing. The doxycycline trial is due to complete recruitment end of 2017. The clinical manifestations and function of patients with NS improve with symptomatic treatments. A definitive cure and preventive intervention is awaited.

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### PUBLIC ENGAGEMENT WITH SCIENCE: A COMMUNITY DRAMA PROJECT AGAINST MALARIA IN CAMBODIA

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Malaria is declining in Southeast Asia but transmission persists in many rural areas and among forest workers and isolated populations. In these remote communities, conventional health services and education are limited. Community and public engagement requires unconventional approaches such as using arts. A participatory drama project was conducted in twenty remote villages in Battambang province, Cambodia with three key messages: to use insecticide-treated bednets, to get early diagnosis and treatment, and learn about risks of forest-acquired malaria. The project combined Cambodian drama, art, music workshops and village concerts and competitions to provide health education in a fun and engaging way. We conducted an evaluation of this project using qualitative and quantitative methods. A total of 29 villagers, community engagement staff and key workers were interviewed while 141 villagers filled in a 5-item questionnaire. Analysis of the interviews resulted in development of the following seven themes: i) exposure to malaria and engagement activities, ii) readiness and barriers to participation, iii) understanding and learning about malaria using drama, iv) entertainment value and engagement method preferences, v) challenges to community engagement, vi) future participation and vii) sustainability. The quantitative data echoed findings of the qualitative analysis, with almost all participants agreeing that the drama has helped them learn more about malaria. The event also saw positive response, with an encouraging average participation rate of 66%. Our assessment demonstrated that the drama project was effective in promoting awareness and understanding of malaria prevention and control. Villagers perceived drama as entertaining and as the preferred choice of engagement activity. Using drama is a promising way for community engagement and could be considered when mobilising communities for malaria elimination.

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### DEMOGRAPHIC SURVEILLANCE TO MONITOR IMPACT OF MALARIA ON PREGNANT WOMEN IN OUELESSEBOUGOU, MALI

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Pregnancy malaria is associated with poor outcomes for pregnant women and their babies. To reduce poor pregnancy outcomes associated with

malaria infection, WHO recommends monthly anti-malarial treatment with sulfadoxine pyrimethamine (SP) during the second and third trimester and the use of insecticide-treated bed nets. However, in East and Southern Africa, SP has lost its efficacy due to the spread of drug-resistant parasites. Therefore, an effective vaccine for PM is needed. Some researchers and scientists are focusing on developing a PM vaccine. Before testing new interventions to improve pregnancy outcomes, it is important to obtain background information on these outcomes in the population that can be used as a baseline to differentiate between adverse outcomes that might be associated with the intervention (vaccine or new drugs) and malaria infection. To investigate the impact of malaria on the outcomes of pregnancy, we are assessing pregnancy outcomes in women in the community with access to the WHO recommended standard of care. Census of the population including child bearing women was conducted and all women presenting for an antenatal visit in health centers in Ouelessebouguou are recruited into the study. Pregnancy outcome information is collected after the birth of the child or pregnancy termination (i.e. miscarriage/stillbirth). To assess the rates of malaria infection and parasite resistance to SP, cross-sectional surveys on a subset of 330 pregnant women and 450 children in the community will be conducted at the peak of malaria transmission (around September-October) for pregnant women and twice a year (July and December) for children age 0-10 years. Results on first malaria transmission season will be analyzed and presented.

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### PEDIATRIC INPATIENT ANTIBIOTIC PRESCRIPTION PRACTICES IN THE CHAIN NETWORK HOSPITALS AT BASELINE

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Antimicrobial resistance (AMR) is a growing global concern. Antibiotic stewardship is being promoted to reduce AMR. However, few studies have documented inpatient antibiotic prescription practices in low-resource settings. We audited the 240 clinical notes of 2-23 month-old inpatient children in Bangladesh (2), Kenya (3), Malawi (1), Pakistan (1) and Uganda (1) as part of a baseline assessment in a study to identify risk factors for mortality in acutely ill inpatients, particularly in undernourished children. This analysis describes adherence to antibiotic guidelines during the first 48 hours of admission. Adherence was defined as a regimen consistent with institutional, national, or international recommendations. The cases reviewed included diagnoses of diarrhea (n:107), severe acute malnutrition (n:101), pneumonia (n:76), malaria (n:35), sepsis (n:25), meningitis (n:12), and shock (n:8). Antibiotics were prescribed to 98% with at least one documented indication for antibiotics (n:173); 80% of these were consistent with recommended regimens. Antibiotics were prescribed in 93% of admitted diarrhea cases, 85% of whom had a comorbidity warranting antimicrobials (n:85) or dysentery (n:1). Among children with malaria noted as a diagnosis and without a documented indication for antibiotics (n:22), those who did not receive a malaria test (n:6) were all prescribed antibiotics. In comparison, 63% of those with a documented positive malaria test (n:16) were prescribed antibiotics without indication. Among those with diarrhea (n:15) and malaria (n:16) without a documented indication for antibiotics, 58% were prescribed an antimicrobial regimen consistent with treatment for a severe bacterial infection. Antibiotics were almost universally prescribed when indicated and adherence to a recommend regimen was comparable to other studies.

Antimicrobials were commonly prescribed for diarrhea and malaria. This may be a response to difficulty excluding severe infections amenable to antibiotics such as sepsis. Improved diagnostic support and education may be critical to improve antibiotic stewardship in many settings.

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### DIAGNOSTIC CHALLENGE OF SKIN LESIONS IN RETURNED TRAVELER FROM IVORY COAST

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A 63-year old Haitian female who migrated to the US over 20 years returned from the Ivory Coast after missionary work for 1 month. In Ivory Coast, 13 days prior to admission (PTA), she first noticed many itchy 1-mm lesions on her buttocks. She denied fresh water exposure, bath tub usage, or insect bites and had a history of impaired glucose tolerance. The lesions became larger and more painful and she took ciprofloxacin for 5 days to no effect. She visited a dermatologist 3 days PTA for painful furuncles with 3 lesions on her buttocks, 7 lesions on upper thighs and 1 lesion on the left arm. She underwent incision and drainage (I&D) and treatment with trimethoprim-sulfamethoxazole. On admission, she had worsening episodic pain and enlarging lesions. On exam, she was in distress but afebrile. There were 11 lesions of 3-cm with erythema and induration and a white dot in the centre draining serosanguineous fluid plus overlying cellulitis. White blood cell count was 4,920 cell/ $\mu$ L, neutrophils 48%, band forms 7% and eosinophils 3%. Creatinine was 1.4 mg/dL (baseline 0.8 mg/dL). C-reactive protein was elevated to 56.1. Vancomycin and cefepime were prescribed for presumed bacterial infection. Surgical I&D was performed and samples were negative on bacterial, mycobacterial and fungal culture. On hospital day 3, larvae were seen emerging from the lesions and 9 were extracted surgically. They were identified as Tumbu fly larvae (*Cordylobia anthropophaga*). Clinical still photographs and video of the larvae were taken. Two lesions did not have living larvae. The patient improved significantly after the extraction and was discharged home without pain. The patient had furuncular myiasis caused by Tumbu fly larvae which is endemic in Tropical Africa. It is rarely seen in the US and could cause a delay in diagnosis and treatment leading to unnecessary use of antibiotics in areas where physicians are not familiar it. It is possible that the female Tumbu fly laid its eggs on the patient's clothes or linens while hanging to dry. As part of pretravel clinic encounters, travelers to endemic areas should be advised to iron all clothes including underwear to kill the eggs and larvae.

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### CLINICAL OUTCOMES OF VENOMOUS SNAKEBITES IN THE ECUADORIAN AMAZON RAINFOREST AFTER IMPLEMENTATION OF A NATIONAL PROTOCOL

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The incidence of snakebite envenomation in Ecuador is not well established. Some reports estimate between 1200-1600 cases annually with mortality rates ranging between 1 and 5.4%. In 2010 the ministry of health of Ecuador (MOH) implemented a national snake envenomation management protocol. We collected retrospective data from January 2012 through December 2014 on all venomous snakebites admitted to Hospital Basico Sucua a rural tertiary medical center, located in the town of Sucua which is found in the Ecuadorian Amazon rainforest. Epidemiological data and clinical outcomes were analyzed. During the study period we identified 211 venomous snakebites. 59.7% were male and 74.4% were farmers of indigenous origin. 64% were between the ages of 15-65. Incidence ranged from 183 to 224 per 100,000 inhabitants per year. 97%

were bitten in the extremities. 27.5% (58/211) of these venomous snake bites were deemed without clinical evidence of envenomation. Among the 153 cases of envenomation, 42.4% (65/153) were classified as mild upon presentation to the hospital, 52.2% (80/153) moderate, and 5.2% (8/153) severe. The pit viper known as "equis" in Ecuador (*Bothrops spp.*) accounted for 40% of the total envenomation cases. After initiating the MOH national snake envenomation management protocol in the hospital, only 21.5% (33/153) suffered further complications. There were no recorded fatalities. Only 3 patients developed permanent neurological disability. Hospitalization days averaged between 4-7 days. Time from bite to hospital ranged from 1 to 96 hours. 64% were transported by ambulance. Total vials administered per patient was between 4 and 12. The cost of each vial ranged from 42-100 US dollars. The incidence of snakebite envenomation in southeast Ecuador is high. This retrospective analysis is the first attempt to analyze the clinical outcomes of the MOH national snake envenomation protocol. Our preliminary results show improving clinical outcomes, with low complication rates and long term disability among snake envenomation cases. Further observational studies are need to fully validate this protocol.

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### THE EFFECTS OF MALNUTRITION AND DIARRHEA TYPE ON THE ACCURACY OF CLINICAL SIGNS OF DEHYDRATION IN CHILDREN UNDER FIVE: A PROSPECTIVE COHORT STUDY IN BANGLADESH

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The World Health Organization (WHO) recommends assessing dehydration in children with diarrhea based on standard clinical signs. However, no prior studies have compared the accuracy of these clinical signs of dehydration in children based on nutritional status or diarrhea type. This study is a planned secondary analysis of data from two prospective cohort studies of children with acute diarrhea in Bangladesh. Dehydration was determined using percent weight change with rehydration. Malnutrition was calculated using mid-upper arm circumference. The effects of malnutrition and diarrhea type on the accuracy of nine clinical signs of dehydration were assessed using the Cochran-Mantel-Haenszel test. The accuracy of three clinical diagnostic models of dehydration was compared in children by nutritional status and diarrhea type using the area under their receiver-operator characteristic curve (AUC). Of the 1,282 patients included in the final analysis, 685 (53%) had dehydration, 240 (19%) had malnutrition, and 446 (35%) had rice-water (cholera) diarrhea. There was no significant association between malnutrition and any of the clinical signs, but seven signs were significantly less predictive of dehydration status among those with rice-water diarrhea. The AUC for the DHAKA Dehydration Score, the Clinical Dehydration Scale (CDS) and the WHO algorithm were similar in children with and without malnutrition. The CDS was significantly less predictive for dehydration in children with rice-water diarrhea. This study provides evidence that clinical signs of dehydration and dehydration scales predict dehydration with similar accuracy in children with malnutrition, but may be less predictive in children with rice-water diarrhea.

## EVALUATING INTESTINAL PROTEINS IN *BRUGIA MALAYI* ADULT WORMS AS POTENTIAL DRUG TARGETS

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Over 70 million people are infected worldwide with lymphatic filariasis (LF). Clinical disease manifestations include lymphedema, hydrocele, and elephantiasis. The infectious agents include *Wuchereria bancrofti*, *Brugia malayi* and *B. timori*, tissue-invasive nematodes that are transmitted to humans by mosquitoes. Once in humans, these helminths migrate to lymphatic vessels where they mature into adult worms 4-8 cm in length over a period of 2-3 months, mate and release circulating microfilariae (L1 stage larvae) that are infective to mosquitoes. While global mass drug administration has significantly reduced the worldwide prevalence and transmission of LF, the strategy is limited. Current anti-filarial drugs such as albendazole, diethylcarbamazine, and ivermectin are effective against the microfilaria stage but not against adult worms. Development of macrofilaricidal agents would greatly enhance efforts to control filariasis. A key anatomical feature of adult filarial worms is a fully developed mouth to anus intestinal tract. We therefore conducted a proteomic analysis of the *B. malayi* intestinal tract to identify potential drug targets in adult filarial worms. A total of 396 proteins were identified as being solely expressed in the intestinal tract. Of these proteins, we identified a subset that have high homology to *W. bancrofti* and other pathogenic filarial worms, low homology to humans, and are predicted to be expressed at the luminal surface of the filarial intestinal tract. A group of 10 candidate protein targets were selected that are involved in cellular adhesion, proteolysis, waste removal and metabolism. To determine whether these candidate proteins are essential for adult *B. malayi* survival, we are using siRNA interference to knock down protein expression. We have recently optimized siRNA knockdown of alpha-actin in *Brugia* worms and are currently assessing the effects of siRNA knockdown on our candidate proteins. Outcomes to be measured include worm viability, motility and fecundity.

## THE MOLECULAR BASIS OF *LOA LOA* CROSS-REACTIVITY IN THE RAPID DIAGNOSTIC TEST FOR LYMPHATIC FILARIASIS

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The Global Program to Eliminate Lymphatic Filariasis relies on rapid diagnostic tests such as the filariasis test strip (FTS) and immunochromatography card test (ICT), which detect circulating filarial antigen via a carbohydrate epitope termed the AD12 epitope. Although the AD12 epitope is not unique to *Wuchereria bancrofti*, it was not initially found on circulating antigens of other filarial infections of humans. The recent recognition of ICT and FTS cross-reactivity in some patients with *Loa loa* suggests the presence of the AD12 epitope on *L. loa* antigens. To identify which *Loa* antigens might be responsible for ICT/FTS cross-reactivity, we immunoaffinity purified AD12 epitope-containing antigens from the sera of an FTS-positive patient with loiasis. Surprisingly, western blot analysis detected many AD12 epitope-containing antigens in the cross-reactive *Loa* sera. Proteomic analysis of FTS+ *Loa* sera identified over 80 cross-reactive *Loa* proteins. These proteins are functionally diverse and predicted to localize to all regions of the cell including the plasma membrane, cytosol and nucleus. This pattern of antigenemia suggests that the origin of the cross-reactivity is worm lysis and release of cellular content, rather than secretion of a specific filarial antigen by adult worms (as seems to be the case for bancroftian filariasis). Why antigen is released in some loiasis patients but not others is not clearly understood and is the focus of ongoing research.

## WOLBACHIA REGULATES *BRUGIA MALAYI* MICRORNA TO MAINTAIN THEIR MUTUALISTIC INTERPLAY

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Human lymphatic filariasis is caused by the filarial parasites *Wuchereria bancrofti*, *Brugia malayi*, and *B. timori*, and is the second leading cause of global disability, affecting 120 million people in 73 countries. As with most filarial nematodes, *B. malayi* has evolved a mutualistic association with its bacterial endosymbiont, *Wolbachia*. *Wolbachia* is crucial for parasite development, fertility, and viability. It is known that the bacteria and its filarial host share essential metabolic and biosynthetic processes (such as glycolysis, nucleotide biosynthesis, iron metabolism). However, how these two organisms can orchestrate the major bioprocesses is still poorly known. Recent studies in *Wolbachia*-arthropod systems demonstrated that *Wolbachia* influences host gene expression by altering the host microRNA (miRNA) profile. Additionally, deep-sequencing studies of small RNAs in *Brugia* and other nematodes have identified several miRNAs found only in filariae that harbor *Wolbachia*. This suggests that the filarial parasites may encode miRNAs that regulate functions unique to the *Wolbachia*-host endosymbiosis. Our study is focused on understanding the role of *Brugia* miRNAs in the molecular interdependency between the bacteria and its filarial host. We identified 33 *B. malayi* miRNAs that show differential expression in response to the elimination of *Wolbachia* by antibiotic treatment. The differential expression of four *Wolbachia*-responsive *Brugia* miRNAs was confirmed in adult *B. malayi* females treated with doxycycline *in vitro* using a specifically designed qPCR assay. We also established that *Brugia* miRNAs potentially regulate apoptotic pathway, suppress autophagy and regulate the expression of proton/amino acid symporters in the filarial host. Future studies will characterize the potential roles the *B. malayi* miRNA target genes have in the *Wolbachia*-parasite interplay. We demonstrate that a defined subset of *B. malayi* regulated genes could constitute one aspect of the molecular foundation for *Wolbachia*-*Brugia* symbiosis, and could be therefore considered as potential drug targets for anti-*Wolbachia* and filaria treatment.

## ASYMPTOMATIC *LOA LOA* INFECTION IN EQUATOGUINEAN ADULT SUBJECTS IN A WHOLE SPOOROZOITE MALARIA VACCINE TRIAL: WHAT IS A RESEARCHER TO DO?

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The filarial nematode *Loa loa* is common in West and Central Africa and can cause Calabar swellings and eye worm in its human hosts. Loiasis is of particular concern in onchocerciasis-endemic areas, since ivermectin used in mass treatment programs can cause potentially fatal adverse reactions in people with a high *Loa loa* parasite load. *Loa loa* microfilariae were incidentally found on routine malaria blood smears in 5 of 248 (2.0%) individuals aged 6 months to 65 years undergoing screening for,

or already enrolled in, a *Plasmodium falciparum* whole-sporozoite malaria vaccine trial on Bioko Island, Equatorial Guinea. All were adult males, ranging in age from 21-61 (mean=32) years. All were asymptomatic and had normal physical exams. Microfilariae were detected in one of the affected individuals on their first screening blood smear, but were not seen in the other four until their second (2), fourth (1) or fifth (1) smears, 2-12 weeks after the initial negative smear. Blood smears were generally done around mid-day (the period of highest sensitivity for microfilaria detection). Eosinophilia was common, ranging from 0.81 to 1.93 X 10<sup>3</sup>/mm<sup>3</sup> (14.1-31.4%). Creatinine levels were all normal. The five men were referred to the national onchocerciasis control program, where the diagnosis of loiasis was confirmed by repeat blood smear and a 21-day course of diethylcarbamazine (DEC) prescribed per program guidelines. At the time of writing, one subject had completed a course of DEC and required a second course due to persistent microfilaremia. The other four subjects were either in treatment or had yet to begin it. The plan was to follow all subjects until microfilariae cleared. Three of the men had already received a malaria vaccine dose; they were deemed ineligible for additional doses of vaccine. The other two subjects were also excluded from the trial. Complete details will be presented, as well as a discussion of the rationale for treating incidental, asymptomatic loiasis discovered during an unrelated clinical trial.

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### REPORT OF THE FIRST INTERNATIONAL WORKSHOP ON ONCHOCERCIASIS-ASSOCIATED EPILEPSY: A CHALLENGE TO THE SCIENTIFIC AND MEDICAL COMMUNITIES AND A RESEARCH AGENDA GOING FORWARDS

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From 12 to 14 October 2017, the first International Workshop on Onchocerciasis-Associated Epilepsy (OAE) will have been held in Antwerp, Belgium. This workshop follows the first conference on nodding syndrome (NS), organized in 2012 by the World Health Organization and the Ugandan Ministry of Health in Kampala, and the NS conference organized by Gulu University, Uganda in 2016. Since then, substantial progress has been made in understanding NS. Recent studies suggest that NS is only one of various clinical presentations of OAE and that *Onchocerca volvulus* (the parasite causing onchocerciasis) is potentially the aetiological trigger of this type of epilepsy. Although the exact pathophysiological mechanism(s) of OAE remain unknown, there is increasing epidemiological evidence indicating that by eliminating onchocerciasis, the incidence of this form of epilepsy will likely decrease. To this end, it is crucial that onchocerciasis control programmes be strengthened, especially as those already affected will continue suffering even after onchocerciasis elimination has been achieved. Consequently, more advocacy is needed to obtain funding to organize the treatment, care and support for persons with OAE. By joining forces and expertise through partnerships between communities, advocacy groups, health care workers, ministries of health, NGOs, pharmaceutical industry and funding bodies, we hope to prevent children from developing OAE and improve the affected patients' quality of life. It is urgent that the global scientific community joins this partnership to eliminate this major cause of epilepsy in resource-poor countries, specifically in Africa. A summary of recommendations of the

Antwerp workshop will be presented including a research agenda aimed to: (i) identify the pathophysiological mechanism(s) of OAE; (ii) prevent incidence of and improve surveillance for OAE; (iii) determine the disease burden caused by OAE; (iv) improve access to treatment/care for persons with OAE and their families and (v) fund an OAE policy plan (including prevention of stigma, discrimination and gender violence associated with OAE).

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### ASSESSING THE AVAILABILITY, READINESS AND QUALITY OF MORBIDITY MANAGEMENT AND DISABILITY PREVENTION SERVICES FOR CLINICAL LYMPHATIC FILARIASIS IN BANGLADESH

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The Bangladesh Filarial Elimination Programme (FEP) has made great strides forward in the elimination of lymphatic filariasis (LF) infection, with the absence of transmission confirmed in all endemic districts where initially 70 million people were at risk. The FEP is now focussed on morbidity management and disability prevention (MMDP), and recent patient searching activities in endemic areas confirmed over 43,000 clinical cases of lymphoedema and hydrocele. To fulfil requirements of the Global Programme to Eliminate LF (GPELF), the Bangladesh FEP must provide evidence of the availability of the minimum package of care, and readiness and quality of services for MMDP in all areas of known patients. This work specifically aimed to address these requirements by conducting a series of patient and health system surveys including i) access to care of lymphoedema patients assessed through patient surveys ii) readiness and quality of lymphoedema care assessed through health facility inspections at community clinics iii) quality of MMDP training assessed through community health workers pre- and post-training surveys and iv) quality of hydrocele surgery assessed through patient pre- and post-surgery quality of life surveys. The work related to the access to- and readiness and quality of- lymphoedema care, and community health worker training (n=3468 trained) is being conducted in seven districts and the full results will be presented. The patient pre- and post- surgery survey was conducted in Panchagar District where a total of 143 men received surgery, and a subset of 39 men were assessed pre- and 3 months' post-surgery to determine change in quality of life across different aspects of their lives. Results showed 85% of men experienced improvement in levels of pain, 92% in mobility and 100% in psychological aspects of life post-surgery. This work will provide evidence of the access, quality and readiness of services for patients with clinical LF in the most endemic districts in Bangladesh, and will support the FEP in the documentation of elimination of LF as a public health problem in Bangladesh.

## MULTI-COUNTRY PROSPECTIVE COHORT TO MEASURE THE IMPACT OF SURGERY ON MEN WITH HYDROCOELE CAUSED BY LYMPHATIC FILARIASIS

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Hydrocoele is the most common clinical manifestation of lymphatic filariasis (LF), affecting approximately 19.4 million men worldwide. The condition can have significant negative physical, social, psychological and economic impact on men and their families. The recommended treatment is simple, surgical repair (hydrocoelelectomy), and in recent years the UK Department for International Development has committed funding to address this burden by supporting National LF Programmes to scale up access to surgery in 12 countries (10 African; 2 Asian). To determine how surgery affects men's lives, a prospective cohort impact assessment was initially conducted in Malawi where the survey tool was refined, and expanded to Bangladesh and Nepal. As part of the campaigns in each country, men enrolled for hydrocoelelectomy were randomly selected to take part in the survey. A semi-structured questionnaire was administered pre-surgery, and 3- and 6-months post-surgery to compare demographic and socio-economic information, LF knowledge, barriers to surgery, and quality of life information; the latter relating to pain, mobility, usual activities, self-care, social participation and psychological health domains, measured using a model disability scoring system (1=no problem, 2=mild, 3=moderate, 4=severe). In Malawi (Chikwawa and Nsanje district), 137 men from 6 hospitals were surveyed; in Bangladesh (Panchagar district) 39 men were surveyed, and in Nepal, surveys are planned to include approximately 300 men from 10 hospitals. Results from Malawi and Bangladesh indicate a statistically significant improvement in men's lives 3-months post-surgery with the majority (>80%) experiencing a change from 'moderate-severe' problems pre-surgery to 'mild-no problems' post-surgery across all domains. A significant reduction in the proportion of men having to take days off work due to their condition was also found in Malawi (pre-surgery 52%; 3-months post-surgery 16%; 6-months post-surgery 0%) and Bangladesh (pre-surgery 64%; 3-months post-surgery 7%). This study shows that hydrocoelelectomies are life changing for men.

## SAFETY OF CO-ADMINISTRATION OF A SINGLE DOSE OF IVERMECTIN, ALBENDAZOLE AND DIETHYLCARBAMAZINE IN SUBJECTS WITH AND WITHOUT WUCHERERIA BANCROFTI INFECTION IN CÔTE D'IVOIRE

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Recent studies in Papua New Guinea and Côte d'Ivoire have shown that a single co-administered dose of ivermectin (IVM), diethylcarbamazine (DEC), and albendazole (ALB, IDA) cleared microfilaria (Mf) more effectively than current two-drug regimens of DEC+ALB or IVM+ALB. However, the safety of IDA in subjects infected with lymphatic filariasis (LF+) versus uninfected participants (LF-) is unknown. Participants with moderate to heavy *W. bancrofti* infections (>50 Mf/mL, N=32) and individuals from the same communities who were LF- (N=26) were hospitalized for 3 days and actively monitored for adverse events (AEs), which included repeated vital signs, targeted physical examinations, urine and biochemical analysis and for the presence of Mf before and following IDA treatment. Participants were passively monitored between days 4 and 7. All participants had at least one drug-associated AE and 86% participants had more than one AE. Mild AEs were similar between LF+ and LF- participants; however, moderate (level 2) AEs were more common in LF+ individuals (38% vs 21%, p=0.034). There were no severe or serious AEs. The most common subjective AEs were headache, abdominal pain and diarrhea. Scrotal pain and swelling was the only subjective AE that occurred with greater frequency for LF+ (19%) compared to LF- men (0%, p=0.005). Amongst objective AEs, fever, hypotension and hematuria by urine dipstick were most common. For LF+ individuals 15/32 (47%) had a recorded fever at least once (mean=2 episodes, range 1-4) compared to 4/26 (15%, p=0.01) with fewer febrile episodes (mean = 1, range 1) for LF- participants. All other objective AEs were similar between the treatment groups. All AEs resolved without intervention within 72 hours and all LF+ participants cleared Mf at 39 hours and 7 days post-treatment. Triple drug therapy is highly effective and safe in both LF infected and uninfected individuals, making it a viable alternative for mass drug administration in areas that are endemic for LF, but not coendemic for onchocerciasis.

## SUSTAINED RESPONSE CRITERIA FOR CHEMOTHERAPEUTIC STUDIES IN ONCHOCERCIASIS: A SENSITIVE AND CLINICALLY RELEVANT OUTCOME MEASURE

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Clearance and sustained clearance of *Onchocerca volvulus* microfilariae (mf) from skin and eyes is a relevant goal of onchocerciasis treatment: mf cause the disease and skin mf are the reservoir for transmission. Chemotherapeutic efficacy has been historically assessed based on mean skin mf density over time or percent (%) change from pre-treatment density, masking variability in individual response. We propose using sustained microfilariae response (SMR) analyses for comparative drug efficacy, modelled from regulatory-acceptable efficacy outcome measures in other infectious diseases. In two double blind Phase II and III studies conducted to regulatory-standard good clinical practice in areas without community directed treatment with ivermectin (IVM) in Ghana, DRC and Liberia, people were randomized to 8 mg moxidectin (MOX) or 150 µg/kg

IVM. Mean mf density/mg skin (SmfD) was determined based on four snips per person obtained pre-treatment and at least 1, 6 and 12 months (M) post-treatment. Pre-specified analyses of skin snip data (mean SmfD and mean % change SmfD from pre-treatment) over time were complemented by complete SMR (% achieving zero SmfD at M1 sustained to M6 (SMR6) and/or M12 (SMR12)). In Phase III (pre-treatment SmfD 10-299 mf/mg; treated MOX n=978, IVM n=494), M12 SmfD (unadjusted mean  $\pm$  SD) for MOX vs IVM was  $1.25\pm 3.00$  vs  $9.97\pm 12.93$ , respectively; mean  $\pm$  SD %SmfD reduction pre-treatment to M12 was  $97\% \pm 3.40$  vs  $77\% \pm 28.9$ , respectively ( $p < 0.0001$ , mixed model). Complete SMR6 for MOX vs IVM occurred in 76% vs 8% and SMR12 in 37% vs 2% subjects, respectively ( $p < 0.0001$ ). In subjects with pre-treatment SmfD  $\geq 80$  mf/mg, MOX SMR6 was 43% and SMR12 16% vs 0% at both timepoints for IVM. In Phase II (pre-treatment SmfD 0.3-83.1 mf/mg; treated MOX 8 mg n=38, IVM n=45), M12 mean % SmfD reduction from pre-treatment for MOX 8 mg vs IVM was 98.2% vs 84.1%; complete SMR6 occurred in 95% vs 24% (MOX vs IVM,  $p < 0.0001$ ) and SMR12 in 53% vs 13% (MOX vs IVM,  $p < 0.0001$ ). SMR criteria is a more sensitive, clinically and onchocerciasis control/elimination-relevant analysis method of SmfD data for anti-onchocercal drugs than mean or percent change-based methods.

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#### SAFETY OF TRIPLE DRUG TREATMENT WITH IVERMECTIN, DEC AND ALBENDAZOLE COMPARED TO STANDARD TREATMENT WITH DEC PLUS ALBENDAZOLE FOR *BRUGIA TIMORI* INFECTION IN INDONESIA

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Recent studies have shown that treatment with ivermectin, diethylcarbamazine and albendazole (IDA) is superior to standard treatment with DEC + albendazole for clearing *Wuchereria bancrofti* microfilariae (Mf). We performed a hospital-based study and a community study to compare the safety profiles of these two regimens for treating *Brugia timori* infections in Indonesia. The hospital study was a randomized, open label trial. Microfilaremic adults were treated with IDA (N=28) or DA (27). Adverse reactions (AEs) were actively assessed for 2 days after treatment and passively assessed through day 7. Mf were detected by membrane filtration of night blood before and 24 h after treatment. Mf were completely cleared in 89.3% and 29.6% of subjects after IDA or DA treatment ( $p < 0.0001$ ). AEs were more common after IDA (53.6 vs. 37.0%,  $p > 0.05$ ). However, almost all AEs in both groups were mild (grade 1); two subjects in the IDA group had grade 2 fevers. We then conducted a community trial in 18 neighborhoods (dusuns) in endemic areas in Sumba and Flores islands that were randomly assigned to receive IDA or DA. 3,880 eligible (not pregnant and age  $> 5$  years) subjects were enrolled and tested for Mf. Mf rates in the dusuns (mostly *B. timori* with lower rates of *W. bancrofti*) were between 1% and 9%. 99% of subjects treated with IDA and 94% of subjects treated with DA were assessed for AEs on days 1 or 2 after treatment. AE rates were similar after IDA and DA (5.9% IDA vs 5.8%), and rates were no higher in persons with Mf. Two all-cause grade 3 AEs occurred after IDA, but no serious adverse events were observed. The most common AEs were headache, abdominal pain, dizziness, fever and myalgia. Thus, IDA was more effective than DA for early clearing of Mf in the hospital-based trial (where all participants were Mf positive) with more frequent mild AEs. In contrast, AE rates were very low in the community study, and both treatments were well tolerated. Our results suggest that IDA is approximately as safe as standard DA in community settings, and it appears to be a reasonable option for programs to eliminate brugian filariasis.

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#### DOXYCYCLINE FOR THE TREATMENT OF ONCHOCERCIASIS: A DAILY DOSE OF 100 MG FOR 6 WEEKS SHOWS REDUCTION OF FERTILE FEMALE *ONCHOCERCA VOLVULUS* EQUIVALENT TO 200 MG/D

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As recently stated by the WHO, alternative treatment strategies are needed for the treatment of onchocerciasis. One of these strategies are drugs or drug combinations that have a permanently sterilising or macrofilaricidal effect. In order to refine existing regimens with known activity against *Wolbachia*, a randomised double-blind trial was carried out in Ghana by members of the A-WOL consortium, with the aims to reduce treatment duration by combination of doxycycline (DOX) plus rifampicin (RIF), or to reduce the daily dosage of DOX. In total 508 participants were treated with either 1) the current best macrofilaricidal regimen of DOX 200mg for 6 weeks (DOX200, N = 153), 2) DOX 100mg for 6 weeks (DOX100, N = 100), 3) DOX 200mg plus RIF 10mg/kg for 3 weeks (DOX+RIF, N = 102), 4) RIF 10mg/kg for 6 weeks (RIF, N = 102) or 5) Placebo (N = 51). Participants were additionally treated with IVM 150 $\mu$ g/kg after 6 months. At 6 months 102 participants got part of their onchocercosmata surgically removed for histological analysis. This procedure was repeated at 20 months for all participants present (411/508, 80.9%). Analysis of the primary endpoint "onchocercosmata with embryogenesis in female worms" revealed 1.2% in both DOX regimens, 12.7% in the DOX+RIF group as well as 35.2% in the RIF and 28.8% in the placebo group, showing that the two DOX regimens were superior to all other groups, and DOX+RIF superior to RIF and placebo. The proportion of dead female worms in onchocercosmata was highest in the DOX200 group with 58%, followed by the DOX100 group with 48% (not significant). Both DOX regimens showed complete absence of *Wolbachia* by immunohistology in  $> 96\%$  of worms, corresponding to the anti-parasitic effect.

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#### DESIGNING ANTIFILARIAL DRUG TRIALS USING CLINICAL TRIAL SIMULATORS

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Lymphatic filariasis and onchocerciasis are targeted for elimination by mass drug administration (MDA) of antifilarial drugs. These drugs are predominantly active against the microfilarial progeny of the adult worms (macrofilariae) of these neglected tropical diseases (NTDs). New drugs are needed both to improve patient therapies and outcomes in the clinical setting and to enhance the effectiveness of public health interventions where elimination is unfeasible by 2020/2025 with current treatment strategies. Several novel and repurposed therapies for the treatment of filarial infections are currently in both pre-clinical and clinical testing. Clinical trial simulators project patient outcomes to assist with the planning and design of clinical trials. They are used in the pharmaceutical sector but are underdeveloped and underused in the NTD domain, where their resource-optimising payoffs could be highly beneficial to the research

and development pipeline. Using onchocerciasis as an example, we demonstrate the utility of clinical trial simulation with illustration from an individual-participant simulator of a novel (hypothetical) macrofilaricidal drug. We show how predicted participant outcomes based on desired pharmacodynamic properties inform the target product profile; the choice of where to conduct trials; how many patients should be recruited; which parasite stages should be sampled from the patients, and when these patients should be followed up to maximise statistical power for demonstrating superiority over existing treatment(s). We also consider how simulations can help to resolve the unique complications that arise when designing clinical trials that are to be conducted in a backdrop of MDA and ongoing transmission.

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### THE ADDITION OF ALBENDAZOLE TO IVERMECTIN DOES NOT REDUCE FEMALE WORM FERTILITY IN ONCHOCERCIASIS

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A randomised open-label clinical trial was performed in an endemic area of Central Ghana to address the question whether ivermectin (IVM) combined with albendazole (ALB) at higher doses and given more than once per year might generate sustained reduction in microfilariae (Mf) by reducing female fertility or by killing adult worms in onchocerciasis. In total 272 Mf-positive participants, with at least one palpable onchocercoma were treated with either 1) IVM 200µg/kg annually (0, 12, 24 months; N = 68), 2) IVM 200µg/kg biannually (0, 6, 12, 18, 24 months; N = 68), 3) IVM 200µg/kg plus ALB 800mg annually (N = 70) or 4) IVM 200µg/kg plus ALB 800mg biannually (N = 66). 76 participants had not taken part in previous mass drug administration (MDA) of annual IVM, the other 198 had done so in at least one round (median 2 (1 – 10)). 36 months after treatment start, 218 patients (80%) got their onchocercomata surgically removed. Skin snips were taken at 0, 6, 18 and 36 months. Histological analysis showed normal embryogenesis in 15/135 (11% [7-18, 95% CI]) adult female worms in the IVM annual group, compared to 22/155 (14% [10-21]) in the IVM biannual, 35/154 (23% [17-30]) in the ALB+IVM annual and 20/125 (16% [11-23]) in the ALB+IVM biannual group (p = 0.1229, comparison over all 4 groups). With a range of 55 - 59% the proportion of dead worms did not differ between the 4 groups. The proportion of individuals that completely cleared Mf at 36 months (after 3 annual/5 biannual treatments) was 35/56 (63% [49-74]) in the IVM annual, 42/59 (71% [59-81]) in the IVM biannual, 39/64 (61% [49-72]) in the ALB+IVM annual and 43/53 (81% [69-89]) in the ALB+IVM biannual group. In the subgroup without prior IVM, there was a trend to more Mf-negative individuals in the ALB+IVM biannual group. In conclusion, addition of ALB to IVM did not improve the efficacy against female worm fertility or the macrofilaricidal effect. However, the increase from annual to biannual drug administration resulted in a sustained increase of Mf negative individuals (annual: 62% [53-70], biannual: 76% [67-83], p = 0.024). It will be interesting to compare these results to an ongoing trial with a similar design in Eastern Ghana.

### DETECTING ALBENDAZOLE METABOLITES IN SERUM AND URINE: A FIRST STEP IN DEVELOPING AN INDICATOR OF MDA COMPLIANCE IN HUMANS

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The neglected tropical diseases are a group of pathogens affecting individuals in the poorest regions of the world. Among them, Soil Transmitted Helminth infections directly impact nutritional status, educational development, individual productivity, and physical and mental development in human populations. Currently, these infections are controlled through mass drug administration (MDA) programs using albendazole (ABZ) or mebendazole. However, not all programs have demonstrated expected impact on prevalence or intensity of infections. These failures may be related to poor programmatic coverage, suboptimal adherence or the exposure of parasites to sub-therapeutic drug concentrations due to poor drug dissolution, insufficient gastrointestinal absorption and/or systemic availability of the active ingredient. Accordingly, improved knowledge of the basic pharmacokinetics of ABZ in treated people is critical. As part of the DeWorm3 project, we sought to characterize the serum disposition kinetics and pattern of urinary excretion of ABZ and its metabolites (ABZ sulphoxide (ABZSO) and ABZ sulphone (ABZSO<sub>2</sub>)) in human volunteers. In addition, we sought to determine the duration and optimal timepoint where ABZ/ metabolites can be measured in urine as an indirect assessment of an individual's adherence to treatment. Venous blood and urine samples were collected from eight (8) volunteers between 2 and 72 h (serum) and 4 and 72 h (urine) for HPLC analysis following administration of a single postprandial oral dose of ABZ (400 mg Glaxo SmithKline). The ABZSO was the main analyte recovered either in serum and urine samples. ABZSO serum concentrations reached its peak concentration (C<sub>max</sub> = 1.20 ± 0.44 µg/mL) at 4.75 h post-treatment. In urine ABZSO C<sub>max</sub> value was 3.24 ± 1.51 µg/mL, reached at 6.50 h post ABZ administration. The urinary AUC value, resulted higher (2.3 fold) compared to that measured in serum. The overall, PK-based information reported here demonstrates that the measurement of ABZSO concentrations both in serum and urine could be useful to confirm compliance to ABZ treatment and an objective measurement of program coverage.

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### FURTHER EVIDENCE OF COLLATERAL IMPACT OF CDTI ON STH PREVALENCE AND INTENSITY: IMPLICATIONS IN DEWORMING STRATEGIC PLAN AND GLOBAL ELIMINATION

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Soil-transmitted helminthiasis (STHs) are among the most prevalent afflictions of the developing world, responsible of physical and mental growth retardation and hindrance of economic development. Periodic deworming with Albendazole or Mebendazole of high-risk groups (school-age children, preschool children, and pregnant women) can significantly lower the levels of infections below the threshold associated with morbidity. Control efforts are mostly focused on school aged children. However, it was shown that age groups other than school-age children

can have similar exposure risk, and might contribute to the persistence of these diseases despite repeated treatments. Mathematical models also revealed that it would be better to broaden treatments across all age classes if one needs to interrupt transmission. After more than 10 years of Mebendazole-based MDA in Cameroon, the disease still persist with relatively high prevalence and intensities of infections in some areas. In order to investigate factors associated with this persistence, we collected at the district level, baseline (1985-1987) and 26-years follow-up (2010) nationwide prevalence data, together with environmental (type of soil, climate, vegetation) and demographic (occupation/habits of populations) factors, as well as mass interventions against other diseases. We found that although environmental factors can explain reduction in prevalence, the persistence of the disease was negatively associated with the implementation of community directed treatment with ivermectin (CDTI) against onchocerciasis (ivermectin alone) and/or lymphatic filariasis (combination of ivermectin and albendazole). This study supports the important collateral impact of CDTI in deworming adults who are not taken into account in the Albendazole/Mebendazole-based deworming strategy, and appears as an advocacy for drug donation to the up to now not targeted age groups that can contribute in the persistence of the disease, thus slowing the momentum towards elimination.

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#### JOINT WATER, SANITATION AND HYGIENE (WASH) AND NEGLECTED TROPICAL DISEASES (NTD) MONITORING: A PRACTICAL EXAMPLE

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The intersection of and collaboration needed between Water, Sanitation and Hygiene (WASH) and Neglected Tropical Diseases (NTDs) has been identified, prioritized and recently emphasized by Ministries of Health and development partners. As outlined in the Joint WASH and NTD strategy published by the WHO in 2015, one of the key objectives focused on the development of joint WASH and NTD indicators. In conjunction with the development of the WHO strategy experts from both sectors underwent a Delphi consultation to identify joint indicators for WASH and NTDs. As part of this consultation, many diverse experts across both sectors participated in 3 rounds of surveys to rank possible joint indicators, their order of importance, and feasibility of measurement. A fourth round was held for disease specific indicators. Using the final list from the Delphi Process and specifically looking at facial cleanliness and environmental sanitation (F&E) interventions for trachoma control, the indicators were adapted into monitoring and evaluation (M&E) frameworks across five countries: Ethiopia, Kenya, Malawi, Tanzania, and Uganda. The DFID SAFE program and the Queen Elizabeth Diamond Jubilee Trust Trachoma Initiative grants supported the development of a project wide F&E M&E (FEME) framework to be implemented at country level. These FEME frameworks include process indicators on joint WASH and NTD coordination, community level indicators on the presence of hand and face washing stations, latrines and clean faces as well as school indicators. Between January and March 2017, Malawi, Tanzania and Uganda conducted baseline surveys to collect these indicators. Data analysis is currently underway. Ethiopia and Kenya are using routine monitoring visits and existing WASH data to report on these indicators. The experience of these 5 country programs' use of data collection for M&E provides examples of different methods of using joint WASH NTD indicators in a NTD program.

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#### COLLATERAL BENEFIT OF INDOOR RESIDUAL SPRAYING FOR MALARIA VECTOR CONTROL ON THE TRANSMISSION OF CUTANEOUS LEISHMANIASIS IN THE DISTRICT OF BAROUELI, CENTRAL MALI

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Cutaneous leishmaniasis (CL) is an endemic neglected tropical disease prevalent in areas with seasonal malaria transmission in Mali. We assessed the effect of the introduction of indoor residual spraying (IRS) for malaria control on sand fly population diversity and abundance, and its impact on the risk of *Leishmania* transmission in the district of Baroueli, Mali. We conducted entomology surveys from March to September 2016 to determine sand fly species composition, density and infection rates using PCR. We also carried out a leishmanin skin test (LST) survey to determine the incidence of *Leishmania* infection. Historical data collected before the introduction of IRS (2005-2008) were compared with data collected in 2016, after five continuous years of IRS in Baroueli, to determine the effect of IRS on CL transmission. We found a substantial reduction in sand fly density after IRS of nearly 70% (9532 versus 2936). Sand fly species composition remained unaltered with *Sergentomyia schwetzi* continuing to be the most abundant sand fly species in the area. The density of sand flies of the *Phlebotomus* genus was significantly decreased compared with *Sergentomyia*. Despite the reduction in the density of *P. duboscqi*, *L. major* parasite infection rates did not vary significantly between the two study periods, 2.6% in 2005 and 3.5% in 2016 (P= 0.40). However, the incidence of exposure to *Leishmania* parasite as measured by LST in the human population was reduced by 50% (from 9% in 2008 to 4.2% in 2016). The substantial reduction in the density of *Phlebotomus duboscqi*, and in the incidence of exposure to *Leishmania infection* in human is most likely attributable to the collateral benefit of the 5 years IRS campaign. Interestingly, the lack of change in *L. major* infection rates in sand fly vector suggests that the reservoirs of disease remain available and that disruptions in the IRS campaign can lead to new outbreaks in this region.

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#### UNDERSTANDING ADHERENCE/COMPLIANCE TO NEGLECTED TROPICAL DISEASE MASS DRUG ADMINISTRATION PROGRAMS: AN IMPORTANT TOOL FOR THE ENDGAME

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Mass drug administration (MDA) has been ongoing for years in many areas endemic for helminth neglected tropical disease (NTD) infections. While coverage of MDA is regularly reported and documented, compliance/adherence to treatment is, by comparison, poorly understood. Using data collected in two villages in southern Myanmar (2015-16), we examine associations between non-adherence to MDA (specifically for soil-transmitted helminths and lymphatic filariasis) and various demographic factors, as well as identifying the primary reasons for non-adherence (as reported by study participants). 9% of the study population reported refusing drugs offered through the government MDA programme in the past year, with the most commonly cited reasons for this being distrust of the drugs (17.6%), not needing the drugs (13.2%), and fear of side-effects (9.5%). It is worth noting that overall compliance, as well as the

reasons behind it, varied between our two study villages (despite relatively similar demographic and socioeconomic profiles). Additionally, it was found that a proportion (approximately 7%) of the population had been engaging in self-treatment outside of the study and/or government-led MDA. Inadequate levels of compliance to MDA have a substantial effect both on the individual's morbidity as well as on the community, as individuals who remain untreated can act as reservoirs of infection and continue to contribute to the transmission cycle. Many MDA programmes do not require directly observed therapy (DOT), and as such must rely on individual reporting of past compliance - which is problematic for a number of reasons (e.g. recall bias, reporting bias). Regular and consistent reporting of compliance/adherence to MDA will be essential as some endemic areas shift their goal from morbidity control to elimination of helminth NTD infections. This is particularly true in areas of low prevalence, as targeted treatment may be required to reach the goal of elimination. It is important to understand levels of non-compliance, as well as the reasons behind it, in order to maximise the efficiency of MDA programmes.

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#### IMPORTANCE OF INTEGRATED VECTOR MANAGEMENT IN VECTOR CONTROL AGAINST VECTOR BORNE DISEASES IN THE DISTRICT OF VATOMANDRY MADAGASCAR

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Vector-Borne Diseases are the infectious diseases transmitted by insect vectors; these diseases are heavy burdens in Madagascar. The main VBD in Madagascar are malaria, lymphatic filariasis and dengue. Integrated vector management is the rational decision-making process for the optimal use of resources for vector control. It is characterized by evidence-based decision-making, intersectoral collaboration, integration of chemical and non-chemical methods, advocacy and social mobilization, and legislation. In Madagascar, IVM is located in a pilot area in Vatomandry and which involves the Afro I project. The objective of this study is to demonstrate the effectiveness of 3 strategies including LLINs + IRS, LLINs + Social mobilization and LLINs alone to reduce the dependence on the use of DDT in the fight against malaria. A distribution of the LLINs in universal campaign mode was carried out in all the communes of Vatomandry, 4 Communes carried out an IRS of Actellic 300CS, some Fokontany of the District implemented a social mobilization and training for the capacity building of the different actors on IVM was carried out in this District. The results of these strategies are: for the IRS, 99.4% of the census structures are treated, 99.55% of the census populations are protected, 2037 children under 5 years old and 366 pregnant women are protected. For the LLINs distribution campaign, 103,500 nets distributed in this district, 195,606 populations and 49,245 households are protected. For the entomological study, the malaria and other VBD vectors are present in the 4 study communes: *Anopheles gambiae sl*, *Aedes albopictus* and *Culex sp. An. gambiae sl* is rather exophilic. Rice fields and stagnant waters are the potential larval breeding sites. The detection of vector resistance to insecticides in study sites is not yet very significant. In order to design an appropriate vector control program, a comprehensive assessment of VBD is essential, such as the systematic collection of epidemiological data, the development of entomological research and the follow-up of all IVM training.

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#### MASS DRUG ADMINISTRATION IN CROSS-BORDER COLLABORATION: CASE OF MALIAN REFUGEES IN NIGER

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Since 2007, Niger and Mali have been implementing annual integrated mass drug administration (MDA) for the elimination and control of lymphatic filariasis (LF), soil-transmitted helminths (STH), schistosomiasis (SCH) and trachoma with the support of technical and financial partners. During the political crisis in Mali, Niger welcomed more than 50,000 refugees from the endemic regions in Mali, and these refugees settled in the Tillaberi, Tahoua and Niamey regions. To protect the health of the refugees and the program impact of both countries, in 2013, with funding from the END Fund, the Ministry of Public Health, Niger organized an integrated MDA campaign for Malian refugees, supported by Helen Keller International (HKI)-Niger in collaboration with HKI-Mali that provided information on refugees' places of origin and treatment history, UNHCR that advised on access procedures in the camps, the Ministry of Interior that authorized the intervention within the camps and provided military escorts for the field teams, and the regional governor and directorate for Tillaberi region. MDA with ivermectin/albendazole (IVM/ALB), praziquantel (PZQ), and Zithromax and 1% tetracycline eye ointment (TEO) was conducted in 7 out of 8 refugee camps in the Tillaberi and Tahoua regions from June to August, 2013. A total of 16 supervisors and 86 drug distributors were used. During the MDA, drug distributors recorded data on distribution registers for each drug package and the data was subsequently summarized by health center chiefs and district NTD focal points. The LF/STH MDA with IVM/ALB treated 21,541 people out of targeted 29,761 (72% coverage: 46.9% male and 53.1% female). The SCH MDA with PZQ treated 23,741 people out of 29,761 (80% coverage: 50.4% male and 49.6% female) and the trachoma MDA with Zithromax/TEO treated 14,456 people out of 20,271 (71% coverage: 50.4% male and 49.6% female). The results show that good therapeutic coverage was achieved for all three drug packages despite the challenges in refugee camp settings. The intervention ensured that the refugees had not missed treatment and minimized the risk of cross-border transmission of the diseases.

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#### A COMMUNITY STUDY OF THE IMPACT OF SEMIANNUAL ALBENDAZOLE ON LYMPHATIC FILARIASIS AND SOIL-TRANSMITTED HELMINTH INFECTIONS IN THE DEMOCRATIC REPUBLIC OF THE CONGO

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Implementation of mass drug administration (MDA) with ivermectin plus albendazole (Alb) for lymphatic filariasis (LF) has been delayed in Central Africa, because ivermectin can induce serious adverse events in people with very high *Loa loa* microfilaraemia. In 2012, the WHO recommended use of Alb MDA together with vector control to combat LF in areas with co-endemic loiasis. This strategy has been supported by the results of a 3-year community trial conducted in the Republic of Congo, where baseline circulating filarial antigenaemia (CFA, assessed using the immunochromatographic card test - ICT) and microfilaraemia (Mf) rates were 17.3% and 5.3%, respectively. In June 2014, we started a parallel trial in an area with higher baseline infection rates (31.6% for antigenemia

and 11.8% for microfilaremia) in the Democratic Republic of the Congo. Therapeutic coverage for the population > 2 years of age was ~ 75% at all treatment rounds. Evaluation at year 1 and 2 showed that the circulating filarial antigen (assessed using Filarial Test Strip - FTS) rate in the community decreased to 28.7% in 2015 and to 20.6% in 2016. Among 530 individuals who were examined both in 2014 and 2016, 185 were positive at baseline; 52 of those 185 (28.1%) cleared their antigenemia in 2016. Mf prevalence in the community decreased to 8.1% in 2015 and to 3.7% in 2016. Mf density (geometric mean of positive microfilariae (mf) counts) decreased from 171 mf/mL in 2014 to 104.4 mf/mL in 2015 and to 68.1 mf/mL in 2016 (60.2% reduction from baseline value). A total of 63/97 (65%) microfilaremic individuals at baseline have cleared their microfilaremia by 2016. Soil-transmitted helminth infections were monitored using Kato-Katz method. Between 2014 and 2016, prevalence of *Ascaris lumbricoides* infection in the community decreased from 14% to 2.3%, prevalence of hookworm infection from 58.6% to 38.3%, and prevalence of *Trichuris trichiura* from 8% to 3.6%. Year 3 results from that study will be presented at the meeting. This study should provide additional evidence regarding the use of semiannual MDA with Alb for elimination of LF in central Africa.

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### STRENGTHENING THE TRANSMISSION ASSESSMENT SURVEY FOR LYMPHATIC FILARIASIS AND ONCHOCERCIASIS IN MUHEZA DISTRICT, TANGA, TANZANIA

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Transmission Assessment Surveys (TAS) are used by lymphatic filariasis (LF) elimination programs to determine whether LF transmission has been interrupted and mass drug administration (MDA) can be stopped. In Tanzania, TAS have been conducted in 76 out of 186 LF endemic districts, and have resulted in LF MDA being stopped in 74 districts. A programmatic TAS conducted in Muheza district in 2014 showed that 13 of 1664 tested children were antigen positive. Though the number of antigen-positive children was below the critical cutoff of 20, the results raised a concern that transmission could be ongoing in some areas within the district. In 2016, a community-based TAS was conducted in Muheza with the aim of testing the sensitivity of the TAS for detecting evidence of recent LF transmission. The study design was modified to accommodate assessment of antibodies to *Onchocerca volvulus*, allowing assessment of program impact for both onchocerciasis and LF. A total of 1,439 children aged 6-7 years and 3,115 individuals with ≥8 years of age were tested for *Wuchereria bancrofti* circulating filarial antigen (CFA) using the Filariasis Test Strip (FTS), and 4,502 individuals were tested for antibodies to *O. volvulus* by Ov16 rapid diagnostic test (RDT). Five children (0.35%) aged 6-7 year and 80 individuals (2.6%) with ≥8 year old were positive by FTS. Overall, 98 (2.18%) individuals were positive by Ov16 RDT and the prevalence for OV16 antibodies among children aged <10 years 0.14%. The FTS results among young children support the TAS results from 2014 while the Ov16 RDT results among children suggest that MDA has had a significant impact on transmission of onchocerciasis in the district. This study demonstrated the feasibility of integrating onchocerciasis and LF elimination program activities. However, there was clear evidence of LF antigenemia and antibodies to *O. volvulus* among older individuals. These findings confirm the decision of stopping Mass drug administration for Lymphatic Filariasis in Muheza district. However due to presence of onchocerciasis in adult population, there may be a need for additional rounds of Mass drug administration.

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### CHALLENGES AND PROSPECTS FOR TAKING DRUGS DURING MASS DRUG ADMINISTRATION WITH PRAZIQUANTEL AND ALBENDAZOLE: CASE OF THREE HEALTH DISTRICTS IN TILLABERI REGION, NIGER

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The Niger Ministry of Health Neglected Tropical Disease (NTD) Program organizes annual mass drug administration (MDA) campaigns with the support of Helen Keller International through funding from USAID's END in Africa project. In 2017, the first round of MDA with praziquantel/ albendazole was held between February and March 2017 in four regions: Niamey, Diffa, Dosso and Tillaberi, where schistosomiasis is highly endemic. Supervision activities were conducted in four selected districts along the Niger River in the Tillaberi region, which is characterized by population displacement around Kandaji dam and insecurity in the northern area. A rapid survey was conducted at the end of drug distribution to assess compliance with taking the drugs. A convenience sample of 200 people aged ≥five years were surveyed in 10 villages (20 per village) of 8 health areas in Tillaberi, Tera and Kollo districts. The age range of 200 respondents was 5-85 years. Overall, 145 (72.5%) people received drugs and 130 (65%) ingested drugs. Of those aged 5-14 years (92), 64 (69.6%) received drugs and 63 swallowed them (68.5%). Of those aged ≥15 years (108), 81 (75%) received drugs and 67 swallowed them (62%). Among women (95), 72 (75.8%) received drugs and 63 swallowed them (66.3%). Among men (105), 73 (69.5%) received drugs and 67 swallowed them (63.8%). In those not receiving drugs the reasons given included absence (44%), the community drug distributor (CDD) did not come (25%), and stock-out (22%). Those who received but did not ingest the drugs reported being sick (6/15) and fear of side effects (6/15). The results showed that only 68.5% children and 62% adults actually ingested drugs (66.3% in women and 63.8% in men), lower than the minimum 75% required, though such rapid surveys do not reflect the true coverage. The factors affecting the receipt and ingestion of drugs reflect the challenges in MDA in these areas. More efforts for improving coverage include reducing CDDs' workload and providing training for key community actors such as public town criers and female volunteers.

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### A COMPREHENSIVE SUSTAINABILITY FRAMEWORK FOR NEGLECTED TROPICAL DISEASE ELIMINATION PROGRAMS

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Great progress is being made in the push to eliminate Lymphatic Filariasis (LF) by 2020. However, additional guidance is required to ensure that elimination is sustained. A typical LF elimination program cycle has three phases: program initiation to identify infection prevalence; treatment to at-risk populations for 4-6 years to suppress transmission; and validation of elimination. This qualitative research was conducted in two steps using a multi-case study methodology: a literature review of sustainability frameworks to identify the optimum approach for LF, and the testing of the new sustainability framework on the Kenya National Programme for Elimination of Lymphatic Filariasis (NPELF). The research aimed to answer the following: (1) what elements of an LF elimination program need to be sustained?; (2) what factors influence sustainability of these elements?; and (3) how does decentralization of health services impact sustainability?. In-depth interviews were conducted with key stakeholders, programmatic documents were reviewed and key meetings attended. Data were subsequently arranged into the two key themes of interest- programmatic and contextual factors affecting sustainability. Within each of these, data were coded to specific programmatic or contextual factors and to the

phase of the LF program. The main findings of this research were, (1) sustainability of elimination programs is influenced by: technical factors related to the intervention; implementation factors; and those related to the broader environment in which the program operates; (2) the relative dominance of the three categories of sustainability factor varies with a program; (3) the goal of integrating NTD activities into national health systems needs to be rethought as health systems are inherently weak and their capacity to handle the intricacies of NTD surveillance is questionable; and (4) funding remains a constant factor that influences sustainability. With three years left to reach the 2020 elimination target, this key decision making tool is timely not only for Kenya but for NTD elimination programs at large.

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### SCHOOL AND COMMUNITY BASED DEWORMING IN KENYA: WHAT ARE THE BARRIERS AND ENABLERS FOR SUSTAINING LONG-TERM IMPLEMENTATION?

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In recognition of the substantial negative impact of soil transmitted helminths (STHs) on health and productivity in Kenya, where approximately 15 million people are infected, a national School-Based Deworming Programme (SBDP) was launched in 2009. To expand coverage to members of the community not reached by the SBDP, a door-to-door Community Based Deworming Programme (CBDP) is being tested through a randomised controlled trial in one high STH burden county, Kwale. While effectiveness of these two models for deworming are being assessed in terms of STH infection rates, less is known about factors facilitating or hampering programme implementation. These factors can be intrinsic (programme-specific) and extrinsic (related to the wider health system). Data were obtained from 43 in-depth interviews with respondents involved in design and implementation of the two programmes: community health workers (CHWs), teachers, government health and education officers at the county and subcounty level in Kwale, and national policy makers in Nairobi. A thematic analysis revealed that strong support and coordination from high-level health and education policymakers, association of deworming with improvements in school performance, and students' comfort levels in accepting deworming tablets from teachers, worked in favour of the SBDP. However, the SBDP relies on continued support from teachers during deworming campaigns, and misses children who are not in school. In contrast, interviewees reported that the CBDP reaches a wider population and that visible excretion of worms led to growing community support. The need for investment in CHW training and transport allowances, and in consistent engagement with community leaders and members to ensure acceptance of the CBDP were identified as critical factors for ensuring long-term success. We conclude that assessments of the fit of programmes with existing systems and policy processes such as decentralisation, informed by experiences of local implementers and planners, should complement evaluations of impact on parasitological outcomes in informing deworming strategies.

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### EVALUATION OF SD BIOLINE ONCHO/LF IGG4 BIPLEX AND SD BIOLINE LF IGG4 SCREENING TOOLS IN FILARIAL-ENDEMIC REGIONS OF CAMEROON

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Lymphatic filariasis (LF) is a disfiguring disease caused primarily by *Wuchereria bancrofti*. In central Africa, LF can be coendemic with the filarial parasites *Loa loa*, *Mansonella perstans*, and *Onchocerca volvulus*. The main strategy to eliminate onchocerciasis and LF in Africa is mass drug administration (MDA) with ivermectin alone or in combination with albendazole, respectively. The elimination programs have a need for rapid point-of-care diagnostics for mapping, monitoring and post-MDA surveillance. In *L. loa*-endemic regions, ivermectin can lead to serious adverse events in individuals heavily infected with *L. loa* and treatment may need to be prevented. Furthermore, previous studies have shown that commercial antigen-detection tests for *W. bancrofti* cross-react with sera from individuals heavily infected with *L. loa*. The Wb123 antigen, a specific biomarker for detection of IgG4 antibodies against *W. bancrofti*, was identified and first evaluated National Institute of Allergy and Infectious Diseases at the NIH, Bethesda, MD, USA. Two new IgG4 rapid tests incorporating Wb123 are available from Standard Diagnostics, Inc.: the SD BIOLINE LF IgG4 rapid test (Wb123 RDT) and SD BIOLINE Oncho/LF IgG4 biplex rapid test (Ov16/Wb123 RDT). In order to evaluate these tests in areas with high prevalence of loiasis, fingerstick blood samples from 5000 individuals residing in 50 villages in 5 areas in Cameroon were screened with the Wb123 RDT and the Ov16/Wb123 RDT. No evidence was found for the presence of *W. bancrofti* by microscopy or qPCR of night blood of antigen. Only 0.2% of total samples were IgG4-positive by Wb123 RDT and 0.4% by Ov16/Wb123 RDT. Positive Wb123 tests did not cluster in any area. In contrast, the prevalence of Ov16 positive samples as measured by Ov16/Wb123 RDT varied between the areas from 0.1 to 28.8%. The results of the both RDTs were compared to ELISA. This study provides key evidence of performance of Wb123-based RDTs in areas endemic for multiple filarial diseases and results may be informative to NTD programs interested in adopting new tools and facing similar coendemicity.

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### IMPROVING MASS DRUG ADMINISTRATION PERFORMANCE USING MHEALTH: FINDINGS FROM NORTHERN NIGERIA

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Onchocerciasis and lymphatic filariasis are slated for elimination by 2025 and 2020 respectively. Mass drug administration (MDA) is the core strategy to eliminate the two, but there are known challenges with how MDA is done. Lack of visibility means issues in the field cannot be identified in time to remedy them; data collected at the community directed distributor (CDD) level is rarely checked for errors; drugs are often allocated based on incorrect census numbers and there is little drug tracking in the field. A smarter process and better tools are needed to counter these issues if we want to reach elimination. In August 2016 Sightsavers, with the UNITED Project, piloted MDA monitoring in the Bungudu LGA of Zamfara State in Nigeria. This involved a change in process 1) census done before treatment and drugs allocated based on that census and 2) health facility workers sending in MDA data (census, treatment, drug use/waste) over their own mobile phones weekly, working towards personalized targets. These data were made immediately available for view online by LGA, State and National teams on custom reports made to monitor progress

against targets and access stock reports. Taking into account the census increase, Bungudu's oncho/LF campaign performance from the year before was compared with 2016. Therapeutic coverage increased 2.96%. Mectizan and Albendazole waste decreased 70% and 90% respectively, saving an estimated \$20,520 in drug waste for 237,875 persons treated. For a qualitative evaluation, extensive feedback sessions were held with participating health facility workers. During these newly prescribed weekly visits to collect data they reported that: 58% fixed mistakes on CDD registers; 15% saw a migrational increase in population and directed CDDs on what to do; 13% identified and regulated drug shortages in certain communities. Sightsavers, with UNITED, plan to scale-up monitoring in additional states in 2017. Sightsavers will also use this new platform and process to monitor both oncho/LF and trachoma MDAs in multiple countries in 2017.

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### **PARTNERSHIP FOR NTD ELIMINATION: THE TANZANIA FIXED OBLIGATION GRANT APPROACH WITH LOCAL GOVERNMENT AUTHORITIES**

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Neglected tropical diseases (NTDs) which include schistosomiasis, soil transmitted helminths (STH), onchocerciasis, trachoma and lymphatic filariasis are still a public health concern in Tanzania. Control and eventual elimination of NTDs require multi-sectoral collaboration to address the key determinants of these diseases. Effective management and coordination of elimination efforts in Tanzania has relied on clear collaborative mechanisms among stakeholders. The councils (local government authorities (LGAs)) are the implementation units (IUs) for disease control and elimination activities. IMA World Health, through the ENVISION Project works with the central government and LGAs to manage funds related to NTD activities. In the past, activities had been delayed due to the time required to reconcile expenses against planned activities. Currently, the Tanzania NTD Control Program through its partner NGO provides fixed obligation grants (FOGs) to all supported IUs, a framework through which planning, implementation and reporting is streamlined. Annual plans are developed with all key stakeholders and reviewed prior to approval. Once plans are finalized, deliverables are set and upon approval, implementation units are funded based on implementation plans. Councils are reimbursed after completion of planned activities. In 2017, 142 district councils and 20 region councils received funding via FOGs ranging from US \$8,433 to \$92,250 based on the district population size. The use of FOGs has improved financial accountability by reducing delays in financial reporting. All districts (100%) have a dedicated accountant for NTD activities and 96% (n=136) of councils report timely access (within 7 days) to funds from the LGA finance system. Overall, there has been reduction in implementation and reporting delays. The use of FOGs at the LGA level has improved transparency, accountability and financial management practices, as well improving local ownership, thus positively impacting implementation of NTD elimination activities. This mechanism can be scaled up for use in other LGA funded activities beyond the NTD program.

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### **STRENGTHENING THE SUPPLY CHAIN FOR NEGLECTED TROPICAL DISEASE PREVENTIVE CHEMOTHERAPY AND TRANSMISSION CONTROL MEDICINES IN TANZANIA**

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In Tanzania, preventive chemotherapy (PCT) for neglected tropical diseases (NTDs) has been undertaken as an integrated national program since 2009. The integrated Tanzania NTD Control Program (TZNTDCP) evolved from several vertical programs and each program had a separate supply chain. After integration, NTD medicine supply chain management became more complex and system improvements were needed. In 2014, the NTD program and partners undertook a logistics assessment in four districts and found a number of challenges with the PCT medicines supply chain. These included poor inventory management, incomplete MDA data forms, lack of reverse logistics and inadequate understanding of quantification formulas. The assessment revealed that better coordination of supply chain for NTD medicines was needed at the district level. The TZNTDCP developed guidelines documenting the supply chain management of NTD medicines, including: inventory management; quantification of medicines for the community; reverse logistics; completion of summary data forms; and reporting of adverse events. The guidelines were initially piloted with 30 district pharmacists who were trained as trainers for frontline health workers (FLHWs) and community drug distributors (CDDs). Routine supervision revealed that inventory records were kept at the district and health facility level; quantification of medicines sent to health facilities and schools was more accurate; left over medicines after MDA were minimized; summary data forms were completed appropriately; and medicines remaining after MDA were returned to districts stores. After the pilot, training on the supply chain was integrated into the routine training on MDA for district pharmacists. As of the end of 2016, an additional 158 district pharmacists have been trained on supply chain for PCT based on the national guidelines. Improved efficiency and reliability of supply chain management is important to reduce wastage of medicines and improve outcome of MDA campaigns. Based on the findings from the pilot, future activities will focus on FLHWs and CDDs nationwide.

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### **SUPPORTIVE SUPERVISION FOR MASS DRUG ADMINISTRATION IN TANZANIA**

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Two neglected tropical diseases (trachoma and lymphatic filariasis) are targeted for elimination by 2020. Mass drug administration (MDA)

for preventive chemotherapy and transmission control (PCT) are key interventions for disease elimination. In Tanzania, supportive supervision with a cascade approach was designed to allow a supervisor to work closely with colleagues to establish goals, monitor progress and identify opportunities for improvement of MDA. To implement effective supportive supervision a checklist was developed in 2011, and reviewed in November 2015. The checklist comprises the following components: 1) advocacy, sensitization and mobilization 2) training activities 3) availability of PCT medicines; 4) availability MDA supplies; 4) availability of funds for MDA; 5) pre-MDA activities such as food preparation for school-based MDA; 6) timeliness of submission of programmatic and financial reports; and 7) success stories. Use of the checklist was shown to increase health worker motivation, improve service quality and increase uptake of interventions, particularly MDA. In 2016, supervision was carried out for school-based MDA in 102 councils and community-based MDA in 71 councils. This approach resulted in increased awareness and participation of council members in MDA activities; improved training at regional, districts, health facility and community levels; better commitment by MDA implementers at all levels; improved coordination of MDA activities; and better involvement of key decision makers and stakeholders. In addition, the proportion of councils achieving coverage targets increased from 71% in 2014 to 81% in 2016. Further, MDA activities were included in Council Comprehensive Health Plans (CCHP), and some councils allocated funds to top up MDA activities in their budgets. Supportive supervision is key for optimal implementation of MDA. Continuous improvement of the checklist as well as supervision before and during MDA activities will help the program reach its control and elimination goals for NTDs.

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### DISSECTING THE ROLE AND PATHOGEN BENEFITS OF TRYPANOTHIONE SYNTHETASE OVEREXPRESSION IN *TRYPANOSOMA CRUZI*

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Chagas disease is a tropical neglected illness caused by *Trypanosoma cruzi* that remains to be endemic in Latin America. This pathogen has to deal with different oxidant challenges such as the respiratory burst triggered inside the macrophage. Thus, it has an effective antioxidant system capable of overcoming the host barriers and maintaining the redox balance. The parasite antioxidant network utilizes trypanothione (TSH), a low MW dithiol, as substrate. The trypanothione synthetase (TryS) enzyme (produces TSH metabolite) is uniquely present in kinetoplastids, and so it is a good candidate for drug design. In order to characterize its role in the host-parasite interaction, we have overexpressed pTREX encoding TryS in *T. cruzi* SylvioX10 isolate by electroporation. Recombinant parasites (TryS+) exhibited stable overexpression (>2-fold increase) of the TryS protein and a significant increase in TryS enzymatic activity as compared to controls. The TryS+ showed a higher rate of metacyclogenesis, and ~20% more of infective forms were obtained in TryS+ cultures compared to the cultures of parasites transfected with empty pTREX. Furthermore, transfectant parasites tolerated higher doses of benznidazole (IC50 value: 21.3 µM and 11 µM, TryS+ vs. controls, respectively, p<0.05). To get insight into TryS role in host-parasite interaction, HL-1 cells were infected. After 36 h incubation, cells infected with TryS+ (vs. control) *T. cruzi* exhibited 2-fold lower levels of intracellular ROS, determined by H2DCFDA fluorescence (p<0.05). Our results suggest that *T. cruzi* utilizes TryS to promote differentiation from insect stage to infective forms and to maintain the redox balance in the host cells that support intracellular survival of the parasite.

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### SPIROPLASMA PREVALENCE IN *GLOSSINA FUSCIPES FUSCIPES* IN UGANDA

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Tsetse flies (*Glossina* spp.) are vectors of African trypanosomes, which cause human and animal African trypanosomiasis (HAT and AAT respectively). In Uganda, *Glossina fuscipes fuscipes* (*Gff*) is the primary vector species and displays high genetic diversity, currently revealing four genetic units. While three endosymbiotic bacteria (i.e. *Wigglesworthia*, *Sodalis* and *Wolbachia*) have been described from *Gff* in Uganda, here we describe a fourth endosymbiont *Spiroplasma* from one of the *Gff* populations. *Spiroplasma* in *Drosophila* has been shown to protect its host against parasitic nematode as well as induce male killing. Bayesian phylogenetic analysis carried out on *Spiroplasma glossinidius* in comparison with other *Spiroplasma* strains show that it is close to pathogenic *S. Penaei*. Individuals collected from four genetic units were screened for *Spiroplasma* infection. An overall infection frequency of 23% (n=936) was detected. The trypanosome infection in the four genetic units was 5% (n=792). The regions with high *Spiroplasma* infection revealed a lower trypanosome infection and vice versa. A model of analysis of the deviance indicate that the source of the fly collection (genetic region) and trypanosome infection status were the main variables structuring *Spiroplasma* infection P < 2e-16 P (0.001). The *Spiroplasma* infection is detected in flies associated with 15% of the haplotypes (nine haplotypes out of the total 62 haplotypes). Ongoing work is focusing on infecting *Glossina morsitans* with *Spiroplasma poulsonii* to enable *in vivo* studies of its fitness effects and studying the effect of *Spiroplasma* on *Trypanosome* infection. *Spiroplasma* infections prevalence was high in the northwest genetic unit; this genetic unit recorded the lowest trypanosome infection. We detected a strong association of infection status with mitochondrial haplogroups A and B. Better understanding of the *Spiroplasma* infection prevalence in different *Gff* populations can help advance knowledge on its role in the evolution of the genetic patterns observed in host species in Uganda. This knowledge can help towards development of new vector control strategies.

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### GENETIC BACKGROUND OF AN ATYPICAL *LEISHMANIA DONOVANI* CAUSING CUTANEOUS LEISHMANIASIS IN SRI LANKA

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*Leishmania* protozoan parasites cause a complex spectrum of diseases in many tropical countries. *Leishmania donovani* causes visceral leishmaniasis, the most severe form of this disease. An atypical *L. donovani* is responsible for mostly cutaneous (CL-SL) and few visceral (VL-SL) cases in Sri Lanka. Here, we explain some genetic factors possibly underlying these different disease tropisms. Genomes of six CL-SL and two VL-SL clinical isolates were sequenced by Illumina MiSeq. Reads were mapped to *L. donovani* reference (BPK282A1). Read depth data was used to infer chromosome copy number and copy numbers of protein-coding genes. Tandem gene arrays were identified using OrthoMCL. Most genes with high SNP counts in CL-SL were kinases. Protein kinase networks have a potentially important role in virulence. Additionally, midasin and hypothetical proteins were found. In VL-SL most high SNP count genes were hypothetical. A Tm vesicle-mediated sorter implicated in regulating host immunity, ubiquitin protein with a potential role in pathogenesis and concanavalin A-like lectin were also found. In CL-SL, most chromosomes were disomic, except for monosomic chromosome 2 and trisomic chromosomes 22 and 26. Chromosome 31 showed pentasomy. In VL-SL, most chromosomes demonstrated disomy or intermediate somy (E.g. 2, 3, 5, 8, 21, 22, and

26). Supernumerary chromosome 31 was tetrasomic. Some differences may have a role in altered pathology. We found 192 and 241 tandem gene arrays in CL-SL and VL-SL respectively with 42 arrays with at least a 2-fold change in gene copy number. Among them D-lactate dehydrogenase-like protein involved in pyruvate metabolism, branched-chain amino acid aminotransferase involved in amino acid metabolism, HUS 1 protein which helps the parasite to cope with replicative stress, GP63/leishmonolysin, a virulence factor were observed with a higher copy number in VL-SL arguing that these genes may have an important role in causing different pathologies. These differences between the CL-SL and VL-SL provide insights in to understanding the genetic background for attenuated visceralizing ability of Sri Lankan *L. donovani*.

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### IDENTIFICATION OF *TRYPANOSOMA CRUZI* LINEAGES ASSOCIATED WITH CONGENITAL CHAGAS IN THE POPULATION OF SANTA CRUZ, BOLIVIA

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Congenital transmission is one of the main problems of Chagas' disease, involving endemic and non-endemic countries. In Bolivia Chagas disease is a serious public health problem. Currently, the implementation of control programs for Chagas disease by WHO allowed the control and interruption of vector transmission of *Trypanosoma cruzi*, but in urban areas Chagas' disease is maintained due to the non-vectorial transmission of the disease like congenital transmission. The objective was identifying the lineages of *T. cruzi* associated with congenital Chagas. We analyzed 11 samples of Umbilical Cord Tissue of newborns congenitally transmitted from the population of Santa Cruz (Bolivia). The diagnosis and quantification of parasites was determined by the Real Time technique (qPCR) using the Taqman probe directed to the *T. cruzi* nuclear DNA. Identification of lineages was determined by the PCR-RFLP technique using three molecular markers: 1) CoxII + AluI, 2) rDNA 24S $\alpha$  and 3) Mini-exon SL-IRac. The results obtained from the 11 samples determined an average parasite load of 19x10<sup>6</sup> parasites / mg and an average of 9x10<sup>5</sup> parasites / mg. The PCR-RFLP technique identified the presence of the DTU V Lineage in the 11 umbilical cord tissue samples. Concluding that was possible to determine the parasitic load in Umbilical Cord Tissue specimens and it was also possible to identify the presence of the DTU V lineage from umbilical cord tissue of newborns congenitally infected with *T. cruzi* from the population of Santa Cruz- Bolivia.

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### MAPPING METABOLOME ALTERATIONS IN *LEISHMANIA AMAZONENSIS* PROMASTIGOTES INDUCED BY LONG TERM AXENIC CULTIVATION, THROUGH A MULTIPLATFORM METABOLOMIC FINGERPRINT APPROACH

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Leishmaniasis are widespread neglected endemic diseases with an incidence of 1.6 million new cases and 40 thousand deaths per year. *Leishmania* parasites may show distinct, species-specific patterns of virulence leading to different disease manifestations. It is well known that successive *in vitro* passages (SIVP) leads to attenuation of virulence, but neither the metabolism nor the pathways involved in these processes are well understood. Here, a virulent *L. amazonensis* (R0) strain was compared to three SIVP attenuated strains, R10, R40 and R60, submitted to the 10, 40 and 60 of axenic passages, respectively. *In vitro* assays and *in vivo* tests were performed to characterize and confirm the strains attenuation profile. By applying capilar electrophoresis, liquid chromatography and gas chromatography, coupled to mass spectrometry, a metabolomic fingerprint approach was performed, comparing R0, R10 and R60. The identified metabolites were clustered in biochemical categories and mapped in metabolic pathways: 67 metabolites were associated to 8 metabolic pathways. Metabolism of fatty acids was considered the most significant pathway altered post-SIVP. The metabolomic approach met correlations with previous proteomic findings of related to these same same SVIP strains. The biochemical pathways' analysis allowed the identified metabolites to be mapped in several biochemical pathways potentially involved in *L. amazonensis* infectivity and virulence.

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### MULTISYSTEM METABOLOMIC FINGERPRINT ANALYSIS, ASSOCIATED TO IN VIVO CELLULAR DIFFERENTIATION PROCESS OF A WILD TYPE STRAIN OF *LEISHMANIA AMAZONENSIS*

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*Leishmaniasis* are widespread neglected endemic diseases with an incidence of 1.6 million new cases and 40 thousand deaths per year. *Leishmania* parasites may show distinct, species-specific patterns of virulence leading to different disease manifestations. Their processes of cellular differentiation are induced by their host's physical-chemical microenvironments, culminating in two main stages, promastigote and amastigote. Despite their importance as potential therapeutic targets or as research material for the development of vaccines, little is known about the biochemical and metabolic phenomena associated to the cellular differentiation in *Leishmania*. This research proposed to identify metabolites and metabolic pathways related to the *in vitro* cellular differentiation process, using a multi-analytic system based in Gaseous Chromatography (GC-MS), Liquid Chromatography (LC-MS) and Capillary Electrophoresis (CE-MS). Said multi-analytic system was able to compare polars, non-polars and low molecular weight metabolites simultaneously. After 3, 6, 12, 24, 48 e 96 hours of amastigogenesis, parasites' metabolomes were compared and the ions identified on their particular times of retention submitted to multivariate (PCA, PLS, OPLS) and univariate (t-Student, U Mann-Whitney) statistical analyses. Those showing statistical significance were selected to the putative identification of metabolites; those identified were confirmed by MS-MS or by

comparative analysis to standards and mapped in metabolic pathways with the help of bioinformatics. CE-MS and LC-MS identified 63 metabolites and confirmed 16. GC-MS analyses, the mapping and association of the identified metabolites to metabolic pathways have been concluded. The research only remains to be biologically validated.

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### PATHOGENIC INFECTION ANALYSIS IN VITRO AND IN VIVO OF TRIPOMASTIGOTES: EVALUATION OF AREQUIPA STRAIN IN COMPARISON WITH COLOMBIANA AND CL BRENER TRYPANOSOMA CRUZI STRAINS

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*Trypanosoma cruzi* is a complex and heterogeneous parasite, including strains with intraspecific variations (pathogenic, antigenic and genetic variability). In this work we evaluated *T. cruzi* isolate from "La Joya", Arequipa-Peru and compared to the Colombian and CL Brener *T. cruzi* strain. The genotyping analysis showed that Arequipa strain is from DTU TcI, similar to Colombian strain and different from CL Brener strain (DTU TcVI). Analysis *in vitro* of proliferative capacity within peritoneal macrophages obtained from BALB/c mice showed that the Arequipa strain was the least pathogenic with the lowest rates of infection and multiplication. The effector responses of the macrophages were analyzed and the results showed that the Arequipa strain induces higher percentages of the oxidant molecules eROS and NO when compared to the Colombiana and CL Brener strains, on the other hand, the evaluation of the antioxidant defenses of the enzymes TcAPX, TcCPX, TcMPX, TcTrS, TcTrR, TcSodA and TcSodB, showed that the genome of these *T. cruzi* strains had similar gene copy numbers, although they had a differential repertoire in expression levels. The CL Brener strain presented higher expression of TcAPX, TcMPX, TcSodA, TcSodB and TcCPX and the Colombiana and Arequipa strains, higher expression of TcSodA, TcSodB and TcCPX, respectively. Likewise, the nonpathogenic Arequipa strain was reactivated (RE) into the triatomine vector, which resulted in increased expression of the enzymes TcAPX, TcMPX, and TcCPX. Finally, *in vivo* infection of C57BL/6 mice naturally resistant to *T. cruzi* was evaluated, the results showed that the Arequipa - RE strain had low parasitemia peaks, with a preferentially cardiac and intestinal tropism, although they had a low parasitic load when compared with the Colombian strain that had the highest cardiac and intestinal tropism and the CL Brener strain that had a higher cardiac and skeletal muscle tropism. Cytokine expression analyzes showed the same immunological profile during infection with the three *T. cruzi* strains evaluated.

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### USE OF IMMUNOGENIC EPI TOPE ALPHA GALACTOSYL (A-GAL) FOR THE DIAGNOSIS OF CHAGAS DISEASE IN CHAGASIC PATIENTS FROM BOLIVIA AND PERU

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Chagas disease affects about 8 million people in Latin America, with 50,000 deaths/year and more than 100 million people living in at-risk areas. This disease can represent a terrible economic loss for the

affected countries because of the high cost of vector control measures, diagnosis and treatment of patients and adverse effects of treatment. For the diagnosis of Chagas disease, up to now, serological methods are more widely used indirect hemagglutination (IHAS), indirect immunofluorescence (IIF) and Enzyme-Linked Immunosorbent Assay (ELISA) assays using total parasite, semi-purified antigenic fractions or non-infectious epimastigote homogenized. These presented variations in the reproducibility and reliability of the results, being necessary to develop new and more effective methods for the diagnosis of Chagas disease. The objective was to develop a immunology test using coupled  $\alpha$ Gal with synthetic viral particle (Q.beta- $\alpha$ Gal) to diagnosis of Chagas' disease based on the use of highly immunogenic epitopes present in glycoproteins and glycolipids on the surface of infectious forms of *T. cruzi*, such as Gal $\alpha$ 1,3Gal $\beta$ 1,4GlcNAc ( $\alpha$ Gal), not normally expressed in human cells, responsible for the induction of protective antibodies (anti-Gal). The humoral immune response to Q.beta- $\alpha$ Gal was tested using genetically modified animal models, such as  $\alpha$ 1,3-galactosyltransferase ( $\alpha$ GalT-KO) knockout mice, these animals, humans and old world monkeys do not express  $\alpha$ -Gal epitope on the surface of cells and tissues, being excellent producers of IgG anti- $\alpha$ Gal. With this methodology, the sensitivity and specificity for the diagnosis was tested with sera of infected and no infected population from Arequipa (Endemic area in Peru), We found a 53% and 90%, sensitivity and specificity respectively. We found moderate agreement with another serological test ( $Kappa=0.44$ ). Because of this, we suggest to increase the sample size to obtain more accurate results.

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### HOST-TSETSE FLY INTERACTIONS IN TRYPANOSOMIASIS ENDEMIC COMMUNITY IN GHANA: IMPLICATIONS FOR HUMAN AFRICAN TRYPANOSOMIASIS (HAT)

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Trypanosomiasis control in Ghana and many other African countries faces constraints as a result of difficulties in assessing the exact epidemiologic situation. To avoid HAT outbreak, control and surveillance of the disease must be continued and sustained. This work aimed at evaluating the current situation of HAT in terms of tsetse fly distribution and density, blood meal sources, and species of trypanosomes in circulation at the New Juaben Municipality (trypanosomiasis endemic community), Ghana. Tsetse flies were trapped using un-baited bi-conical traps deployed in various locations of the study site. The DNA extraction was conducted on blood samples spotted on filter papers from fed flies and mid gut sections using the Qiagen DNeasy kit. Portions of the mid gut samples were used for serological analysis. The results showed that, 176/259 (68%) tsetse flies captured were identified with undigested blood meal that was used in ELISA to determine the blood meal sources. The main sources of blood meal identified were: humans (16.9%), pigs (26.2%), goats (12.3%) and chicken (16.2%). There were 130/259 (50.2%) mid-gut tissues for nested PCR using ITS1 and other primer sets. About 58.5% (76/130) were positive for trypanosomes DNA with 64.5% (49/76) having single infection while 27/76 (35.5%) had mixed infections. The results further showed that, 89.5% (68/76) of the flies carried only *T. congolense* Forest subgroup, 24.1% (19/76) had *T. b. brucei* and none carried *T. b. gambiense*. There were 13/76 (17.1%) flies carrying *T. evansi*, *T. congolense* forest and *T. b. brucei* DNA. The study site was inhabited by a single tsetse fly species, *G. p. palpalis* in higher densities during the dry season, which was noted to have taken most of the blood meal from the pigs. The *T. congolense* Forest

type was the dominant sub-species carried by the tsetse fly. Even though *T. b. gambiense* was not identified, in a situation of unexpected introduction into the Municipality, the high populations of pigs and *G. p. palpalis* would present potential risk of sleeping sickness outbreak. Regular and integrated surveillance system is therefore recommended.

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### EFFICACY OF GENETICALLY MODIFIED LIVE ATTENUATED LEISHMANIA VACCINES AGAINST INFECTED SAND FLY BITES

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During natural transmission, an infected sand fly deposits parasites along with saliva and vector-derived components of the infectious inoculum. The enhanced virulence observed in sand fly-mediated transmission has been attributed to a sustained recruitment of neutrophils. The mechanism underlying this phenomenon remains unknown. In addition, previous studies have demonstrated that several experimental *Leishmania* vaccines protected mice against needle-challenge but failed against vector-mediated challenge. These studies clearly demonstrate that a vector-initiated infection is crucial for understanding pathogenesis, immunity and vaccine evaluation for leishmaniasis. Vector-transmission of *L. donovani* resulted in the intense accumulation of neutrophils and resulted in a 100-fold increased IL1 $\beta$  induction compared to needle injected parasites. Further, our studies demonstrated that IL1 $\beta$  is the main cause of neutrophil persistence following sand fly-transmission. Importantly, neutralizing the effect of IL1 $\beta$  by blocking IL1R abrogated neutrophil recruitment after infected bites and abolished parasite visceralization. To address whether live attenuated parasites can protect against the enhanced virulence of a sand fly-initiated infection, hamsters were immunized with *LdCen*<sup>-/-</sup> and sand fly salivary gland protein rLJM19, a potent immunomodulatory component of sand fly saliva. Five weeks post immunization hamsters were challenged with *L. donovani*-infected sand flies. We monitored these hamsters for up to 14 months post-challenge. During this period 50% of hamsters from the non-immunized group developed severe VL and died. None of the hamsters from the immunized group developed any VL symptoms. Immunized hamsters induced a strong Th1 type of immune response upon challenge with infected sand fly. The protection observed by survival of immunized hamsters was corroborated by a significantly lower splenic and hepatic parasite burden compared to non-immunized animals. The study demonstrates that *LdCen*<sup>-/-</sup> immunization is efficacious in controlling VL initiated by the sand fly vector.

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### EVALUATION OF SAFETY AND EFFICACY OF LEISHMANIA MAJOR CENTRIN DELETED LIVE ATTENUATED PARASITES AS A PROPHYLACTIC VACCINE AGAINST CUTANEOUS AND VISCERAL LEISHMANIASIS

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Protozoan parasites of the genus *Leishmania* are responsible for a spectrum of human diseases known as leishmaniasis. Visceral leishmaniasis (VL) is most fatal if not treated and there is no licensed vaccine. Previous studies have shown that vaccination with low dose of dermatotropic

*Leishmania major* confers protection against reinfection as well as cross-protection against VL. However such method of immunization is not practical because of the greater risk of infection in naïve population. Therefore, genetically modified live attenuated parasites that are non-pathogenic might induce the same protective immunity as leishmanization. Previously, we have shown that centrin gene deleted *Leishmania donovani* induces a protective immunity in various animal models against homologous as well as heterologous challenge. In this study, we have developed centrin-gene deficient *Leishmania major* (*LmCen*<sup>-/-</sup>) using CRISPR-Cas methodology and evaluated the safety and immune stimulating efficacy using hamster model. Our results demonstrated that intradermal immunization of hamster with *LmCen*<sup>-/-</sup> by needle injection did not develop any detectable lesion in the ear of the hamsters up to seven weeks after immunization and there was no parasite persistence compared to *L. major* wild type (*LmWT*) infection. *LmCen*<sup>-/-</sup> immunization significantly induced the expression of IFN- $\gamma$  with a concomitant down regulation of IL-10 in the ear, draining lymph node and spleen compared to *LmWT* infection suggesting induction of a Th1 type of immunity. Compared to *LmWT* infected animals, *LmCen*<sup>-/-</sup> immunized hamsters showed a protective IgG2 dominant response as observed by a higher IgG2/IgG1 ratio further indicating the development of Th1 response. We also showed that *LmCen*<sup>-/-</sup> parasites alone is sufficient to induce a host protective immune response and addition of adjuvant (Glucopyranosyl Lipid Adjuvant, GLA) does not enhance the protective immune response. Studies are underway to demonstrate the efficacy of this vaccine against cutaneous and VL infections. Taken together, the study demonstrates that the *LmCen*<sup>-/-</sup> parasite is safe and immunogenic vaccine candidate.

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### COMPARATIVE STUDY OF VARYING CONCENTRATIONS OF A NIGERIAN PLANT EXTRACT (E557) ON LOCAL AND STANDARD STRAINS OF MYCOBACTERIUM TUBERCULOSIS

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The widespread recurrence of drug-resistant tuberculosis particularly fluoroquinolone-resistant multidrug-resistant and extensively drug-resistant tuberculosis has counterbalanced the Millennium Development Goal of reversing the global spread of tuberculosis by 2015. In this study, antimycobacterial sensitivity testing of a Nigerian plant extract, E557 was carried out to discover its possible effect at both extremely low and mildly high concentrations against clinical isolates (MTB-584) and control strains (H37Rv) of *Mycobacterium tuberculosis* (MTB). The proportion method for drug susceptibility testing on Löwenstein Jensen (LJ) media was utilized for antimycobacterial screening in a Biosafety Level-3 (BSL-3) facility. The extract was also assessed for its phytochemical constituents. The extremely low concentrations of E557 were 1, 40 and 250  $\mu$ g/ml while the high concentration was 100 mg/ml. Löwenstein Jensen media was prepared with respective concentrations of E557 and inspissated. Afterwards, the LJ media were inoculated with 10<sup>-3</sup> and 10<sup>-5</sup> dilutions of both local (MTB-584) and standard (H37Rv) strains of *Mycobacterium tuberculosis*. The positive control for the test was Rifampicin while untreated inoculated media was engaged as negative control. All the prepared LJ media were incubated at 37°C and observed every seven days for six weeks. At the end of six weeks, the result showed that both MTB-584 and H37Rv exhibited resistance (> 250 colonies: ++) to the extracts at 1, 40 and 250  $\mu$ g/ml as revealed by the creamy non-pigmented colonies observed on all the LJ media prepared with extracts. Contrariwise, there was no growth at all (zero colony: 0%) on all the LJ media prepared with 100 mg/ml of E557 when matched with the negative control (150 and 500 colonies respectively for MTB-584 and H37Rv strains). Phytochemicals such as alkaloids, antocyanins and betacyanins, flavonoids, steroids and terpenoids were present in E557. Conclusively, higher concentrations of E557 possibly contain potent bioactive phytochemicals which can be developed into effective antibiotics against *Mycobacterium tuberculosis* strains.

## EVALUATION OF ANTIMYCOBACTERIAL ACTIVITY OF FIVE CAMEROONIAN PLANTS

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Tuberculosis is considered as one of the most important public health problems worldwide. The inappropriate use of existing anti-tuberculosis drugs over the years has led to an increase in the prevalence of resistant strains, hence the need to develop new effective agents. The present study was carried out to evaluate the *in vitro* antimycobacterial activity of ethanolic and aqueous extracts (decoction) of *Spathodea campanulata* (Bignoniaceae), *Newbouldia laevis* (Bignoniaceae), *Canarium schweinfurthii* (Burseraceae), *Zanthoxylum heitzii* (Rutaceae) and *Drypetes gossweileri* (Euphorbiaceae) on *Mycobacterium smegmatis* ATCC 700084. These plants are used in traditional pharmacopoeias in Cameroon and other countries in Africa and Asia to treat people with symptoms of tuberculosis and others respiratory ailments. The ethanolic and aqueous extracts (decoction) obtained after drying and grinding of the bark of different plants enabled us to determine the antimycobacterial activity of the various extracts. This antimycobacterial activity was done using the microdilution method in liquid medium to determine the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC). The results of the antimycobacterial tests showed that the ethanolic extracts of the barks of our plants were active on the strain of *Mycobacterium smegmatis* ATCC 700084 with MIC ranging from 62.5 to 500 µg / ml and a MBC ranging from 125 to 500 µg / ml. However, the most active plant with a MIC of 62.5 was *Zanthoxylum heitzii* (Rutaceae). The MBC / MIC ratio showed that the ethanolic extracts of the various plants had a bactericidal action on *Mycobacterium smegmatis* ATCC 700084. Phytochemical screening revealed the presence of the secondary metabolites that may be responsible for these activities. The results of this study suggest that the aforementioned plants could be important sources for the development of new antimycobacterial molecules.

## TUBERCULOSIS INFECTION IN MYASTHENIA GRAVIS PATIENTS: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Tuberculosis (TB) infection has been documented to exacerbate myasthenia gravis (MG) patients' condition and precipitate myasthenic crisis. Mechanism by which TB occurs and worsen MG patients condition is not fully understood. Therefore, we conducted a systematic review and meta-analysis to explore this phenomenon by collecting clinical and laboratory data on the complications and mortality of patients with concurrent conditions. Electronic databases and manual search were conducted. Retrieved papers were selected against inclusion and exclusion criteria, underwent full-text review, and data extraction. Quality

assessment was done using The Joana Briggs Institute Critical Appraisal Tool for cohort and case report studies. Data synthesized using open meta-analyst software and narrative review approach. Thirteen studies were included in our review: 10 of them were case reports and three retrospective cohorts. Pooled results of three cohort studies, involving 14150 patients, showed that prevalence of TB in MG patients was 0.6% (95% CI 0 to 1.4%). Two cohort studies reported that there is significantly increased rate of pulmonary TB following MG admission. Of interest, this association was absent for extra-pulmonary TB. Age more than 60 years old and corticosteroids administration prone MG patients to higher risk of developing TB. From 10 case reports of concurrent MG and TB, clinical signs and symptoms identified were ptosis associated with generalized fatigue and weakness of the lower extremities, shortness of breath in five cases with persistent productive cough in three of them, dysphagia and food regurgitation in three cases. MG patients are highly predisposed to the risk of developing pulmonary TB. This risk is enhanced by corticosteroids administration. Since TB infection aggravates the symptoms of MG, patients should be followed up with regular investigations for early diagnosis and management of TB.

## ANTIMYCOBACTERIAL 2-AMINOQUINAZOLIN-4-ONES: SYNTHESIS, BIOLOGICAL AND PHARMACOLOGICAL EVALUATION

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Tuberculosis (TB) is a life-threatening infectious disease caused by *Mycobacterium tuberculosis* (*Mtb*). Globally, TB is a major public health burden with an estimated 10.4 million new cases and 1.8 million deaths reported in 2015. Although TB is curable, the treatment options currently available are beset by numerous shortcomings such as lengthy and complex treatment regimens, drug-drug interactions, drug toxicities, as well as emergence of widespread multi-drug resistance. Therefore, there is an urgent and compelling need to develop new, more effective, safer drugs with novel mechanisms of action, and which are capable of shortening treatment duration. 2-aminoquinazolinones with low µM antimycobacterial activity were identified through phenotypic whole-cell *in vitro* screening. In this study, about 90 analogues were synthesized in an effort to deliver an optimized lead compound. When evaluated against H37Rv *Mtb* strain grown on GASTE-Fe media, several compounds exhibited potent activity ( $\leq 10$  µM) and clear SAR trends. In addition, potent compounds had low cytotoxicity ( $> 25$  µM) and high microsomal metabolic stability. Candidates subjected to *in vivo* pharmacokinetics studies in mice exhibited good plasma exposures, favorable half-lives and were well tolerated. However, the representative compound evaluated for *in vivo* efficacy studies in an acute mouse model lacked activity. Further studies, including use of different growth media, showed that 2-aminoquinazolinones killed *Mtb in vitro* via a glycerol-dependent mechanism of action. These findings correlated well with the results from resistant mutant generation and whole genome sequencing studies, which revealed that all strains resistant to the investigational compound had SNPs in glycerol metabolism genes: *glpD2* and *glpK*.

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## THE ROLE OF SOCIAL MEDIA IN A NATIONAL TUBERCULOSIS DRUG RESISTANCE SURVEY: LESSONS FROM AN ONGOING SURVEY IN GHANA

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There is currently no countrywide representative, reliable data on the prevalence of Anti-Tuberculosis drug resistance in Ghana. The National Tuberculosis Control Programme and other stakeholders in collaboration with the Kumasi Centre for Collaborative Research (KCCR) as a technical agency are conducting a nationwide survey on drug resistance TB for the first time. Sputa from selected diagnostic sites some of which are more than 500km have to be transported to the laboratories of KCCR in Kumasi. We report on preliminary experiences of transporting and tracking samples using social media. The ongoing survey begun in April 2016 and involves 32 TB diagnostic sites selected on the basis of a two-stage cluster randomized sampling design on both anticipated yield and probability proportional to size method. We engaged the services of a conglomerate of privately owned vehicles' union, which are accessible to these sites to transport samples to the laboratories of the KCCR for further processing. We then created a mobile social group platform on Whatsapp (an application for social network) consisting of two representatives from each site as well as other stakeholders and named it 'National TBDRS'. The purpose is to notify the team at KCCR, on the following details about the sample; date and time of dispatch, driver's name, car number, estimated time of arrival, and bus terminal name. The group is administered by the KCCR Receptionist and the Coordinator of the survey at KCCR. A total 680 sputum samples have been transported from 32 survey sites. There have been no major delays (11:44±03:50) and all samples arrive within a three-day window from sputum collection sites. Additionally, 2.5% of samples received have been contaminated indicating a possibility of an efficient transport system. Although early days yet, we are of the opinion that the use of Whatsapp platform as well as privately owned transport system is an innovative technique that could be used in transporting and tracking potentially infectious and contagious samples in national drug resistance surveys for TB in resource poor settings.

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## CLINICAL AND RADIOLOGIC FEATURES OF PNEUMONIA CAUSED BY STAPHYLOCOCCUS AUREUS AND STREPTOCOCCUS PNEUMONIAE

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The introduction of conjugate vaccines against *Haemophilus influenzae* type b and *S. pneumoniae* will cause these bacteria to be less common causes of pneumonia and *Staphylococcus aureus* will become a relatively more common cause of pneumonia. Clinicians are often faced with the decision whether empiric therapy should cover *S. aureus*. Even though textbook descriptions of *S. aureus* pneumonia are often quite distinctive, there are few objective data that describe the clinical and radiologic findings in pneumonia due to *S. aureus* compared to *S. pneumoniae*. We hypothesized that pneumonia due to the two different etiologies would exhibit different characteristics. We conducted population-based surveillance (365 days of the year, 24/7) in rural Gambia, West Africa, for

suspected pneumonia, in all health facilities in Basse Health Demographic Surveillance System (population 179,000) from 12 May 2008 to 31 December 2015 and Fuladu West district (population 97,000) from 12 September 2011 to 31 December 2014. Children aged <5 years were evaluated and investigated according to standardized criteria. Surveillance nurses collected blood cultures, doctors performed lung or pleural aspirations and chest radiographs were taken for all patients. We detected 306 cases of pneumonia proven by culture to be caused by *S. pneumoniae* and 72 cases of *S. aureus*. Comparing cases caused by *S. pneumoniae* vs *S. aureus*, 23 (8%) vs 8 (11%) died; median respiratory rate was 63 (IQR 55-76) vs 60 (52-64); median oxygen saturation was 96% (94-99) vs 97% (94-100), lower chest wall indrawing was observed in 168 (55%) vs 34 (47%); consolidation > half the hemithorax was observed in 60 (20%) vs 3 (4%), pleural effusion > half the hemithorax was observed in 39 (13%) vs 3 (4%), abscess was observed in 1 (<1%) vs 3 (4%), and WHO defined end-point consolidation was present in 214 (70%) vs 21 (29%). There is substantial overlap in the features of staphylococcal and pneumococcal pneumonia. With increasing prevalence of staphylococcal pneumonia in sub-Saharan Africa, and the difficulty in its diagnosis, it will be increasingly difficult for clinicians to prescribe targeted antimicrobial therapy.

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## IMPACT OF THE INTRODUCTION OF PCV7/13 ON ANTIMICROBIAL RESISTANCE IN INVASIVE PNEUMOCOCCAL DISEASE OF LESS THAN 5 YEARS CHILDREN IN RURAL GAMBIA

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The routine use of pneumococcal conjugate vaccines (PCV) has been associated with reductions in the incidence of invasive pneumococcal disease (IPD) associated with antimicrobial resistance. We describe antimicrobial resistance in IPD due to all serotypes and NVTs pre- and post-introduction of PCV in The Gambia, West Africa, in the 0-59 month age group. We identified, serotyped and performed Clinical Laboratory Standard Institute guided-antimicrobial susceptibility testing on invasive pneumococcal isolates obtained during standardised population-based surveillance in the Basse Health & Demographic Surveillance System. PCV7 was introduced in August 2009 and PCV13 in May 2011. We report data from 2009 to the end of 2016. Of the 20,616 patients recruited, 301 pneumococcal isolates were screened against five antimicrobial agents. In the 2-23 month age group, incidence of oxacillin, chloramphenicol, and tetracycline resistant IPD fell from 78-96 and 75-106 per 100,000 person-years in 2009 and 2010 respectively to 14-47 /100,000 person-years in 2016. In the 24-59 month age group, there was a large fall in oxacillin and tetracycline resistant cases from 66-54 /100,000 person-years in 2009 to 5-9 /100,000 person-years in 2016. Resistance fell primarily due to reductions in vaccine-serotypes 5, 6A, 14, 19F and 23F. In contrast, the incidence of resistant NVT cases increased over time, particularly in the 2-23 month age group, primarily due to an increase in the incidence of NVT IPD with antimicrobial resistance from 0-20 /100,000 person-years (which is 0-27% of proportion of total resistance incidence) to 7-41 /100,000 person-years (which is 50-100% of proportion of total resistance incidence) in 2016. Tetracycline resistance generally rise mainly in serotypes 12F, 19F, and 14. The isolates were generally sensitive to erythromycin but were 95-98% resistant to cotrimoxazole throughout the study. Overall, antimicrobial resistant IPD is reducing as emerging resistance in NVT is moderately rising in 2016. Ongoing surveillance is important to determine future trends in resistance as it has both clinical and public health importance in PCV era.

## SELECTING A REFERENCE STANDARD FOR EVALUATING RESPIRATORY RATE DEVICES TO DIAGNOSE SYMPTOMS OF PNEUMONIA IN CHILDREN UNDER 5: LESSONS LEARNED FROM RESOURCE-POOR SETTINGS IN SUB-SAHARAN AFRICA AND ASIA

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Manually counting a child's respiratory rate (RR) for 60 seconds using an acute respiratory infection (ARI) timer is the WHO-recommended method for diagnosing symptoms of pneumonia. Evaluating new respiratory diagnostic aids is challenging due to the absence of an appropriate gold standard. A major difficulty is that automated monitors do not measure chest movements but derive the RR from other variables including carbon dioxide (CO<sub>2</sub>), blood oxygen saturation (SpO<sub>2</sub>) and sound. Other difficulties include measuring RR over a simultaneous time period, inconsistencies in medical expert RR counting, logistical difficulties when using videography to record chest movements for retrospective RR assessment, and poor performance of automated and manual RR counting aids. Here, we review evidence from the Pneumonia Diagnostics Project which trialled four RR devices using three reference standards, across four countries in Sub-Saharan Africa and South-East Asia, to share lessons learned about selecting a suitable reference standard for evaluating the performance of new RR counting aids for resource-poor settings. Three reference standards were used: a video recording of the child's chest movements with RR assessment by a 3-person expert panel; medical experts manually counting RR with a stopwatch, and a continuous respiratory patient monitor with a capnography CO<sub>2</sub> module. Results from the video reference standard show that of 120 readable videos, 97 (81%) had panel agreement ( $\pm 2$  breaths per minute (bpm)). The 97 video references were merged with the contemporaneous (<5 minutes) medical expert RR and the simultaneous capnography RR reference standards. For the medical expert, 34 (38.6%) were within  $\pm 2$  bpm and 48 (54.6%) were within  $\pm 5$  bpm from the video reference. Of the 77 matched pairs for the capnography, 31 (40.3%) were within  $\pm 2$  bpm and 45 (58.4%) were within  $\pm 5$  bpm. These results show that agreement with the video reference for both medical expert counting and capnography is low. For future studies, we recommend using the video expert panel reference standard until it can be proven that a simultaneously medical expert count is equally accurate.

## EVALUATION OF THE LOOP MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) METHOD ON THE EARLY DIAGNOSIS OF PULMONARY TUBERCULOSIS IN CAMEROON

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Pulmonary tuberculosis is a bacterial infection caused by mycobacteria belonging to the *Mycobacterium tuberculosis* complex (Mtb). Tuberculosis is a major public health concern in the world. In 2012, 8.6 million persons developed tuberculosis and 1.3 million deaths. More than 95% of deaths from tuberculosis are recorded in low and middle income countries. In 2011 in Cameroon, 25,126 cases of tuberculosis were recorded. An efficient fight against this disease depends on reliable early diagnosis and proper management. Due to the importance of an early and accurate diagnosis, many efforts have been made to improve the management of tuberculosis. Amongst the techniques of nucleic acid amplification, a new method, LAMP (loop mediated isothermal amplification) used in the diagnosis of tuberculosis and easily applicable in limited resource settings has been recently implemented with sensitivity and specificity rates of 80% and 96% respectively. This is why we decided to undertake a study to assess this LAMP method in the early and rapid diagnosis of pulmonary tuberculosis at the Yaoundé Jamot hospital. Our general objective was to

assess the LAMP method (Loop Mediated Isothermal Amplification) for the rapid diagnosis of tuberculosis. We conducted a cross-sectional and prospective study which took place from 14th April to 5th June 2014 at the Yaoundé Jamot Hospital and *Centre Pasteur du Cameroun*. On the basis of an informed consent, we recruited 539 suspected TB (Tuberculosis) patients to assess the lamp method. We compared the LAMP method with microscopy using culture as the reference standard. Results obtained showed that the sensitivity and specificity of LAMP were 83.0% and 96.2% respectively. The sensitivity and specificity of microscopy were 53.9% and 99.1% respectively. We concluded that the sensitivity of LAMP was higher than that of microscopy. In other words, when a patient is sick, it will be easier to diagnose using the Lamp method, than microscopy.

## DIAGNOSTIC, MANAGEMENT AND REFERRAL PATTERNS AMONG PRIVATE HEALTH CARE PROVIDERS FOR PEDIATRIC RESPIRATORY ILLNESSES IN SOUTH INDIA

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Acute Respiratory tract Infections (ARI) cause approximately 30% of all pediatric deaths and is the leading cause of morbidity in developing countries. The Integrated Management of Newborn and Childhood Illness (IMNCI) focuses on reducing pediatric morbidity and mortality. Understanding diagnosis, management and referral patterns among health care providers promotes effective use of health care resources and understand antibiotic usage. A survey identified private health care providers in one block of Vellore district of whom a stratified subset representing allopathic, non-allopathic providing primary and secondary level care (65/312) were interviewed. Of those surveyed, 38/52 (73%) had diagnosed pediatric Acute Lower Respiratory Tract Infection (ALRI) in the last year. A majority 45/53 (85%) preferred supportive care for Upper Respiratory Infection (URI) while 21/53 (40%) preferred antibiotics. For severe ALRI 19/47 (39%) preferred chest X-Ray, 18/47 (37%) blood tests with 83% (44) choosing to treat with oral antibiotics along with bronchodilators (36, 68%). Penicillins were commonest for URI (18, 32%) while a combination of antibiotics was preferred for non-severe ALRI (10, 20%) and severe-ALRI (15, 27%). High respiratory rate (45, 83%), grunting (34, 63%), X-Ray findings (34, 63%), seizures and cyanosis were commonest causes for referral to higher centers and most (41/47, 87%) chose to refer children with severe-ALRI. Preferred referral centers were Government Vellore Medical College (30, 56%) and Christian Medical College Hospital (26, 48%). While most had diagnosed ALRI among children, most preferred supportive therapy for URI with some choosing to give antibiotics. While most preferred supportive therapy for URIs some added antibiotics, a quarter did not provide nebulization for severe and very-severe ALRI. Half the providers preferred not observe or admit children with ALRI but provided out-patient antibiotic therapy, which may have been due to their primary care setting. Understanding the basis of antibiotic therapies followed by private health care providers will improve tackling antibiotic resistance.

## APTAMERS SPECIFIC FOR PYRAZINOIC ACID AS A NEW TOOL TO DETERMINE PYRAZINAMIDE RESISTANCE DIRECTLY FROM SPUTUM SAMPLES CULTURES

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Tuberculosis (TB) is still an important infectious disease that affects low-income countries. The most important problem related to TB is the increase of multidrug-resistant and extensively drug-resistant

tuberculosis. Susceptibility tests exist for Rifampin and Isoniazid, including microbiological and molecular methods. However, for pyrazinamide (PZA) there are not any standard test to evaluate susceptibility. The detection of PZA resistance shows several disadvantages, i.e. necessity of an acid pH or the variable correlation index between PZA resistance and pncA mutation. Previous studies in our laboratory have demonstrated that the detection of pyrazinoic acid (POA) can be used as a biomarker to predict PZA resistance. PZA susceptibility of *Mycobacterium tuberculosis* strains can differentiate by the production of POA. Sputum samples cultures in presence of PZA can produce POA in the supernatant. In order to detect POA in culture, DNA aptamers were identified through SELEX. Each SELEX cycle included an amplification, a transcription, and a retro-transcription step. A library of 20N random aptamers was used in the process. A complex of POA linked to sepharose beads was target for positive rounds of SELEX. Sepharose beads were target for negative rounds of SELEX. To evaluate the enrichment, a Nuclease 1 assay was performed using dsDNA of each SELEX round. Libraries of different SELEX rounds were sequenced in Illumina MiSeq Platform. Sequences present in the negative round were discarded from all positive round sequence populations before to evaluate the abundance and enrichment across the SELEX process to identify the aptamers candidate. At least four sequences were identified. Currently, the binding capacity and specificity of the selected aptamers are being evaluated by ELISA and Cyclic voltammetry. This is the first time to use aptamers to detect POA. The use of aptamers can let to develop fast and specific susceptibility tests to determine PZA resistance directly from primary cultures of clinical samples, contributing in this way to increase the adherence treatment by a fast and correct susceptibility drug profile for the patients.

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### BACTERIAL PROFILE AND DRUG RESISTANT PATTERNS IN PNEUMONIA SUSPECTED HIV PATIENTS AT ART CLINICS IN NORTHERN ETHIOPIA

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HIV infected patients are susceptible to infectious agents. Pneumonia is one among the most frequently encountered diseases, where bacterial pneumonia are believed to be one of the most common causes of co-morbidity and mortality in HIV patients. In Ethiopia, there is scarcity of data which shows real burden of bacterial pneumonia and their antimicrobial susceptibility and associated risk factors. A cross-sectional study design was conducted from August- December, 2016 at ART clinics in Northern Ethiopia. A total of 252 HIV positive individuals who presented with signs and symptoms of pneumonia were consecutively included. Structured questionnaire was used to collect socio-demographic, clinical and associated risk factors data. Sputum specimens were collected, transported, and processed using standard operating procedures. The data was analyzed by descriptive statistics, bivariate and multivariate logistic regression. P-value < 0.05 and corresponding 95% confidence interval were considered for statistically significance. Out of the 252 samples from HIV patients, significant bacterial pneumonia was observed in 110 (43.7%) samples. The predominant bacterial isolates were *K. pneumoniae*, 23.6 %, *S. pneumoniae*, 15.5 % and *E.coli*, 14.5 %. Age, number of CD4+ cell count, alcohol consumption, and WHO stage of HIV infection, were potential risk factors to have bacterial pneumonia. Bacterial isolates showed resistance for penicillin, 9 (81.8%), followed by co-trimoxazole, 40 (39.8%) and tetracycline, 26 (24.5%). In addition to this, MDR was observed among 17.9% of the isolates. Significant number of bacterial pneumonia and high resistance for co-trimoxazole was observed in this study. Therefore, identification of the etiologic agent and performing the antimicrobial susceptibility pattern should be done to select the appropriate antimicrobial agent for the management of bacterial pneumonia in HIV patients.

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### MULTI-DRUG RESISTANT TUBERCULOSIS TREATMENT OUTCOMES IN A HIGH HUMAN IMMUNODEFICIENCY VIRUS (HIV) PREVALENCE PROGRAMMATIC COHORT IN UGANDA

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Multidrug-resistant (MDR) tuberculosis (TB) is a threat to TB control and a public health concern. We present treatment outcomes of the first patients treated for MDR TB at Mbarara regional referral hospital, Uganda. MDR TB suspects are referred from peripheral health facilities where X-pertMTB/RIF is performed. If positive with resistance to rifampicin, patient is immediately started on a standardized MDR TB treatment regimen. The sample is then transported to the national TB reference laboratory where drug resistant testing, TB microscopy and culture are done. Data were collected retrospectively from the MDR TB register and hospital files. Only patients' data who had completed the treatment period were analysed. A total of 53 MDR TB patients were initiated on MDR TB treatment from the 14th January 2013 to 11th November 2015. Majority were male 39 (73.6%) and HIV co-infected, 29 (55%) with a CD4 cell count of 240 IQR [63-551] and median was of 35 years [30-40]. Out of these, 45 (89%) were analysed. Treatment success was registered in 35 (77.8%) patients, 9 (20%) died and only 1(2.2%) was a treatment failure. There was no association with HIV status  $p=0.37$ . Median times to culture conversion and death were 104 [62-112], and 122 IQR [21-82] days respectively. Out of 38 available patient files, 36 (95%) recorded 115 drug related adverse events of which the majority were gastrointestinal symptoms 31 (27%). Others were; hearing impairment 17(14.8%), other central nervous system toxicities; 21(18.3), dysaesthesia and arthralgia; 12 (10.4%) and 10 (8.7%) respectively, hypothyroidism and body itching; 6 (5.2%) each, gynaecomastia; 5(4.3%), loss of libido and renal impairment, 2(1.7%) each, and others 3 (2.6) were TB IRIS, weight loss and deteriorating condition leading to death in a patient with severe malnutrition. There was very good treatment success with only one failure despite the several drug toxicities and long treatment duration in the context of high HIV prevalence and this was enhanced by the short conversion time (4months). Treatment experience and outcomes could be improved by use of less toxic TB drugs or shorter MDR TB regimens.

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### 13-VALENT PCV AGAINST INVASIVE

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In 2011 a 13-valent pneumococcal conjugate vaccine (PCV13) was introduced in The Gambia to replace the 7-valent vaccine (PCV7). The effectiveness of PCV7 against invasive pneumococcal disease (IPD) is well established. However, PCV13 was licensed based on the non-inferiority of immunological correlates of protection compared with PCV7, so the effectiveness of PCV13 against IPD still needs to be confirmed, particularly in countries with a high disease burden. We conducted a population-based case control study to estimate the effectiveness of PCV13 against IPD. Cases of IPD (i.e. *Streptococcus pneumoniae* isolated from the blood or cerebrospinal fluid), were recruited from two hospitals in the Upper River Region and Central River Region of The Gambia, and four community controls, matched on date of birth and season, were identified for each case. We used conditional logistic regression to estimate the effectiveness (1-adjusted odds ratio) of PCV13. We recruited 159 cases (28 vaccine type) and 628 controls. The median age of cases and controls was 58 weeks. For three compared to zero doses of PCV13, effectiveness was 49% (95% CI: -36%, 81%) against all IPD and 64% (95% CI: -130%, 94%) against

vaccine-type IPD. Current results suggest that PCV13 is effective against IPD in The Gambia, although study power is limited by high vaccine coverage. Enrolment is ongoing.

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**ENTAMOEBA SPP IN BANGLADESH**

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Diarrheal diseases are a leading cause of mortality and morbidity in low and middle income countries. Two members of the *Entamoeba* genus (*E. histolytica* and *E. moshkovskii*) are known to cause diarrhea. However other morphologically similar non-pathogenic members of the genus also infect humans. Diagnosis by microscopy of fecal specimens therefore can lead to false-positive results. To determine the prevalence of four of the *Entamoeba* species known to be present in the Bangladesh population (*E. histolytica*, *E. dispar*, *E. moshkovskii* and *E. bangladeshi*) a new *Entamoeba* species specific real time PCR assay was developed. Fecal samples (7134) were collected from Bangladesh children aged between 1 and 11 years were screened for the presence of *Entamoeba* cysts and trophozoites by direct microscopy and fecal culture. DNA was extracted from microscopy positive samples (334; 4.7%) and the infecting *Entamoeba* species was identified in 180 cases (54%). *E. dispar* was present in 107 of the 180 samples (60%); *E. histolytica* in 59 (33%), *E. bangladeshi* in 27 (15%). *E. moshkovskii* infected only one child in this study. Co-infections result in a total higher than 180 and as might be expected these most commonly involved the *Entamoeba* species with the highest prevalence (*E. histolytica* and *E. dispar*) although co-infections with *E. bangladeshi* were also observed. In conclusion: *E. bangladeshi* and *E. dispar* are both common in our study population and can significantly inflate the numbers in microscopy based diagnosis of amebiasis. The frequency of *E. dispar*, *E. bangladeshi* and *E. histolytica* co-infections support the hypothesis that cross-species immune protection does not occur in the *Entamoeba* genus. Interestingly, *E. moshkovskii* highly prevalent in previous studies was infrequent in the current study, suggesting that in the Bangladesh population outbreaks with this species may only occur periodically. Additional surveillance studies will be required to understand the epidemiology of *E. moshkovskii* associated diarrhea.

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**ENTAMOEBA BANGLADESHI IN SOUTH AFRICA**

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*Entamoeba bangladeshi* is an anaerobic amoebozoan. It was firstly described in Bangladesh. *Entamoeba* species have been identified in different endemic regions of the world. In a recent study in S. Africa stools positive for amoeboid organisms by microscopy were now commonly PCR negative for the *E. histolytica* pathogen. This led to the hypothesis that other species of *Entamoeba* were now more frequent in the South African population. Stool samples were collected from November 2013 to June 2015 (rural=227) and (urban=257) from diarrheal and non-diarrheal patients. DNA was extracted and a highly sensitive qPCR assay for the and amplicon sequencing were used to detect and characterize infecting *Entamoeba*. *Prevotella copri* and *Enterobacteriaceae* were also detected by qPCR. The broad range *Entamoeba* probe however identified only 129 of the 484 as members of the *Entamoeba* genus (26.6%). Of these 49 were not able to be initially identified at the species level by the qPCR assay. The *Entamoeba* identified were *E. dispar* (8%), *E. histolytica* (6.4%), *E. bangladeshi* (4.5%) (co-infections result in a number higher than 26.6%). The amplicons of the 34 of the 49 unassigned *Entamoeba* were purified

and sequenced. Of these 10 were *E. histolytica* (adjusted prevalence 8.5%) and one *E. bangladeshi* (adjusted prevalence 4.75%) the remainder proved to be derived from *E. hartmanni* (2.6%), which was not discriminated against by the *Entamoeba* genus probe. *E. moshkovskii* was not identified in this population. A high parasite burden and expansion of the *P. copri* level was associated with diarrhea due to *E. histolytica*. In conclusion, *E. bangladeshi*, first discovered in an urban cohort in Bangladesh, was identified in urban and rural S. Africa. This is the first description of *E. bangladeshi* outside of Bangladesh. We were also able to observe changes in the host microbiome and the parasite burden associated with *E. histolytica* infections in S. African diarrhea cases versus infected asymptomatic controls but not with *E. bangladeshi* or the non-pathogenic *E. dispar*.

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**IMPACT OF INTESTINAL PROTOZOA INFECTIONS ON CYTOKINE PROFILES OF INDIVIDUALS INFECTED BY FILARIAL AND/OR INTESTINAL HELMINTHS IN DIFFERENT AREAS OF GABON**

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Filarial and Intestinal Protozoa Infections are prevalent in Gabon, an endemic area of Malaria. Thus, coinfection with these infections is not an exception. Complex Immunological interactions are implicated on the outcome of many infections. Therefore, this study estimated the level of Proinflammatory (IL-6, TNF-alpha) and down regulatory (IL-10) cytokines. Patients were included between January 2013 and June 2016, in Urban and Rural areas through a partnership between the Red Cross and Department of parasitology-Mycology of Université des Sciences de la Santé of Libreville, the capital City. Filaria and IPI were detected by using direct microscopy examination and Merthiolate Iode Formaldehyde concentration technics. Coproculture was used for the identification of helminths larvae. Cytokines were detected by flow cytometry. A total of 154 patients were included. Prevalence of Filarial, Protozoa, Helminthes+Protozoa, Filarial+Protozoa, Filarial+Helminthes infections were 20.8%, 25.3%, 20.8%, 7.1%, 3.2% respectively. The frequency of uninfected ones was 22.7%. Significantly lower levels of IL-6 were found in individuals infected by protozoa only compared to uninfected group (P=0,0024). Also, lower IL-10 levels were detected in group of individuals coinfectes by IPI and Filarial or Helminthes (P=0,0072). Whereas, TNF-alpha levels were higher in the group of individuals coinfectes by Filarial+Helminthes and Protozoa+Helminthes. TNF-alpha levels were lower in the group with Filarial+Protozoa. The ratio IL10/IL6 was significantly elevated in the group of individuals infected by protozoa and lower in the Filarial+Helminthes groups. Our results suggest that IPI reduce proinflammatory cytokines which play an important role in the protection against several infection such acute intracellular pathogen like *Plasmodium*. Coinfection with IPI+Helminthes also reduces down regulatory cytokine levels implicated in the protection during inflammation.>

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**EVALUATION OF A NEW RAPID TEST FOR AMOEBIASIS, THE E. HISTOLYTICA QUIK CHEK™**

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*Entamoeba histolytica* and *E. dispar* are intestinal parasites that infect approximately half a billion people worldwide annually. Of the huge number of persons infected, most are infected with *E. dispar*, which has not been associated with disease. *E. histolytica* is well recognized

as a pathogenic amoeba, associated with intestinal and extraintestinal infections. *E. histolytica* is responsible for 100 million cases of amoebiasis annually, causing diarrhea, dysentery, and colitis. It is necessary to distinguish between the two species, as inaccurate diagnosis may result in unwarranted and unnecessary treatment. The most common method used to diagnose amoebiasis has been wet mount microscopy, which has poor sensitivity and specificity. Trophozoites and cysts are not easily identified in a single fecal specimen, and only rarely can *E. dispar* and *E. histolytica* be visually distinguished using light microscopy. The *E. HISTOLYTICA QUIK CHEK™* test is a rapid membrane enzyme immunoassay for the qualitative detection of pathogenic *E. histolytica* in human stools. This test offers an alternative to labor-intensive microscopy and PCR, and provides results in less than 30 minutes with simple and clear interpretation. A total of 851 human fecal samples including 755 fresh and 96 frozen specimens from male and female subjects aged from less than 1 year to 100 years were evaluated with the *E. HISTOLYTICA QUIK CHEK™* test at three geographically distinct clinical sites, including two US sites and one endemic site at Bangladesh. A Composite Reference Method (CRM), including microscopy, Luminex xTAG® GASTROINTESTINAL PATHOGEN PANEL test, and PCR with sequencing for the identification of *E. histolytica*, was used for analysis of the performance of the *E. HISTOLYTICA QUIK CHEK™* test. The test showed 91.4% sensitivity and 100% specificity, with positive and negative predictive values of 100% and 99.6%, respectively. The overall correlation with the CRM is 99.6%. In conclusion, the *E. HISTOLYTICA QUIK CHEK™* test, the only rapid device specific for *E. histolytica*, accurately detects this protozoan pathogen in human fecal specimens.

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### EVALUATION OF A DIAGNOSTIC SCREENING TEST, THE TRI-COMBO PARASITE SCREEN, FOR DETECTION OF ENTAMOEBA.HISTOLYTICA, GIARDIA, AND CRYPTOSPORIDIUM PARASITES IN HUMAN FECAL SPECIMENS

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Diarrheal diseases account for 1 in 9 child deaths worldwide, making diarrhea the second leading cause of death among children under the age of 5. The three most common causes of protozoan diarrheal infections are *Giardia spp.*, *Cryptosporidium spp.*, and *Entamoeba histolytica*. The traditional screening method, microscopic ova and parasite (O&P) examination of stool, is laborious, suffers from poor sensitivity and specificity, and requires expertly trained personnel to interpret results. In addition, it is very difficult to distinguish between pathogenic and non-pathogenic species of *Entamoeba* using microscopy. The TECHLAB® TRI-COMBO PARASITE SCREEN test, an enzyme immunoassay, qualitatively detects *Giardia spp.*, *Cryptosporidium spp.*, and *Entamoeba histolytica* rapidly from a single stool sample in a single reaction. This test is designed as the primary screening method for fecal specimens submitted for parasite analysis. The format allows the rapid screening of large numbers of clinical specimens. A panel of 850 human fecal specimens including 754 fresh and 96 frozen specimens from male and female subjects aged from less than 1 year to 100 years was tested with the TRI-COMBO test at two US sites and one endemic site at Bangladesh. The performance of the TRI-COMBO PARASITE SCREEN test was compared to a Composite Reference Method (CRM) which included microscopy, Luminex xTAG® GASTROINTESTINAL PATHOGEN PANEL test, and PCR with sequencing for the detection of *Giardia spp.*, *Cryptosporidium spp.*, and *E. histolytica*. The TRI-COMBO test exhibited a sensitivity of 91.6%, a specificity of 98.6%, a predictive positive value of 91.6%, a predictive negative value of 98.6%, and an overall correlation of 97.6% with the CRM. The results indicate that the TRI-COMBO test is a highly sensitive and specific ELISA that can be used as a primary screening assay to eliminate negative specimens

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and identify specimens that require additional testing for *Giardia*, *Cryptosporidium*, and *E. histolytica*. The TRI-COMBO PARASITE SCREEN test makes parasite screening simple to perform, more cost efficient, and accurate.

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### THE SEARCH FOR A SMALL MOLECULE THERAPEUTIC FOR THE TREATMENT OF GRANULOMATOUS AMEBIC ENCEPHALITIS

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*Balamuthia mandrillaris* is a pathogenic free-living amoeba found in soil that exists in two forms. The trophozoite form is proliferative while the non-proliferative cyst form is resistant to harsh physical and chemical conditions. In rare instances, these protists cause granulomatous amebic encephalitis (GAE), a central nervous system disease that is almost always fatal. Among the few survivors of GAE, a multi-drug regimen including miltefosine, an anti-leishmanial agent and antifungals such as fluconazole, albendazole and flucytosine have been reported. However, studies evaluating the susceptibility of *B. mandrillaris* to these compounds and other potential therapeutics are limited. Here, we examined the *in vitro* efficacy of 1,134 FDA approved compounds for amoebicidal activity at high, moderate and low micromolar concentrations. Viability was determined using CellTiter-Glo reagent post-incubation with drug. Our most promising candidates include an antidepressant and an anticancer drug that achieve EC50s (the concentrations at which each drug gives half the maximal response) at moderate micromolar concentrations. Current work involves validation of amoebicidal activity and determining the interaction between these compounds and amoeba forms. Ultimately, we seek amoebicidal agents that are effective against both cyst and trophozoite forms to serve as pre-clinical leads for further medicinal chemistry optimizations. Additional future work includes the determination of cyst and trophozoite protein profiles to identify distinct surface proteins that interact with human cells. Such proteins may serve as candidate targets for novel treatments of GAE.

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### IMPACT OF GASTROINTESTINAL PARASITES ON GROWTH USING QUANTITATIVE PCR IN A LONGITUDINAL ECUADORIAN BIRTH COHORT

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Gastrointestinal (GI) parasites are a public health concern with over 600 million school-age children and over 270 million pre-school children affected worldwide. These fecal-oral transmitted parasites may influence childhood growth. Previous studies investigating GI parasites and growth largely use poorly sensitive microscopy-based assays with few epidemiologic data on GI parasitic infections in pre-school children from rural Ecuador. To investigate the effects of GI parasites on growth, longitudinal data from a birth cohort in rural Ecuador were analyzed, correlating impact of GI parasites on anthropometric measures. Stool from a random sample of 400 children was collected at 1, 2, 3 and 5 years and analyzed using high throughput, multi-parallel qPCR assay for helminths (*Ascaris l.*, *Ancylostoma d.*, *Necator a.*, *Strongyloides s.*, and *Trichuris t.*) and protozoa (*Cryptosporidium spp.*, *Entamoeba h.*, and *Giardia l.*). At these time points, anthropometric measures (height, weight, head circumference) were collected. Overall prevalence of GI parasites peaked at 3 yrs. ( $p = 0.01$ ). Prevalence for *Giardia* (31.5%, 45.6%, 52.1%, and 43.3%) and *Ascaris* (6.8%, 12.9%, 16.4%, and 14.4%) was greatest at 1, 2, 3 and 5 yrs., respectively. *Trichuris* prevalence increased >200%

with peak burden by 2 yrs. ( $p = 0.013$ ). *Ascaris* burden was greater at 1 to 3 yrs. (0.72 fg/ul to 2.3 fg/ul,  $p = 0.001$ ), peaking at 2 yrs. with more moderate-to-heavy burden ( $p = 0.01$ ). *Giardia* burden increased with age and was greatest at 5 yrs. ( $p = 0.0001$ ). Infected children subsequently re-infected with higher burden of *Ascaris* and *Giardia* ( $p < 0.05$ ). GI parasites were associated with low anthropometric measures compared to WHO growth curves. At 5 yrs., infected children had lower height ( $p = 0.003$ ) and at 2 and 3 yrs., children with polyparasitism had lower height ( $p = 0.02$ ). At 3 and 5 yrs., infection with both helminth and protozoa was associated with reduced height ( $p = 0.006$ ). Our multi-parallel qPCR assay provided accurate epidemiologic data on GI parasitic infections in rural Ecuadorian pre-school children further, that GI parasitic infections may affect growth in early childhood.

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### SEROLOGICAL DIAGNOSIS OF PARAGONIMIASIS USING A RECOMBINANT *PARAGONIMUS KELLICOTTI* ANTIGEN

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Paragonimiasis (lung fluke infection) affects about 23 million people in Asia, Africa and the Americas. *P. kellycotti* is enzootic in North America and it occasionally causes human disease. While the infection is easily cured with praziquantel, diagnosis is difficult and often delayed. Paragonimiasis is commonly mistaken for community acquired pneumonia, tuberculosis or lung cancer. We have previously reported results from a study that used proteomics to identify *P. kellycotti* adult worm antigens with diagnostic potential such as a cysteine protease (Pkc-6) that were affinity purified with serum antibodies from infected patients. Full-length Pkc-6 was expressed in *E. coli* and purified and its diagnostic value was compared to crude total *P. kellycotti* extract. A IgG4 Western blot detected antibodies to Pkc-6 in 27 of 27 serum samples from subjects with either proven *P. kellycotti* or *P. westermani* (Philippines) infection. The recombinant antigen did not cross-react with sera of 30 subjects with other trematode or cestode infections or with sera from healthy U.S. volunteers. A Western blot with crude worm extract had similar results but higher background than the Pkc-6 assay. We evaluated the Pkc-6 assay with 161 serum samples from 3 villages in South West Cameroon that are endemic for paragonimiasis. We detected IgG4 antibodies to Pkc-6 in 12.4% of these samples, while microscopic detection of eggs in sputum or stool identified only 2 positives (1.2%). Both egg-positive subjects were also strongly positive by Western blot. Thus, the Pkc-6 Western blot appears to be sensitive diagnostic tool for *Paragonimus* infections in Africa, in Asia, and in the Americas. Additional studies are needed to further define the value of this new tool for clinical diagnosis and to better understand the epidemiology of paragonimiasis in different endemic settings.

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### CLINICAL STAGES, EPIDEMIOLOGICAL AND LABORATORY CHARACTERISTICS OF FASCIOLIASIS IN CHILDREN POPULATION AT ANTA COMMUNITY IN CUSCO, PERU

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*Fasciola hepatica* is a hyperendemic zoonosis in the Andes of Peru and Bolivia. The clinical course of the infection in the community has not been well described. We conducted a cross-sectional analysis of information from a fascioliasis community cohort of children 3-16 years old from Anta

province in Cusco. The aim was to define the clinical differences between fascioliasis phases in a community. Demographic and clinical information was collected from parents through household surveys. Each participant provided 3 stool samples that were tested by rapid sedimentation and Kato Katz tests and 1 blood sample that was tested for *F. hepatica* antibodies (ELISA), complete blood counts, and transaminases. Fascioliasis phases were defined as acute (stool (-), ELISA (+), and eosinophilia or elevated AST/ALT), subacute (stool (+), ELISA (+), and eosinophilia or elevated AST/ALT), chronic (stool (+), any ELISA result, and any eosinophils and AST/ALT result excluding subacute phase), and non-classified (ELISA (+) only). Chronic phases were categorized as C-ELISA (+) and C-ELISA (-). We compared clinical characteristics of the phases excluding the unclassified cases. The overall prevalence of *Fasciola* was 10% (250/2497). Four cases lacking blood results were excluded, 77 (31.3%) were non-classified, 18 (7.3%) acute, 26 (10.6%) subacute, 41 (16.7%) were C-ELISA (-) and 84 (34.1%) C-ELISA (+). C-ELISA (+) children were older than subacute children (11.2 ± 3.1 vs. 9.0 ± 3.0 years,  $p=0.001$ ) and were also more likely to present cough ( $p=0.007$ ), history of treatment for anemia ( $p=0.027$ ) or malnutrition ( $p=0.004$ ). Children with C-ELISA (+) were more likely than children with C-ELISA (-) to have fever ( $p = 0.022$ ), cough ( $p=0.031$ ), diarrhea ( $p=0.01$ ) or infection with *H. nana* ( $p=0.018$ ). No significant differences were found between acute vs. subacute phases, possibly due to limited statistical power. The sex and socioeconomic status distribution was similar between phases. Chronic disease was the most frequent phase in this community. Few clinical differences were significant between children in different phases of the infection which may hinder diagnosis.

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### REGULATION OF GENE TRANSCRIPTION BY JNK AND P38 MAPK SIGNALING PATHWAYS IN *SCHISTOSOMA MANSONI*

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The identification and characterization of mechanisms and molecules involved in cell signaling are essential to the understanding of the *Schistosoma mansoni* parasite's biology. The *S. mansoni* genome encodes 252 eukaryotic protein kinases (ePKs), however, only 32 have some experimental functional evidence. Our group has demonstrated through functional studies that MAPKs are involved in parasite development, reproduction and/or survival and may therefore be considered potential targets for the development of new drugs. Due to the data scarcity on the *S. mansoni* ePKs function, this work has the main motivation to contribute to their experimental characterization by correlating expression data with *S. mansoni* development and reproduction. For this, SmJNK and Smp38 ePKs were knockdown in schistosomula by RNA interference, including three biological replicates. After two days, extraction of total RNA and evaluation of transcript levels by RTqPCR were performed. For all genes selected, we observed approximately 75% reduction on transcript levels. The extracted RNA was used to construct paired-end libraries sequenced on Illumina HiSeq 2500 platform. The reads obtained were mapped to the latest version of the *S. mansoni* reference genome and differentially expressed genes (DEGs) were identified by comparing each MAPK knockdown relative to untreated parasites. We identified 390 DEGs in SmJNK knockdown schistosomula and 810 DEGs in Smp38 knockdown schistosomula. UniProt, KEGG and GO databases were used to elucidate the functional roles of DEGs. This work allowed a better understanding of SmJNK and Smp38 signaling pathways, elucidating the functional roles of these MAPKs and the targets they regulate.

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### A SYSTEMS BIOLOGY APPROACH LINKING GENETIC, EPIGENETIC, CYTOPLASMIC AND HOLOBIONT INHERITANCE TO UNDERSTAND RAPID ADAPTATION OF PARASITIC FLATWORMS (*SCHISTOSOMA SP.*)

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The GxE concept, meaning that genotype x environment interactions bring about the phenotype is widely used to describe adaptation phenomena. We propose to extend the initial notion of the GxE concept, and to replace G by "Inheritance system". This system is composed of several elements: the genotype, the epigenotype, cytoplasmic components but also microorganisms. They interact as an inheritance system with the environment, leading to the development of a phenotype. The elements of this system can be defined using their molecular composition, for instance the DNA as genetic information carrier and then the bearers of epigenetic information such as the chromatin marking system. However, it is not the system itself that generates the phenotype but the developmental process that produces over time and in interaction with the environment a phenotypic trait. In each of these processes, genetic, epigenetic, cytoplasmic (e.g. mitochondria) and holobiont diversity can change and result in ephemeral, fluctuating or stable, i.e. heritable phenotypic variations that are important for adaptation. To understand how parasitic worms can sometimes rapidly adapt to changing environments the parasitologist must 'simply' define (i) the units or elements of interactions i.e. the boundaries of these elements, and (ii) the types of interactions that interrelates them. The major consequence of our systems approach to inheritance is that if one wishes to understand the heritability of a trait, all elements of the inheritance system must be analyzed comprehensively using a range of different genotypes, epigenotypes, haplotypes and holotypes of the Inheritance System. However, we believe it is legitimate to exclude some of the elements from the experiment as long as one does not exclude them from the conclusions and generalizations, e.g. the finding that genetic variants have a strong association with a phenotype does not exclude similar or even stronger epiallelic associations and vice-versa. Our approach will be illustrated using the human parasite *Schistosoma sp.* a tropical platyhelminth that recently invaded the European continent via Corsica (France).

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### KNOCKDOWN OF A *SCHISTOSOMA MANSONI* TRPML CHANNEL (SMTRPML) DISRUPTS ADULT WORM TEGUMENTAL STRUCTURE

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Praziquantel is currently the only drug available for treatment and control of schistosomiasis, a disease affecting over 200 million people. There is thus an urgent need for new antischistosomal. Several current anthelmintic drugs target ion channels of the parasite neuromuscular system. TRP channels are a large superfamily of non-selective cation channels that display an extraordinary diversity of functions and activation mechanisms. They play important roles in sensory signaling and other functions. Members of the TRPML (mucolipin) channel family are non-selective, predominantly intracellular channels that play key roles in endolysosomal physiology, including vesicular trafficking and biogenesis. They also function in nutrient acquisition and in regulating and maintaining neuronal development and integrity (in humans, mutations in one of the three TRPML subtypes causes mucopolipidosis type IV, a childhood neurodegenerative disorder). *Schistosoma mansoni* has a single predicted TRPML gene (SmTRPML; Smp\_198800). Knockdown of SmTRPML using RNAi in adult worms results in large, bubble-like sub-tegumental vacuoles

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along with tegumental blebbing, effects similar to those found for TRPML loss in other organisms, and consistent with a role for SmTRPML in regulating the parasite endolysosomal system. MLSA1, a potent and selective cell-permeable agonist of TRPML channels, evokes an increase in adult worm motility, perhaps indicating a potential role for these channels in neuromuscular activity as well. These results indicate that SmTRPML may be a useful target for disrupting parasite endolysosomal regulation and nutrient acquisition.

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### QUANTITATIVE PROTEOMIC ANALYSIS OF THE HUMAN URINE FOR NOVEL PROTEIN BIOMARKERS FOR SCHISTOSOMIASIS AND ITS ASSOCIATED BLADDER PATHOLOGIES USING LABEL FREE MASS SPECTROMETRY

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The failure to elicit an adequate immune response to adult *Schistosoma haematobium* is characterized by continuing inflammatory challenges to the exposed population, and it has been established as a possible risk factor in the aetiology of bladder cancer. Improved diagnosis of schistosomiasis and its associated pathology is possible through the use of a mass spectrometry approach for biomarker discovery among the infected population, which will influence early detection of the disease and its subtle morbidity. A high-throughput proteomic approach was used to analyse human urine samples for 49 volunteers from Eggua, a schistosomiasis endemic community in South-West, Nigeria. The individuals were previously screened for *Schistosoma haematobium* and structural bladder pathologies via microscopy and ultrasonography respectively. Samples were categorised into schistosomiasis, schistosomiasis with bladder pathology, bladder pathology and No pathology and schistosomiasis. These samples were analysed for potential protein biomarkers. A total of 1306 proteins and 8752 unique peptides were observed in this study (FDR = 0.01). Fifty-four human proteins were found to be potential biomarkers for schistosomiasis and bladder pathologies due to schistosomiasis by label free quantification. 36 parasite derived potential biomarkers were also identified, which include some existing putative schistosomiasis biomarkers which had been previously reported. Some of these proteins include Elongation factor 1 alpha, phosphopyruvate hydratase, histone H4 and heat shock proteins (HSP 60, HSP 70). These findings provide an detailed analysis of schistosoma and human host protein biomarkers for diagnosis of chronic schistosomiasis due to *Schistosoma haematobium* and its pathogenesis.

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### *SCHISTOSOMA MANSONI* EXOSOMES AS MODULATORS OF THE HOST IMMUNE SYSTEM

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Schistosomiasis is a parasitic disease that currently affects over 240 million people worldwide. In humans, schistosomiasis develops from infection with one of five *Schistosoma* species, including *S. mansoni*. As is the case for most parasitic helminths, the host response is generally not sufficient on its own to clear the infections with *Schistosoma* species, due, at least in part, to the ability of the parasite to evade the host immune system. Although many evasion mechanisms employed by *Schistosoma* species have been described, the potential contribution of parasite-derived extracellular vesicles (EVs) in immune evasion remains unexplored. EVs are known to transfer molecules from one cell to another via membrane vesicle trafficking, thus explaining the broad array of functional activities

attributable to them. Importantly, there is increasing evidence of the release of EVs in parasitic diseases, with roles both in parasite-parasite inter-communication as well as in parasite-host interactions. Here we describe a protocol for the isolation and purification of *Schistosoma mansoni*-derived exosomes-like vesicles and show that the vesicles elicit a response in bone marrow-derived macrophages (BMDMs) and alter the BMDMs' response to LPS. We also show that *S. mansoni*-derived exosomes elicit a humoral immune response in mice.

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### IDENTIFICATION OF A CD193+ SUBPOPULATION OF B CELLS IN PRE-ADOLESCENT CHILDREN WITH SCHISTOSOMIASIS

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CD193 is a chemokine receptor expressed on inflammatory cells and has been associated with the migration and activation of eosinophils and T helper 2 (Th2) cells at sites of allergic diseases and helminth infections. In the spleen follicles, B cell CD23-bound IgE is thought to augment protective Th2 responses through induction of CD193+ T cells. However, to date, CD193 expression on B cells has not been reported. We sought to evaluate whether B cells express CD193 in human schistosomiasis. Seventy five children aged 10-12 years old were enrolled from five primary schools located within 5 kilometers of the shores of Lake Victoria in western Kenya. *S. mansoni* infection was determined prior to blood collection. Whole blood was stained for CD19, CD3, CD193 and CD23 and their expression levels measured using flow cytometry. Overall, the percentage expression of CD193 on CD19+ and CD19+CD23+ B cells were 50.44% (95% CI= 44.6256.27%) and 56.20% (95% CI=49.9362.47%) respectively while those on CD3+T cells and eosinophils were 15.95% (95% CI=11.6920.20%) and 91.18% (95% CI=88.7193.65%) respectively. Overall comparisons showed that the expression of CD193 was significantly higher on CD19+CD23+B cells than CD19+B cells, P<0.0001. The expression of CD193 was also shown to be significantly higher on B cells than on T cells (P<0.0001), but significantly lower than on eosinophils (P<0.0001). Similar findings were observed in the *S. mansoni* infected and uninfected groups. However, comparisons between *S. mansoni* infected and uninfected groups, and among children with different infection intensity levels did not show significant differences in the expression of CD193 on B cells. No correlation was observed between CD193 expression by B cells with the expression of CD193 on eosinophils and T cells. We show that CD193 expression is evident on CD19+B cells and that the expression is neither associated with *S. mansoni* infection status nor infection intensity. We are currently investigating the functionality of the CD193 expressed on B cells.

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### IPSE, A UROGENITAL PARASITE-DERIVED HOST MODULATORY PROTEIN, INDUCES GENE EXPRESSION CRITICAL FOR HOST PATHOGENESIS AND PARASITE EGG SURVIVAL AND EXPULSION

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Schistosomiasis continues to be a burden of disease for over 260 million people. *Schistosoma haematobium* and *S. mansoni*, the two most prevalent human-specific species, cause urogenital and hepatoenteric schistosomiasis, respectively. Eggs laid by *S. haematobium* worms cause the immunopathology associated with urogenital schistosomiasis, which include pronounced bladder fibrosis, urothelial hyperplasia, and

is associated in increased risk of bladder cancer; egg-associated lesions caused by *S. mansoni* infection can lead to portal hypertension and liver failure. Egg secreted proteins have been identified to play a large role in balancing host pathogenesis with egg passage through the urine and feces to continue the parasite life cycle. Interleukin-4 inducing Principle of *S. mansoni* Eggs (IPSE) is the most abundant egg secreted protein and has three important functions: 1) bind immunoglobulins, 2) sequester chemokines, 3) translocate into the host cell nucleus to modulate gene expression. Preliminary work has shown that the *S. haematobium* homolog of IPSE, or H-IPSE, has species-specific host modulatory functions. To elucidate the potential of H-IPSE for therapeutic exploitation or its conceivable role in carcinogenesis, we studied H-IPSE in two formulations: 1) intravenous administration and 2) delivered in a suspension of Matrigel. Effects on bladder gene expression were assayed through RNA-Seq, revealing upregulation and downregulation of several critical pathways, such as genes related to cell cycle, immune signaling, and tissue repair. These findings hint at the roles of H-IPSE in host pathogenesis and parasite egg survival and expulsion.

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### A STUDY OF SALMONELLA PREVALENCE IN FROZEN MEATS AND CORRELATION IN TESTS USED FOR SURVEILLANCE TO ASSIST IN A MORE EFFECTIVE FOOD INSPECTION GUIDELINES AT THE FOOD AND ENVIRONMENT LABORATORY IN AJMAN, UAE

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Foodborne diseases continue to be a major problem despite advances in detection and prevention measures due to ingestion of food contaminated with microorganisms. *Salmonella enterica* tops the list of causative organisms that can cause foodborne disease. *Salmonella* outbreaks are commonly associated with eggs, meat and poultry. This study was undertaken to estimate the prevalence of *Salmonella* in the meat products for the first time in the emirate of Ajman. Meat products form the main stay of the regions' food intake, so it is of crucial importance to ensure it is free of *Salmonella* and other related pathogens. The aim of this study is to determine the presence of *Salmonella* in the meat products and find if there is correlation between detection by culture, rapid tests and PCR analysis. This study is a prospective study conducted in the Food and Environment laboratory Ajman in 2015 to investigate the prevalence of *Salmonella* in frozen meats. A total of 133 samples of frozen meat samples were collected randomly. From each sample, 25 g was separated and treated with 225 mL of buffered peptone water, homogenized and incubated at 37°C for 24 hours. Three different methods were used to detect *Salmonella*, namely; manual method by routine culture, BAX system- Ready Reference for Standard PCR Assays method and RapidChek Select for *Salmonella* By PCR assay, 8 out of 133 frozen meat samples (6%) were found to be contaminated with *Salmonella*. By manual culture result 6 out of 121 (4.5%) were positive. This is a high rate of contamination and samples were declared unfit for consumption as per the UAE standards. In conclusion, these results indicate that the prevalence of the *Salmonella* sp. is of concern in the samples obtained by the Food and Environment laboratory in Ajman. The regions high ambient temperatures throughout the year, questionable storage facilities at small outlets and transport conditions in hot and humid conditions can be a potential source of bacterial growth and food borne diseases. The study also confirms that routine culture techniques cannot be relied upon alone to ensure food free of *Salmonella* contamination

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### ABERRATION IN IRON AND MEAN CORPUSCULAR HEMOGLOBIN METABOLISM CAUSED BY LAMBDA CYHALOTHRIN AND AFLATOXINS IN FISH DIET FROM SELECTED AQUATIC SOURCES IN KENYA

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Fish is high in iron that the human body needs to stay healthy. Excess or deficiency of minerals like iron may seriously disturb biochemical processes and upset internal homeostasis, leading to various diseases and disorders. This is occurred in organisms of various fish species due to deficiency or excess of micro and macro elements which are caused by improper nutrition, avitaminosis or poisoning. The specific objectives of the study were to determine the iron levels and mean corpuscular hemoglobin affected by lambda cyhalothrin and aflatoxins in fish diet from selected aquatic sources in Kenya. The *Oreochromis niloticus* and *Clarias gariepinus* used were bred in Kenya marine and fisheries research institute at Sagana whereas other fish were obtained from River Nyando. Laboratory procedures for fish from both sources to determine iron levels were carried out at Kenya Industrial Research and Development Institute and measured using atomic absorption spectrophotometer (AAS) analyst 800 (Parkin Elmer Instrument, USA). Mean Corpuscular Hemoglobin measurements were done at Moi Teaching and Referral Hospital laboratory. The results showed that iron availability was lower on treatment with aflatoxin compared to lambda cyhalothrin with a mean of  $3.66 \pm 0.84$  mg/kg. The mean corpuscular hemoglobin (MCH) levels was higher in both fish species obtained from Sagana with a mean of  $42.9 \pm 10.28$  (SEM 1.98 n=27) in *Clarias gariepinus*. The MCH on treatment with lambda cyhalothrin were elevated at 53.21 pg with both having means higher than in the control group. The iron levels were lower than the normal values in both treatments but aflatoxins caused lower iron levels. The reverse was true for MCH. Natural occurring toxins as well as synthetic toxicants affect the nutritional value of food and food quality tests be conducted from farms before and after processing to prevent disease transmission through diets.

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### DRINKING WATER-SPECIFIC RISK FACTORS FOR MODERATE-TO-SEVERE DIARRHEA IN YOUNG CHILDREN IN THE GLOBAL ENTERIC MULTICENTER STUDY — SUB-SAHARAN AFRICA AND SOUTH ASIA, 2007-2012

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Diarrhea is a leading cause of childhood illness in developing countries; waterborne diseases are important contributors. We examined associations between drinking water practices and moderate-to-severe diarrhea (MSD) in children <5 years old participating in the Global Enteric Multicenter Study (GEMS) in rural (The Gambia, Kenya, Mozambique, Bangladesh), urban (Mali, India), and peri-urban (Pakistan) sites. Cases seeking care for MSD (defined as  $\geq 3$  loose stools in 24 hrs with one or more of the following: sunken eyes, skin tenting, dysentery, IV rehydration, or hospitalization) were enrolled at health facilities. Age-, gender- and community-matched controls were enrolled at home. Site-specific conditional logistic regression models were used to explore associations between water practices and MSD; proportions in cases and controls respectively, matched Odds Ratios (mOR) and 95% Confidence Intervals are reported. From Dec, 2007 to Nov, 2012, 12,109 cases and 17,290 matched controls were enrolled. Urban (Mali: 86% v. 88%; India: 98% v. 98%) and peri-urban (Pakistan: 61% v. 64%) sites predominantly used piped water as their main source. Recent use of an unimproved main drinking water source [Kenya: 42% v. 36%; mOR 1.3 (1.1-1.5); Bangladesh: 48% v. 41%; mOR 1.4 (1.2-1.7); Pakistan: 36% v. 32%; mOR 1.3 (1.1-1.7)], and recently giving the child stored drinking water [Gambia: 84% v. 63%; mOR 5.7 (4.5-7.2); Mozambique: 94% v. 87%; mOR 3.5 (2.7-4.4); Kenya: 91% v. 89%; mOR 1.8 (1.4-2.4)] were associated with MSD. Recent intermittent daily water availability was a risk factor for MSD in Mali [3% v. <1%; mOR 4.3 (2.6-7.4)] and Pakistan [32% v. 26%; mOR 1.4 (1.2-1.7)]. Round-trip water fetching times >30 min were associated with MSD in Gambia [10% v. 6%; mOR 1.8 (1.4-2.4)] Mali [3% v. 1%; mOR 3.0 (2.0-4.7)] and Kenya [26% v. 16%; mOR 1.9 (1.6-2.3)]. Reported household chlorination was <3% at all sites and confirmed use rare, with the exception of Kenya (48%), where confirmed use was not associated with MSD [16% v. 17%; mOR 1.1 (0.7-1.6)]. Water practices were highly variable across sites, however the impacts on MSD were relatively consistent across rural sites.

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### SANITATION, WATER AND INSTRUCTION IN FACE-WASHING FOR TRACHOMA (SWIFT): THE CLUSTER-RANDOMIZED CONTROLLED TRIAL'S PROTOCOL AND RATIONALE

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Trachoma is the leading infectious cause of blindness worldwide and a focus of elimination efforts. The WHO recommends the SAFE strategy for the elimination of trachoma. Numerous randomized clinical trials have demonstrated the efficacy of mass azithromycin distributions, but in areas with hyper-endemic trachoma antibiotics alone do not appear to be sufficient for elimination. Many believe that the "F" and "E" components of SAFE are crucial for preventing the return of trachoma after mass azithromycin distributions have ended. However, the evidence base suggesting efficacy of non-antibiotic measures for trachoma is extremely weak. The objective of SWIFT study is to determine the efficacy of non-antibiotic measures for trachoma control. SWIFT is a series of 3 cluster-randomized trials designed to assess several alternative strategies for trachoma control in Ethiopia. The first trial (named WUHA) compares 20 clusters that receive a comprehensive WASH package to those that receive no intervention in the other 20 clusters. The second trial (TAITU-A) compares 16 clusters randomized to targeted antibiotic treatment versus

those 16 clusters randomized to mass antibiotics for trachoma, and the third trial (TAITU-B) compares 16 clusters randomized to targeted antibiotics versus those 16 clusters randomized to delayed antibiotics. Our primary outcome is the prevalence of ocular chlamydia in 0-5 year old children at 36 months. We will also monitor for several secondary outcomes, including facial cleanliness, anthropometry, nasopharyngeal pneumococcus, and soil-transmitted helminths. We will perform an intention-to-treat analysis.

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### HANDWASHING WITH SOAP PRACTICES AMONG CHOLERA PATIENTS AND THEIR ACCOMPANYING FAMILY MEMBERS IN A HOSPITAL SETTING

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There are estimated to be 3 to 5 million cases of cholera annually. Without treatment the case fatality rate for cholera can be higher than 50%. For severely dehydrated cholera cases hospitalization is required with intravenous and oral rehydration solution. A hospital setting is a high risk environment for the transmission of enteric infections. The objective of our study was to observe the hand washing practices of patients and their accompanying family members in a Dhaka hospital, Bangladesh which is treating more than 140,000 patients a year. Informed consent was obtained from participants. Trained assistants conducted three hours structured observation and administered a questionnaire to patients and their accompanying family members at hospital. It focused on observing hand washing with soap at the following key events: during food preparation, before eating and feeding, after toileting, after cleaning the child anus and removing child feces. In addition spot checks were performed of the presence of soap at the bathroom area. There were a total of 55 cholera patients and 93 household contacts observed during the 3 hour structured observation period. There were 17 male and 38 female patients and 34 male and 59 female household contacts. There were different age categories cholera patients and attendants (less than 5 years to older than 14 years old) under this observation study. Overall, 4% (4/103) of key times involved handwashing with soap among patients and household contacts during the structured observation period. This was 3% (1/37) among patients, and 5% (3/66) for household contacts. For toileting events, this was 7% (3/46), and 7% (1/14) for patients and 6% (2/32) for household contacts. For food related, overall this was 2% (2/93) for patients and 3% (2/59) for household contacts. During spot checks, 7% (4/55) of observations had soap present at handwashing station. Observed handwashing with soap at key times among patients and accompanying family members was very low (4%). These findings highlight the urgent need for intervention to target this high risk population of Bangladesh.

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### IMPACT OF INDUSTRIAL FOOD PROCESSING EFFLUENT ON MAJOR DRINKING WATER SOURCES IN TECHIMAN MUNICIPALITY, GHANA: WATER POLLUTION AND POTENTIAL HUMAN HEALTH RISKS

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Many water bodies are losing their capacity to host aquatic fauna and flora because of the alarming extent of pollution. Due to the possible human and aquatic health risks associated with untreated industrial effluent discharges, we assessed the microbiological and physicochemical impacts of effluent discharges from a major Ghanaian nut processing factory (Ghana Nut Limited (GNL)) on nearby water body, Tano River (TR). Six sampling stations (the GNL effluent discharge point, two upstream, two downstream sites and the point where the effluent joins the river)

were studied monthly for four consecutive months in terms of their physico-chemical quality (pH, Alkalinity, Turbidity, Total Dissolved Solids (TDS), Total Soluble Solids (TSS), Biological Oxygen Demand (BOD), Oil and Grease, Chemical Oxygen Demand (COD), phosphorus and nitrate) and microbiological quality (Total Coliforms (TC), Faecal Coliforms (FC) and *Escherichia coli* (*E.coli*)). These parameters of the effluent were compared to standards of the Environmental Protection Agency of Ghana (EPA-Ghana) to determine suitability for surface-water discharge. Whilst those of the stream and river samples were compared to the WHO drinking water quality guidelines. The BOD, COD, TSS, Fe and Oil and Grease values for the effluent were significantly above EPA Ghana standards with 1292.00 mg/l, 3156.00mg/l, 1242.00 mg/l, 13.38 mg/l and 6.31mg/l respectively. The BOD, COD, Fe and Turbidity values for the midpoint which was the tributary of the river and the effluent exceeded the WHO water quality guideline as 24.93mg/l, 75.83mg/l, 6.15mg/l and 43.08mg/l respectively. The tributary of the river and the effluent on the TR recorded significant values of TC, FC, and *E.coli* which were above the WHO guidelines of 0cfu as 29 x 10<sup>4</sup> cfu, 4.70 x 10<sup>1</sup> cfu, and 1.3 x 10<sup>1</sup> cfu. The contaminant levels reduced as the river flowed from the effluent discharge point to the downstream sites. This suggests that industrial effluent discharges were causes of pollution of drinking water sources and could pose waterborne health risks. Thus, industrial effluents must be properly treated before discharge.

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### HIGH THROUGHPUT DETECTION OF 37 ENTERIC PATHOGENS WITH TAQMAN ARRAY CARDS IN ENVIRONMENTAL SPECIMENS

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Diarrhea is one of the leading causes of mortality in children under five years of age in low-income countries. Prior studies of diarrhea etiology using our custom developed enteric Taqman Array Cards (TAC) have shown that multiple enteric pathogens are often present from the first months of life not only in diarrhea specimens but also in asymptomatic stool specimens. This suggested that early exposure was the norm but the route of exposure to these pathogens in these infants who are mostly exclusively breastfed is unknown. We conducted a pilot study testing drinking water, stored water, tap water, sewer, and soil in and around the households for a range of 37 enteric viruses, bacteria, helminthes, and parasites by TAC molecular method in Mirpur, Bangladesh. On average 2 pathogens (range 0 to 4 enteropathogens) were found in water used for drinking, bathing, and washing including *Aeromonas*, *Blastocystis*, *Campylobacter*, *Entamoeba histolytica*, and pan-*Entamoeba*. A broad range of pathogens (average 10 pathogens; range 5-13) including *E. coli*, bacteria, viruses, and protozoa were detected at a high burden level in the samples from the open sewer near the households. Soil samples on average had 9 pathogens (range 5 to 15) with included similar pathogens found in sewer samples with additional detections of helminthes such as *Ancylostoma duodenale*, *Ascaris lumbricoides*, and *Trichuris trichiura*. This study confirms the presence of ubiquitous enteric pathogens in the environment in and around the households in the urban slums of Dhaka. Further studies are needed to understand the route of transmission and infection from the environment to infants living in this low-income setting.

## ASSESSMENT OF WATER, SANITATION, HYGIENE AND INFECTION, PREVENTION AND CONTROL CONDITIONS IN FIFTY TWO HEALTHCARE FACILITIES IN HOIMA DISTRICT OF UGANDA

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There is a dearth of knowledge of water, sanitation, hygiene (WASH) and infection prevention and control (IPC) conditions in healthcare facilities of developing countries. Absence of these services or conditions are associated with healthcare associated infections that disproportionately burden neonates and their mothers. A 2015 WHO/UNICEF report states that about 38% of healthcare facilities in lower and middle income countries do not have safe supply of water and 19% lack adequate toilets. The sustainable development goal six calls for a concerted effort to identify and improve these conditions. The aim of the study was to conduct an assessment of water, sanitation, hygiene and infection, prevention and control conditions in 52 healthcare facilities in Hoima District of Uganda to identify priority areas for improvement, guide plans for interventions and advocate for action. In November 2016, an assessment tool was deployed in 52 healthcare facilities in 10 sub counties of Hoima District. The tool consists of interviews with directors and administrative staff of the facilities, observation and water quality sampling and analysis of key wards. The data was collected on a mobile device and analyzed in SAS software program. A score card for each facility was produced based on whether the facility provides a basic, improved or unimproved or no service. Overall, only about 15% met basic standards for managing waste, about 8% met the basic standards for sanitation, about 10% had basic water supply systems and approximately 11% had handwashing with soap and water facilities at the time of visit. In addition, only about 29% carried out regular cleaning routines. Approximately 69% and 4% of the sampled water from all the healthcare facilities met the national standards for drinking water for *Escherichia coli* and free chlorine respectively. The study shows that majority of the healthcare facilities have poor IPC conditions and do not provide basic WASH services. These results have dire implications for healthcare associated infections. The results from this work is being used to inform interventions and advocate for action.

## DETECTION AND QUANTIFICATION OF ROTAVIRUS IN SEWAGE USING DROPLET DIGITAL PCR

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Globally rotavirus (RV) causes severe diarrhea in children < 5 years of age. The detection and quantification of RV in environmental samples is cumbersome and its recovery requires collection and concentration of a large volume sample. Molecular detection methods such as quantitative polymerase chain reaction (qPCR) and conventional reverse transcriptase (RT-PCR) have been used to detect and characterize RV in environmental samples. The goal of this study was; i) to evaluate droplet digital PCR (ddPCR) as a tool to detect and quantify RV in untreated sewage, ii) to optimize the sampling preparation method that can be able to quantify RNA viruses in untreated sewage. Untreated sewage samples were collected from a Kenyan lagoon (5 L, n=10), a US lagoon (10 L, n=10) and influent samples in the US (2 L, n=18) from a wastewater treatment plant (WWTP). The samples collected from Kenya lagoon (5 L) and from the US lagoon (7.5 L) were concentrated using an adsorption-elution

method (ViroCap), while 2 L of the US lagoon and 2 L of WWTP samples were concentrated using polyethylene glycol (PEG)/sodium chloride (NaCl) precipitation. Nucleic acid for RV (dsRNA) was extracted using commercially available kit (QIAamp Viral RNA Mini Kit) and RV was detected and quantified using ddPCR. Rotavirus was detected in 100% (10/10) in the Kenya, 100% (10/10) in the US lagoon and 100% (18/18) in the US WWTP samples. In the Kenya lagoon RV was detected at a mean concentration of  $1.09E+05 \pm 1.90E+05$  genome copies/L. In the US lagoon, the samples concentrated using ViroCap had a mean of  $7.68E+02 \pm 9.41E+02$  genome copies/L, while the one concentrated using PEG had a mean of  $1.48E+04 \pm 8.97E+03$  genome copies/L. The mean difference of RV concentration between the 2 viral concentration methods (ViroCap Vs PEG) in the US lagoon samples were statistically significant  $p < 0.05$ . The US WWTP had a mean concentration of  $4.92E+05 \pm 8.19E+05$  genome copies/L. In conclusion, the droplet digital PCR is an easy and promising technology for detection, identification and quantitation of RV in untreated sewage and the method was easily used with PEG or ViroCap concentrates.

## EFFECT OF SANITATION ON PHYSICO CHEMICAL QUALITY OF GROUNDWATER

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The paper investigated physico-chemical quality of groundwater, considering the relative proximity of hand dug wells to coastal environment, but most importantly close to insanitary facilities like refuse dumps, septic tanks and pit latrines; this was done in selected coastal communities in the Keta. Thirty vials of water samples from selected hand dug wells in two seasons were tested for pH, Total Dissolved Solids (TDS), Conductivity, Turbidity, Calcium, Magnesium, Nitrate, Sulphate, and Phosphate; WHO standards of drinking water were adopted to validate the results obtained and statistical analysis run. Statistical analysis of results showed a statistical significance in Turbidity, Nitrate, Phosphate, Calcium and Magnesium values at  $p < 0.05$ , between the two seasons. There was a weak but significant correlation between Calcium and distance ( $r=0.398$ ) and a strong correlation between Magnesium and distance ( $r=0.763$ ). The study revealed that all the hand dug wells sampled recorded relatively high concentration of conductivity, turbidity, TDS, Calcium, Magnesium and Phosphates, irrespective of their proximity to the insanitary facilities and coastal environment, apart from nitrates when compared to WHO standards of drinking water.

## ASSESSING THE EFFECT OF A NOVEL HOUSEHOLD WATER PASTEURIZATION INTERVENTION ON CHILD DIARRHEA: EVIDENCE FROM A RANDOMIZED CONTROLLED TRIAL IN THE PERUVIAN AMAZON

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In low- and middle-income countries, diarrheal disease is a leading cause of morbidity and mortality among children under 5. Household water treatment generally reduces childhood diarrhea risk, but water boiling is the only method of household treatment to have reached scale in any country. Barriers to effective boiling such as time and indoor air pollution may be addressed by the use of a water pasteurization indicator (WAPI). We conducted a randomized controlled trial to evaluate the effect of a low-cost WAPI on diarrhea in children <5 and fecal contamination of

household drinking water in the Peruvian Amazon. In the intervention arm, 141 households received a water pasteurization indicator to treat their drinking water. All households received a 20 liter water storage container. Twelve weekly follow-up visits were conducted to measure the longitudinal prevalence of health outcomes and the prevalence of *Escherichia coli* contamination in household drinking water. We deployed temperature data loggers in a random subset of households to assess intervention use. Follow-up data from 377 children <5 (183 control group; 194 intervention group) were analyzed in an intention to treat analysis. The water pasteurization intervention did not reduce the longitudinal prevalence of diarrhea among children <5 (LPR=0.99, 95% CI=0.72, 1.38) or reduce the prevalence of *E. coli* contamination of household drinking water (PR=0.94, 95% CI=0.75, 1.18). Among intervention households monitored with a data logger, 7% used the indicator at least twice a week during the follow-up period. A water pasteurization intervention did not reduce diarrhea among children <5 or fecal contamination of household drinking water, consistent with low adherence to the intervention. Future research aimed at understanding the factors influencing the adoption of this treatment method would be valuable to inform the design of interventions.

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### PSYCHOSOCIAL FACTORS MEDIATING THE EFFECT OF A HEALTH FACILITY BASED HANDWASHING WITH SOAP AND WATER TREATMENT INTERVENTION IN BANGLADESH (CHOBI7 TRIAL)

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Inadequate hand hygiene is estimated to result in nearly 300,000 deaths annually, with the majority of deaths being among children younger than 5 years. In an effort to promote handwashing with soap and water treatment behaviors among highly susceptible household members of cholera patients, we recently developed the Cholera-Hospital-Based Intervention-for-7-Days (CHoBI7); chobi means picture in Bengali. This 1-week handwashing with soap and water treatment intervention is delivered by a promoter in the hospital and the home to cholera patients and their household members. In our randomized controlled trial of this intervention, we observed a significant reduction in symptomatic cholera infections during the 1-week intervention period compared to the control arm and sustained high uptake of observed handwashing with soap behaviors up to 12 months post-intervention. The aim of the present study was to assess the underlying mechanism of change that led to the high handwashing with soap behavior observed among participants who received the CHoBI7 intervention. Handwashing with soap was measured using 5-hour structured observation, and psychosocial factors were assessed using a structured questionnaire among 170 intervention and 174 control household members enrolled in the CHoBI7 trial. To investigate potential mediators of the CHoBI7 intervention effect, mediation models were performed. Response efficacy was found to mediate the intervention's effect on habit formation for handwashing with soap at the 1-week follow-up, and disgust, convenience, and cholera awareness were mediators of habit maintenance at the 6- to 12-month follow-up. These results support the use of theory-driven approaches for the development and implementation of handwashing with soap interventions.

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### RISK FACTORS FOR HOUSEHOLD TRANSMISSION OF *VIBRIO CHOLERAE* IN DHAKA, BANGLADESH (CHOBI7 TRIAL)

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Household contacts of cholera patients are at a 100-times higher risk of a *Vibrio cholerae* infection than the general population. To examine risk factors for *V. cholerae* infections and investigate intervention strategies among this population, we followed household contacts of cholera patients for the 1-week high-risk period after the index patient obtained care. This study was nested within a randomized controlled trial of the Cholera-Hospital-Based-Intervention-for-7-days (CHoBI7), a handwashing with soap and water treatment intervention in Dhaka, Bangladesh. Rectal swab results were available from 320 household contacts of cholera patients at five time points over a 1-week period. Fecal and water samples were analyzed for *V. cholerae* by culture. All analyses were stratified by study arm. Within the intervention arm, a household median free chlorine concentration below 0.5 mg/L was associated with a three times higher odds of a cholera infection (odds ratio [OR]: 3.0; 95 confidence interval [CI]: 1.32, 6.63). In the control arm, having *V. cholerae* in stored water was associated with a significantly higher odds of a symptomatic cholera infection (OR: 8.66; 95% CI: 2.11, 35.48). No association was found between observed handwashing with soap at food and stool-related events and *V. cholerae* infections. Household water quality and treatment practices were found to be important risk factors for cholera infection among household contacts of cholera patients. These findings emphasize the need for water treatment interventions targeting this high-risk population.

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### NOVEL GAMETOCYTE BIOMARKERS FOR DETECTION OF THE *PLASMODIUM FALCIPARUM* INFECTIOUS RESERVOIRS

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Eradication efforts as well as the optimal use of transmission-reducing malaria interventions requires further knowledge of the submicroscopic infectious reservoirs among asymptomatic individuals. Even sub-microscopic levels of *Plasmodium falciparum* gametocytes can be infectious to mosquitos and promote onward transmission. Most efforts to identify gametocyte carriers rely upon PCR amplification of the gametocyte-specific transcript Pfs25, a sexual stage antigen. We have utilized gene expression profiling microarrays of blood stage and gametocyte stage *P. falciparum* parasites to identify potentially novel gametocyte-enriched transcripts that could be sensitive biomarkers for gametocyte detection in asymptomatic individuals. This has led to identification of over 200 molecules that are uniquely expressed in sexual parasite stages. One candidate in particular, Pfg17, exhibited superior analytical sensitivity against a reference panel of gametocyte-spiked whole blood detecting as few as 10 gametocytes/mL of blood; in comparison Pfs25 detected only 25.3 gametocytes/mL of blood. Pfg17 also exhibited superior clinical sensitivity; identifying 19.1% more samples among blood-film negative Ghanaian children and 40% more samples from asymptomatic adults as gametocyte positive. Cumulatively, our

results suggest that Pfg17 could serve as a novel biomarker to detect asymptomatic infectious reservoirs who would be otherwise missed by the most sensitive molecular method available. Our study has also improved the repertoire of transmission stage antigens available for evaluation as candidate vaccines.

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### PERFORMANCE OF LOOP-MEDIATED ISOTHERMAL AMPLIFICATION FOR THE IDENTIFICATION OF SUBMICROSCOPIC *PLASMODIUM FALCIPARUM* INFECTION IN UGANDA

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Accurately identifying and targeting the human reservoir of malaria parasitemia is critical for malaria control, and requires a reliable and sensitive diagnostic method. Loop-mediated isothermal amplification (LAMP) is increasingly used to diagnose submicroscopic parasitemia. Although most published studies report the sensitivity of LAMP as >80%, they have failed to use a consistent, sensitive diagnostic as a comparator. We utilized cross-sectional samples from children and adults in Tororo, Uganda, a region with high but declining transmission due to indoor residual spraying of insecticides to characterize the sensitivity and specificity of pan-*Plasmodium* LAMP. We compared results from LAMP using primers *PgMt19* targeting a mitochondrial DNA sequence conserved in all *Plasmodium* species, performed on DNA extracted from dried blood spots, to those of a gold standard quantitative PCR assay targeting the *var* gene acidic terminal sequence of *Plasmodium falciparum* (*varATS* qPCR), performed on DNA extracted from 200  $\mu$ L of whole blood. Using LAMP and *varATS* qPCR increased the detection of parasitemia 2- to 5-fold, compared to microscopy. The sensitivity of LAMP was 81.5% for detecting submicroscopic infection > 1 parasites/ $\mu$ L. However, low density infections were common, and LAMP failed to identify more than half of all infections diagnosed by *varATS* qPCR, performing with an overall sensitivity of 44.7% for detecting submicroscopic infections of > 0.01 parasites/ $\mu$ L. Thus, although the LAMP assay is more sensitive than microscopy, it missed a significant portion of the submicroscopic parasite reservoir. These findings have important implications for malaria control, particularly in settings where low-density infections predominate.

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### INVESTIGATING THE KINETICS OF TRANSGENIC *PLASMODIUM FALCIPARUM* HRP2 PROTEIN PRODUCED BY *P. BERGHEI* IN A NOVEL MURINE MODEL

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There is an increasing need for improved diagnostics for subclinical malaria, as these cases act as transmission reservoirs. Low parasitemic, subclinical malaria is difficult to diagnose. One way to better diagnose these cases is increasing the sensitivity of the malaria rapid diagnostic test that detects the *Plasmodium falciparum* histidine-rich protein 2 (PfHRP2). To develop more sensitive diagnostics, we need an understanding of PfHRP2 kinetics *in vivo* during infection and after clearance. We must also determine primary localization of PfHRP2 and its clearance time in plasma and erythrocytes for better targeting and detecting in diagnostic tests. In mice, we determined the average plasma half life of PfHRP2 to be 2.1 hours after intraperitoneal injection of recombinant protein, with a total clearance time of 4 days. PfHRP2 persists in circulation for 1-4 weeks post parasite clearance, presenting an additional challenge to

extending the limit of assay detection for PfHRP2. We established a novel working murine model using a transgenic *P. berghei* parasite expressing PfHRP2 to study this phenomenon. The transgenic parasite produces PfHRP2 in the erythrocyte and exports it into the erythrocyte cytoplasm in a manner similar to *P. falciparum*, which naturally produces the protein. In our model, PfHRP2 persists 5-7 days in the plasma and 8-9 days in the erythrocytes following treatment with artemisinin-based combination therapy. The average plasma half life of PfHRP2 during infection was 8 hours. Parasites were absent by microscopy at day 3 and parasite DNA was absent by qPCR on day 7. Due to the significantly extended persistence of PfHRP2 in erythrocytes, persistence could result from slow clearance from the red blood cells, through the process of erythrocyte pitting, a consequence of increased rigidity of *P. falciparum* infected cells. Preliminary IFA data demonstrated PfHRP2 positive, DAPI negative erythrocytes, corroborating the pitting hypothesis, which will be further explored. In conclusion, we developed a novel murine model to investigate PfHRP2 dynamics *in vivo* to better inform the generation of more sensitive malaria diagnostics.

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### COMPARISON OF SIMULTANEOUS CAPILLARY AND VENOUS PARASITEMIA AND GENOTYPING RESULTS FROM CHILDREN AND ADULTS WITH UNCOMPLICATED MALARIA

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Light microscopy remains the gold standard for diagnosis and follow-up of malaria. In addition, drug efficacy studies rely on molecular genotyping of polymorphic markers to distinguish recrudescence from new infections. While capillary blood remains as the most commonly used matrix for parasite detection and genotyping, clinical trials use results interchangeably from venous and capillary matrices. Importantly, parasitized erythrocytes adhere to capillary endothelium, and several studies support differences in hematologic and chemistry parameters between venous and capillary blood. To our knowledge, there has been no systematic comparison of diagnostic results from these two matrices. We hypothesize that variation between parasitemia and/or molecular genotyping data from these two compartments could have important implications for the conduct of clinical trials. To address this, we conducted a prospective study of uncomplicated *Plasmodium falciparum* malaria in adults and children, with and without HIV infection, in a high transmission region of Uganda. All participants were treated with artemether-lumefantrine. Simultaneous capillary and venous samples for microscopy and molecular genotyping were obtained at various time points: at baseline, over the course of follow-up, and at time of recurrent malaria. Smears were prepared identically with 2% Giemsa, stained for 30 minutes, and were read by 2 expert microscopists, with discrepancies resolved by a third reader. In total, we have simultaneous measurements at baseline in 52 children and 36 adults, at the time of recurrence in 52 children and 6 adults, and additional simultaneous measurements in 186 children and 46 adults over the course of 42-day follow-up. Thus far, baseline parasitemia measurements from both matrices have been compared in 36 adults and 37 children. In addition, paired samples from baseline and the time of recurrent malaria are being compared for 6 polymorphic markers to assess how results could impact the determination of recrudescence versus new infection, and multiplicity of infection. Full results will be presented at the time of the meeting.

### BLUE-LASER TECHNOLOGY FOR RAPID SENSITIVE DETECTION OF *PLASMODIUM FALCIPARUM* PARASITEMIA AND GAMETOCYTEMIA

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Sensitive detection of low-density parasite carriers is a priority for malaria elimination efforts. The rapid detection of low-density malaria parasites and in particular the transmissible gametocytes is a challenge that is not met by the currently available diagnostics. Novel tools for rapid identification of gametocyte carriers may support the planning and evaluation of malaria elimination programs. The Sysmex XN-30 hematology analyzer is able to detect parasitized erythrocytes, as well as gametocytes, using a novel blue-laser technique combined with a specific surfactant and DNA fluorescence dye. This method is quantitative, with the ability to differentiate between parasite subpopulations. We tested the diagnostic performance of this analyzer *in vivo* using a controlled human malaria infection model (CHMI, n=16) where low densities of gametocytes were induced, and *in vitro* using cultured *Plasmodium falciparum* parasites and gametocytes. Blood smear microscopy, 18s qPCR (asexual parasites), and Pfs25 qRT-PCR (gametocytes) were used as reference standard. Quantitative data of the CHMI study showed a strong correlation between 18s qPCR and blue-laser technology for parasitemia ( $r=0.769$ ;  $p<0.0001$ ). *In vitro* experiments showed similar correlations ( $r>0.892$ ;  $p<0.0001$ ) between the blue-laser technology, microscopy, and PCR, using cultured asexual- and sexual parasites. The analyzer detected asexual parasites and gametocytes at densities as low as 10 parasites/ $\mu$ L. The stability of samples was confirmed for at least 6hrs at room temperature. Further work is currently performed to assess performance of the blue-laser in submicroscopic malaria. In conclusion, our analyses show that this high throughput method can detect microscopic malaria in a volume  $<100\mu$ L of whole blood within 60 seconds without any sample preparation. We will present evidence that this hematology analyzer can detect low level parasitemia and gametocytemia *in vivo*. Therefore, this analyzer may be useful for diagnosis of malaria parasitemia, treatment follow-up, detection of gametocyte carriers and mass screening campaigns to support elimination efforts.

### BREATHPRINTING REVEALS MALARIA-ASSOCIATED BIOMARKERS AND MOSQUITO ATTRACTANTS

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Evidence suggests that the malaria parasite manipulates host odors to augment *Anopheles* mosquito attraction to infected hosts. *In vitro* studies have shown that parasite-infected red blood cells release plant-like volatile organic compounds (VOCs) called terpenes, including the monoterpene alpha-pinene, which attract mosquitoes. We investigated whether the attractants found in cultured parasites were also produced during *in vivo* infection. We performed two independent studies of pediatric patients with and without uncomplicated *P. falciparum* malaria in Malawi to evaluate for malaria-induced volatile changes in a natural field setting. We collected breath samples onto sorbent tubes, prior to analysis by thermal desorption gas chromatography-mass spectrometry (TD/GC-MS). Sweat volatiles were also collected and analyzed using nylon sleeves and solid phase microextraction (SPME)/GC-MS. In both cohorts, the malaria-infected group displayed significantly elevated levels of the known mosquito attractants alpha-pinene and 3-carene. Each of these compounds proved to have high specificity for predicting malaria infection ( $>94\%$ ), and moderate sensitivity (41 - 53%). To improve diagnostic

sensitivity, we used an unbiased, correlation-based algorithm to identify a combination of six breath biomarkers that successfully classified infection status with 83% accuracy. Our findings confirm, for the first time, that natural human malaria infection increases levels of known mosquito attractants, providing a likely mechanism for preferential attraction of *Anopheles* mosquitoes towards malaria-infected hosts. Furthermore, we demonstrate a robust "breathprint" of infection and nominate candidate breath biomarkers of malaria in a typical African field setting. Our findings will inform future development of noninvasive malaria diagnostics, as well as advanced measures of mosquito control.

### THE USE OF SMALL PEPTIDE MICROARRAYS TO DETECT MALARIA EXPOSURE

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Highly sensitive diagnostics that measure asymptomatic malaria infections within a region are vital to malaria elimination efforts. Molecular tests that identify parasite nucleic acids enhance our ability to detect very low-density active infections, but they cannot identify people who have recently cleared infections or those with parasite levels below the limit of detection. Serological testing allows us to measure anti-Plasmodium antibodies present in patient sera and may provide measurements of current, recent, distant and cumulative infections. However, current serological tests are based on a few antigens and may miss parasites with variants of these proteins not represented by the reference strain used to create the tests. We designed a diversity-reflecting peptide array to measure antibody levels to variants of 41 malaria antigens. Our goal was to use serological data to determine whether malaria transmission is ongoing in a region and help prioritize targeted interventions to those areas. We probed the array with sera from 10 Malian children before, at the peak of, and after the malaria transmission season and 10 Malian adults before the malaria transmission season. Comparisons of children to adults show that adults with a long history of malaria exposure recognize a greater number of peptides from 22 proteins than from children with limited, recent exposure. Similarly, comparisons between children before the malaria transmission season to children at the peak of or following the transmission season demonstrates that we can differentiate between the seroprofiles of children throughout the course of a malaria transmission season. We used multivariate analysis to identify a set of peptides to represent the signature of an individual's age and malaria exposure history. Seroreactivity against these peptides represents a promising indicator of recent or current malaria exposure that can help prioritize malaria elimination efforts to areas with ongoing or recent transmission. In the future, we will evaluate this new tool's ability to identify *foci* of infection in low transmission setting such as Myanmar.

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**NEW EVIDENCE OF MATING SWARMS OF THE MALARIA VECTOR, *ANOPHELES ARABIENSIS* IN TANZANIA**

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Malaria mosquito mating swarming phenomenon may offer new opportunities for controlling the mosquitoes, for example by targeting the swarms with insecticides. Unfortunately, studies of actual *Anopheles* swarms have been rare in East Africa, the last recorded field observations in Tanzania having been in 1983. Mosquito swarms were surveyed between September 2016 and February 2017, in rural villages south-eastern Tanzania. Identified *Anopheles* mosquito swarms were sampled using sweep nets, and collected mosquitoes killed by refrigeration and identified morphologically. A sub-sample of the *Anopheles* was further identified by PCR, and all females examined to assess mating status. Age of the mosquitoes was estimated by observing the coiling and unwinding of female ovarian tracheoles, and the rotation of male genitalia. A total of 202 *Anopheles* swarms were identified, characterized and mapped in the study villages. During the same period, 9,319 *An. gambiae* s.l and 13 *An. funestus* mosquitoes were collected in the swarms. The *An. gambiae* s.l were 99.6% males and 0.4% females, while all the *An. funestus* collected were males collected in mixed swarms. Of all the male *An. gambiae* s.l analysed by PCR, 86.7% were identified as *An. arabiensis*, while 13.3% were unidentifiable, due to non-amplification in the PCR assays. The median height of the swarms was 2- 4 m above ground. We identified 5 potential swarm markers, of which 90% had confirmed swarm occurrence at least once during the study period. Common types of confirmed swarm markers included rice field areas with heaps of dry grass (65%), open grassland (20), woodpiles (10%) and brick piles (5%). The median duration of swarms was (20-30). Minutes. Visual estimates of swarm sizes closely matched the actual sizes as determined by sweep net sampling (R=0.9402). The study also provides evidence that swarms of *Anopheles* mosquitoes can be identified, characterized and quantified in the field by trained community volunteers. This finding opens up new opportunities for targeting malaria mosquitoes, including targeting of male swarms as an approach to deplete vector densities and reduce disease transmission.

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**THE EFFECTS OF METAPOPULATION STRUCTURE ON FINE-SCALE MOSQUITO POPULATION DYNAMICS AND POTENTIAL CONSEQUENCES FOR THE TRANSMISSION DYNAMICS OF DENGUE AND MALARIA**

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Fine-scale spatial heterogeneity in the transmission intensity of dengue and malaria is primarily caused by variation in the density of female adult mosquitoes. Thus an understanding of the fine-scale dynamics of mosquito populations is needed to understand spatial heterogeneity in disease transmission and persistence at those scales. In any given environment, adult mosquito density is largely determined by the death rate of adult mosquitoes and the rate at which new adult mosquitoes emerge from larval breeding sites. Although larval breeding sites of both *Anopheles* and *Aedes aegypti* mosquitoes are often highly fragmented, mathematical models of dengue and malaria transmission, which consider larval population dynamics, do not generally take account of such fragmentation. Rather, the entire larval population is treated as a well-mixed population coming from a single large breeding site, and density dependent larval mortality is assumed to increase linearly with population size. Here, using a fine-scale stochastic metapopulation model of dengue and malaria transmission, which has been calibrated against

appropriate entomological and epidemiological data, we explore the impact of accounting for breeding site fragmentation when modelling the dynamics of mosquito populations. We observe that, when mosquito population densities are low, fragmentation can lead to a reduction in vector population density, with mosquito population persistence highly dependent on the dispersal behaviour of the adult mosquito and features of the underlying landscape. We show that our results have particular importance for novel vector control measures such as *Wolbachia*-infected *Aedes aegypti* or genetically modified mosquitoes with gene-drive, as by not accounting for fragmentation, existing models are likely to over-estimate the speed of spatial spread of such invasive technologies. Last, we show that landscape fragmentation may have an important impact on the dynamics and attack rate seen in invasions of novel arboviruses, such as Zika virus.

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**CHANGES IN MOSQUITO BEHAVIORS ARE LIKELY TO IMPACT ON THE EFFECTIVENESS OF INDOOR-BASED MALARIA VECTOR CONTROL INTERVENTIONS IN CHIKWAWA, MALAWI**

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Malaria vector control interventions tend to exploit behaviors of mosquito vector populations. Reports from Tanzania and Kenya have indicated changes in behaviors of malaria vectors following the scale-up of insecticide-treated nets (ITNs) and indoor residual spraying (IRS) interventions in recent years. In Chikwawa district, southern Malawi, we studied mosquito biting and resting preferences in 20 randomly selected households in an area of high and perennial malaria transmission along the lower Shire River basin. Mosquitoes were sampled both indoors and outdoors using indoor aspiration, human landing catch and outdoor barrier-screening techniques in order to investigate the species diversity of the indoor-resting and host-seeking populations, and to understand when and where exposure takes place. In total, 1342 anophelines were collected in the study over one month of sampling out of which 44% were intercepted flying outdoors by barrier screening and an equal proportion of 25% each were caught biting indoors and outdoors. Only 6% of the vector population was found resting indoors. These results showed that the majority (69%) of the entire vector population was sampled outdoors (barrier screening, n=593 and outdoor-landing catch, n= 337). *Anopheles arabiensis* made up 80% of the human-landing collections while *Anopheles funestus* s.l and *Anopheles coustani* made up 18% and 2% of the total host-seeking, respectively. *Plasmodium falciparum* sporozoite rate was 1.2% (n=328) determined by quantitative polymerase chain reaction and all positive specimens were *Anopheles arabiensis* collected outdoors. These results indicated that interventions which mainly target indoor-biting and indoor-resting mosquitoes may not be offering adequate protection against this vector population.

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**A NOVEL HIGH-THROUGHPUT SEQUENCING ASSAY TO IDENTIFY AND CHARACTERIZE ALL EUKARYOTIC PARASITES PRESENT IN HUMAN STOOLS AND DISEASE VECTORS**

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Eukaryotic parasites cause many human infectious diseases - including malaria, schistosomiasis, leishmaniasis and Chagas disease - and are responsible for more than one billion cases and over one million deaths worldwide every year. These parasites also cause many animal diseases, that sometimes lead to poorly-characterized zoonoses. Unfortunately, the detection of these parasites and screening of clinical or entomological samples is challenging, time-consuming and expensive, which limits researchers and public health officials to monitoring only a few specific

parasites. Early identification and comprehensive characterization of (potential) parasites in vectors or clinical samples would greatly improve patient treatment and rapid implementation of specific control measures. To address this need, we developed a rapid and cost-efficient screening technique to detect and identify both known and potential parasites present in human stool samples and vectors. We designed several sets of PCR primers, each targeting a different taxon of eukaryotic parasites and use massively-parallel sequencing to obtain the taxonomic information for any positive amplification. Our preliminary data demonstrates that this approach can efficiently recover and identify DNA from Apicomplexa, Platyhelminthes, Nematodes (Spirurida and Trichocephalida), Parabasalia, Kinetoplastida and Blastocystis parasites. This enables screening hundreds of samples simultaneously for all these taxa. Our assay can also easily be modified to provide, in a single sequencing run, information about the vector's species and the composition of its previous bloodmeals. We believe that this approach could complement the efforts of clinicians and entomologists to monitor the presence and spread of diseases and could lead to significant improvement in public health in a variety of settings.

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### NOVEL VECTORS OF THE ZONOTIC MALARIA PARASITE, *PLASMODIUM KNOWLESI*, IN TWO DISTRICTS OF SARAWAK, MALAYSIAN BORNEO

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The zoonotic malaria parasite, *Plasmodium knowlesi*, is the most common cause of human malaria in Sarawak, Malaysian Borneo. It accounted for over 80% of hospitalised malaria cases from 2014-2016. Previously identified vectors of the parasite in nature in Malaysia and Vietnam all belong to the Leucosphyrus Group. Only one study on vectors of *P. knowlesi* has been conducted in Sarawak that incriminated *Anopheles latens* as the vector in the Kapit District. This project was therefore undertaken to identify malaria vectors in other districts of Sarawak. Human landing catches were conducted in forested sites of the Betong and Lawas Districts. The salivary glands of anophelines were removed, DNA was extracted and screened with nested PCR assays for *Plasmodium* and species of *Plasmodium*. The sequences of the small sub-unit ribosomal RNA (SSUrRNA) genes of *Plasmodium* spp. and the internal transcribed spacer 2 (ITS2) and mitochondrial cytochrome c oxidase subunit 1 (CO1) sequences of the mosquitoes were derived from the *Plasmodium*-positive samples. Collectively, 238 anophelines and 2,127 culicines were caught. *An. letifer* (44.5%, n=173) and *An. balabacensis* (47.7%, n=65) were found to be the predominant anophelines in Betong and Lawas districts, respectively. By PCR, 15 anophelines were found to be infected with only *P. knowlesi*, while 8 others were infected with two or more simian *Plasmodium* species (*P. coatneyi*, *P. cynomolgi*, *P. fieldi* and *P. inui*). Phylogenetic analysis of the SSUrRNA genes confirmed the presence of *P. knowlesi* and other simian malaria parasites in 3 *An. barbirostris*, 6 *An. balabacensis*, 1 *An. latens* and 1 *An. letifer*. Phylogenies inferred from the ITS2 and CO1 sequences of *An. balabacensis* and *An. barbirostris* indicate that the former is genetically indistinguishable from *An. balabacensis* in Borneo while the latter is a sibling species of the Barbirostris Subgroup. In conclusion, new vectors of *P. knowlesi* were identified in Betong (*An. barbirostris* and *An. balabacensis*) and Lawas (*An. latens* and *An. letifer*), including 2 species (*An. barbirostris* and *An. letifer*) which do not belong to the Leucosphyrus Group.

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### DIFFERENTIAL EFFECTS OF TIRE LEACHATE ON *Aedes* MOSQUITOES MAY FACILITATE INVASION SUCCESS

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Discarded vehicle tire casings are an important habitat for the developmental stages of numerous vector mosquitoes. The rubber of the tire casings degrade under ultraviolet light and leaches numerous soluble metals (e.g., barium, cadmium, zinc) and organic substances (e.g., benzothiazole and its derivatives, polyaromatic hydrocarbons) that could affect any mosquito larvae inhabiting rainwater that collects within the tire casing. This study examined relationships between Zn, as a marker of tire leachate, and other environmental correlates on mosquito densities in discarded tires in the field and tested the effects of tire leachate concentration on the survival and development of newly hatched *Aedes albopictus* and *Aedes triseriatus* larvae in a controlled laboratory dose-response experiment. *Aedes albopictus* and *Ae. triseriatus* co-occurred in over half (22/42, 52.4%) of tires in the field, and *Ae. triseriatus* was only collected without *Ae. albopictus* in one tire. *Ae. triseriatus* was more strongly negatively associated with zinc concentration than *Ae. albopictus* and another common mosquito, *Cx. pipiens*. *Ae. albopictus* was the most widespread species, being collected in 88.1% (37/42), and was correlated with detritus amount, while *Ae. triseriatus* (23/42, 54.8%) and *Cx. pipiens* (17/42, 40.5%) were less widespread and both were correlated with *Ae. albopictus*. In the laboratory experiment, *Ae. albopictus* and survival steeply declined to zero from 10,000 to 100,000 mg/L leachate. In contrast, *Ae. triseriatus* and survival declined at the lower concentration of 100 mg/L leachate, and was zero at 500, 10,000, and 100,000 mg/L. These results suggest that the invasive *Aedes albopictus* has superior tolerance to tire leachate than the native *Aedes triseriatus*, and that this tolerance may contribute to its success and displacement of *Ae. triseriatus* in urban areas and resultant disease risk.

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### INFLUENCE OF RUBBER AND PALM CULTIVATIONS ON HUMAN EXPOSURE TO *Aedes aegypti* EVALUATED BY USING AN IMMUNO EPIDEMIOLOGICAL BIOMARKER

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In recent decades, numerous cases of arbovirus infections have been reported in Africa, mainly transmitted through infected *Aedes aegypti* bites. Their control is primarily based on anti-vector strategy and its efficient implementation requires an understanding of factors of risk of transmission linked to specific ecological settings. Environmental changes related to agricultural practices can impact upon arbovirus transmission, by influencing the vector species composition and their density which could, in turn, have an effect on the human-vector contact. The present study aims to assess the influence of oil palm and rubber plantations on a human exposure to *Ae. aegypti* bites, by using a new immunological tool which quantifies human IgG antibody (Ab) response to the *Aedes* Nterm-34kDa salivary peptide. Human IgG responses to the salivary peptide was assessed in 582 children living in different agro-ecosystem villages in Côte d'Ivoire: N'Zikro (rubber cultivation), Ehania-V5 (palm oil exploitation) and Ayébo (control village without plantations), in the dry and rainy seasons. In the dry season, specific IgG responses were significantly different between villages (P = 0.0089). The specific IgG level was significantly lower in Ayébo compared to Ehania-V5 (P = 0.0067) and N'Zikro (P = 0.0110). In contrast, specific IgG levels were similar between villages in the rainy season. As a consequence, specific IgG responses remained high in villages associated

with intensive agricultural during both seasons whereas, in the control village, a significant increase of the specific IgG response was observed ( $P = 0.0017$ ) in the rainy season compared to dry season. The present study indicated that rubber and oil palm plantations could maintain a high level of human exposure to *Ae aegypti* bites during both dry and rainy seasons. These agricultural activities could, therefore, represent a permanent factor of transmission risk of arboviruses.

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### MAPPING GLOBAL VARIATION IN DENGUE TRANSMISSION INTENSITY AND ASSESSING THE IMPACT OF CONTROL STRATEGIES

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Reliable local estimates of the intensity of dengue transmission (as characterised by the reproduction number,  $R_0$ ) are required for evaluating the potential impact of control measures and informing public health policy. Published global dengue maps give estimates of the burden of disease but not of transmission intensity. Using age-stratified seroprevalence and incidence data, combined with a geostatistical machine-learning model based on environmental factors which drive suitability of *Aedes aegypti*'s habitat, we have produced the first 1km resolution map of global dengue force of infection. Our force of infection predictions allowed us to also derive local estimates of the reproduction number and number of yearly dengue infections and cases. We predict high transmission rates in South America (Colombia, Venezuela and Brazil), Democratic Republic of Congo and south Asia (India, Indonesia and the Philippines). We estimated ~170 million infections annually, approximately half that of Bhatt et al. (2013). We also estimated an annual incidence of 80 million symptomatic infections. We then assessed the likely impact of two new control measures on dengue transmission intensity and disease burden: release of Wolbachia-infected mosquitoes, and introduction of childhood vaccination with the Sanofi dengue vaccine. The impact of both control measures varies as a function of local transmission intensity. Under the most pessimistic scenario examined, large scale release of Wolbachia might lead to a long-term reduction in dengue disease burden of 95%, rising to almost 100% reduction in more optimistic scenarios. We estimate that wide scale and near optimal use of the Sanofi dengue vaccine in line with WHO recommendations could reduce the global burden of dengue by approximately 20%. The high spatial resolution at which we have quantified dengue transmission intensity will help target and assess the potential impact of control measures.

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### MAPPING THE GLOBAL ESTIMATES OF DENGUE SEROPREVALENCE AND TRANSMISSION INTENSITY

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The first dengue vaccine (CYD-TDV) has been licensed for use in children >9 years of age in several countries. The World Health Organization recommends countries consider vaccination only in areas (national or subnational) with high endemicity corresponding to a seroprevalence of  $\geq 70\%$  in the target age group. Vaccination is not recommended where seroprevalence is  $< 50\%$ . Given these recommendations and the observed safety signal of the vaccine in seronegative recipients, it is important to know the proportion of potential vaccine recipients who might be seronegative. We have previously published work showing that the force of infection can be inferred by fitting catalytic models to age-stratified

seroprevalence and incidence data, and that estimates derived from the two data types are largely comparable. In order to present available estimates and provide some guidance to countries considering introducing the vaccine, a web tool was developed. The web tool maps currently available estimates of dengue transmission intensity at subnational levels, derived from age-stratified data on dengue: i) seroprevalence and ii) incidence, as well as the expected seroprevalence for several countries. The uncertainty around each estimate is also presented. The interactive web tool allows the user to explore how the transmission intensity relates to the expected seroprevalence at the target vaccination age group, or the expected age at which e.g. 70% seroprevalence is reached. The target age group or target seroprevalence can also be determined by the user. Users are also able to export all the estimates visualised on the website. Since the map only presents estimates for regions where data are available, it can also help to identify countries, regions, and sub-regions lacking dengue data which may inform surveillance systems. Although there are limitations such as the spatial heterogeneity observed in dengue transmission even at very small scales, having a baseline estimate of transmission intensity and seroprevalence at subnational level will be an important consideration for vaccine programmes deciding where to deploy the vaccine and who to target.

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### A MULTI-COUNTRY STUDY OF THE ECONOMIC BURDEN OF DENGUE FEVER IN SIX COUNTRIES OF DVI FIELD STUDIES: VIETNAM, THAILAND, COLOMBIA, CAMBODIA, BURKINA FASO AND KENYA

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Dengue is a major public health concern in the tropics and subtropics. With the first dengue vaccine licensed in several countries, it is critical to understand the economic burden of dengue fever to set health policy priorities for effective use of the vaccine. Dengue Vaccine Initiative (DVI) has conducted cost-of-illness studies in six countries: Vietnam, Thailand, Colombia, Cambodia, Burkina Faso, and Kenya. In order to capture all costs during the entire period of illness, patients were followed up on multiple interviews, after confirmation of dengue on rapid diagnostic tests on the first day of clinical visit, until the patients recovered from the current illness. Various cost items were collected such as direct medical and non-medical costs, indirect costs, and non-out-of-pocket costs. These cost components were presented as private, non-private, and societal perspectives. In addition, socio-economic factors affecting disease severity were also identified by adopting a logit model. We found that direct medical costs as patients' private expenditure were the highest in Vietnam and the lowest in Thailand where the universal healthcare system was implemented. The average direct medical costs in Burkina Faso (\$10) and Kenya (\$15) lied in between Vietnam and Thailand. In terms of direct non-medical costs, the two African countries were lower than the other four countries showing less than \$1 and \$3 in Burkina Faso and Kenya respectively. Among the first three countries, total cost per episode ranges from \$141 to \$385 for inpatient and from \$40 to \$158 outpatient, with Colombia having the highest and Thailand having the lowest. The percentage of the private economic burden of dengue fever was highest

in the low-income group and lowest in the high-income group. The logit analyses showed that early treatment, higher education, and better knowledge of dengue disease would reduce the probability of developing more severe illness. Our study indicates that substantial economic burden is caused by dengue and the findings can be used to consider vaccine introduction and prioritize alternative health interventions among competing health problems.

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### THE ROLE OF HETEROTYPIC NEUTRALIZING ANTIBODY IN PROTECTION FOLLOWING TRIVALENT DENGUE VIRUS VACCINATION AND CHALLENGE

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There are four dengue virus (DENV) serotypes, each capable of causing the full spectrum of illness ranging from an asymptomatic or mild infection to life-threatening disease. Typically, infection with one DENV serotype confers long-lived protection against symptomatic re-infection with the same serotype but only short-lived protection against a heterotypic serotype. However, following sequential infection with a second heterotypic DENV, cross-reactive immunity often provides protection against all four serotypes. Is a similar level of cross-protection elicited when multiple serotypes are encountered simultaneously, as would occur during live multivalent DENV vaccination, rather than sequentially, as occurs in nature? We sought to test this paradigm using our controlled human infection model (CHIM) developed for DENV-2. Twenty-four flavivirus-naïve adults were enrolled in a randomized, placebo-controlled trial. Eighteen subjects received a trivalent mixture of the live attenuated candidate DENV vaccines rDEN1Δ30, rDEN3Δ30/31, and rDEN4Δ30 (no DENV-2). Eight subjects received placebo. Six months later, all returning subjects were challenged with our DENV-2 CHIM strain. Following receipt of the trivalent admixture, all subjects seroconverted to DENV serotypes 1, 3, and 4, however, less than 40% of the subjects had evidence of cross-reactive neutralizing antibody to DENV-2. Twenty-one subjects returned for challenge (15 trivalent and 6 placebo recipients). Following challenge with DENV-2, four (27%) of those subjects who had received the trivalent mixture showed signs of breakthrough viremia and all subjects showed a significant boost in DENV-2 neutralizing antibody titer. All placebo recipients were viremic following challenge. Whereas efficacy against DENV-2 challenge viremia following tetravalent vaccination has been shown to be 100%, efficacy provided by the trivalent vaccination was only 73%. The complete clinical, virologic, and serologic responses following trivalent vaccination and challenge will be presented. The role of heterotypic antibody and cellular immune responses will be discussed.

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### VIRAL GENETIC DIVERSITY AND PROTECTIVE EFFICACY OF A CYD-TDV TETRAVALENT DENGUE VACCINE IN A PHASE 3 TRIAL IN ASIA

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CYD14 (NCT01373281) was a Phase 3 placebo-controlled efficacy trial of a tetravalent dengue vaccine (CYD-TDV) evaluated in children aged 2 to 14 years in Asia. It demonstrated a vaccine efficacy (VE) of 56.5% to prevent symptomatic, virologically confirmed dengue (VCD) of any severity during one-year post-vaccination follow-up. We conducted sieve analyses to evaluate variation in the level of VE with genomic features of breakthrough dengue sequences. Nucleotide sequences of the dengue virus (DENV) prM/E region were obtained from VCD endpoint cases via 454 sequencing, translated into proteins, and aligned with the four serotype-specific insert sequences of CYD-TDV. Extensions of the proportional hazards model were used to assess variation in VE with phylogenetic genotypes, amino acid (AA) patterns, and distances of breakthrough sequences to the vaccine strains. Of the four DENV serotype endpoints, VE significantly differed by DENV genetics only for DENV4, with differences restricted to 2–8 year-olds. In this age group, VE was significantly greater against the vaccine-matched DENV4-II (76.3%) than the vaccine-mismatched DENV4-I genotype (23.9%). Correspondingly, VE was significantly greater against DENV4 with a vaccine-matched residue at any of nine signature AA sites (pr73, M65, E46, E120, E160, E203, E329, E461, E478). The VE against DENV4 significantly decreased with an increasing percentage of vaccine-mismatched residues. We hypothesize that greater prior DENV exposure in 9–14 vs. 2–8 year-olds and their resultant broader specificity of immune responses to DENV sequences helped maintain VE against vaccine-mismatched DENV4 at a level that was comparable to that against vaccine-matched DENV4. This finding underscores the importance of improving vaccine-induced immune responses in younger children to achieve broader protection against DENV4. In addition, the similarity of the inserts to circulating viruses may partially explain how CYD-TDV's VE was greater against DENV3 and DENV4 than DENV1 and DENV2. Further investigation of the identified signature sites could increase knowledge of protective epitopes.

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### PROGRESS IN DEVELOPMENT OF TAKEDA'S TETRAVALENT DENGUE VACCINE CANDIDATE

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Takeda's live attenuated tetravalent dengue vaccine candidate (TDV) contains a molecularly characterized dengue serotype 2 virus (TDV-2), and three recombinant viruses expressing the pre-membrane (prM) and envelope (E) structural genes for serotypes 1, 3, and 4 in the attenuated TDV-2 backbone. Following the WHO guidelines for dengue vaccine development, Takeda has investigated different formulations, routes of administration, dosage schedules, and vaccine presentations, in several phase 1 and phase 2 studies in adults and children in dengue endemic and non-endemic countries. In an ongoing phase II placebo-controlled, multi-centre trial (ClinicalTrials.gov: NCT02302066), the safety and immunogenicity of different TDV vaccination schedules is being evaluated in ~1800 subjects from 2 to <18 years of age, living in dengue endemic areas of the Dominican Republic, Panama, and the Philippines. Here we present 18-month safety and immunogenicity results, including febrile illness surveillance, in which all reported febrile illnesses were investigated and DENV infections were laboratory-confirmed by serotype-specific RT-PCR and non-structural protein 1 ELISA. Progress of the phase 3 pivotal efficacy trial (TIDES) will also be presented.

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**CORRELATES OF RISK AND PROTECTION FOR CYD-TDV, THE FIRST LICENSED DENGUE VACCINE IN ENDEMIC COUNTRIES**

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Identifying a correlate of protection (CoP) is a major goal of vaccine research as it can predict vaccine efficacy in a new setting without the need to follow participants for the efficacy endpoint. However, very few CoPs have been identified recently. In Phase 3 efficacy trials of the CYD-TDV dengue vaccine in 2-14 year olds in Asia (NCT01373281) and in 9-16 year olds in Latin America (NCT01374516), estimated vaccine efficacy (VE) against symptomatic, virologically-confirmed dengue (VCD) between Months 13 and 25 post dose one was 57% and 61%, respectively. Case-cohort analyses assessed how dengue incidence and VE against VCD varied with dengue neutralizing antibody titers measured one month post-vaccination (Month 13) in 2848 vaccine and 1574 placebo recipients. Month 13 titers were assessed as correlates of risk using Cox regression accounting for the case-cohort sampling design, and assessed as CoPs using the principal stratification framework that studies how VE varies over vaccinated subgroups defined by Month 13 titers. For vaccine recipients in each trial, risk of dengue of each serotype significantly decreased with Month 13 homologous serotype titer (hazard ratios 0.19–0.43 per 10-fold increase); VE against dengue of any serotype significantly increased with average titer to the four serotypes (P values < 0.001). For 9–16 year olds pooled across trials ( $\geq 9$  is the licensed indication), VE estimates were 34.6%, 50%, 80%, and 97.5% for vaccine recipients with average titer of < 10 (below the assay limit), 82, 500, and 10,000, respectively. Month 13 neutralizing antibody titers positively correlate with vaccine efficacy to prevent dengue. The VE curve analysis concluded that high Month 13 titers predict high VE for all serotypes, pre-vaccination serostatus groups, age groups, and both trials. Other factors, however, may influence vaccine efficacy, especially at lower titers post third vaccination.

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**LOW LOA LOA BLOOD MICROFILARIA DENSITY IN HYPO-ENDEMIC ONCHOCERCIASIS AREAS IN NIGERIA: USING THE NEW LOASCOPE TECHNOLOGY TO DETERMINE WHERE IT IS SAFE TO TREAT WITH IVERMECTIN**

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Ivermectin treatment can rarely result in serious adverse events (encephalopathy and death) in persons with high *Loa loa* microfilariaemia (>30,000 mf/ml). In five states of southern Nigeria we conducted a study to determine whether it is safe to provide ivermectin MDA in onchocerciasis hypoendemic areas that are also *Loa loa* coendemic. Previous RAPLOA (a non-invasive rapid questionnaire assessment to determine history of eye worm) surveys conducted in this area showed many villages with a prevalence >40%, which should correlate with a prevalence of high intensity infections of >2% that would represent unacceptable risk for MDA. Our survey was conducted in 2016 in 110 ivermectin naïve villages located in districts likely to be hypoendemic for

onchocerciasis since they bordered districts currently receiving ivermectin MDA. Of the villages surveyed using RAPLOA prevalences, 28 had a prevalence of 40-60%, 57 had prevalences of 20%-39%, and 25 with prevalences of 10%-19%. We examined 10,605 residents of these villages aged 5 years and above using the "LoaScope" an innovative cellphone-based imaging device for rapidly determining the density of *Loa loa* infections at the point of contact. We found a mean village *Loa loa* prevalence of 8.4% (median 5.8%, 0 – 46.7% range). The maximum individual mf count was 11,429 mf/ml (mean individual mf count 20.1 mf/ml). Of the 2,756 persons sampled from the 28 villages with >40% RAPLOA, we were able to exclude the 2% threshold of high *Loa loa* intensity with high statistical confidence (p<0.0001). These findings indicate that ivermectin MDA can be delivered in these ivermectin naïve areas with low risk of *Loa loa*-related SAEs. We also concluded that in Nigeria the RAPLOA survey methodology is not predictive of high prevalence (>2%) of high density *Loa loa* microfilariaemia.

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**QUANTIFICATION OF VECTOR INFECTION THRESHOLD FOR MAKING PROGRAMMATIC DECISION ON STOPPING OR CONTINUING THE PROGRAM TO ELIMINATE LYMPHATIC FILARIASIS**

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Transmission Assessment Survey (TAS) is the recommended strategy for deciding to stop mass drug administration (MDA) and post-MDA surveillance until certification of lymphatic filariasis (LF) elimination. TAS is based on detecting filarial antigen in children to verify absence of transmission in an evaluation unit (EU). The currently available antigen assays, the immuno-chromatographic card test (ICT) or filarial test strip (FTS), are expensive, besides issues on the size of the EU. Molecular xenomonitoring (MX) could be a potential alternative to TAS especially when human infection levels are very low. However, application of MX, as an alternative to TAS requires a valid sampling strategy and an estimate of the vector infection threshold below which transmission ceases to occur. Parallel data on *W. bancrofti* infection in human and its vector *Culex quinquefasciatus* from 33 villages/wards (sites) from one of the primary health centres, in Thanjavur district, Tamil Nadu, India was used to quantify the relationship between human and vector infection. The study area has undergone 8 MDAs, and stopped MDA after a mass screening survey results showed that the Mf-prevalence is <lt> 1% and Ag-prevalence in children is <lt> 2%. The vector infection prevalence was assessed by detecting parasite DNA in pools of gravids collected from each site. Logistic regression analysis was done to quantify the relationship of Ag-prevalence in community or children with community Mf-prevalence and to derive an estimate of the vector infection threshold. A significant linear relationship was observed between prevalence of vector infection and the Ag-prevalence in children (r=0.44, P<lt>0.01). The value of the intercept, 0.9% (95CI: 0.5-1.3%) provides an estimate of the vector infection threshold below which transmission would cease to occur ('zero' Ag-prevalence in children). The lower 95% CI of 0.5% could be a safe threshold for stopping / continuing MDA. The estimated vector infection threshold of 0.5% indicates that the currently recommended Ag-prevalence threshold of 2% in children for stopping MDA needs reconsideration to prevent the risk of LF-resurgence.

## A SIGNIFICANT STEP TO WARDS LYMPHATIC FILARIASIS ELIMINATION IN CAMEROON: THE DISEASE IS NOT ENDEMIC IN 31 HEALTH DISTRICTS CO-ENDEMIC WITH LOA LOA AND HYPOENDEMIC FOR ONCHOCERCIASIS

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Mapping conducted in 2010-2012 using immunochromatographic tests (ICT) showed 156 health districts (HDs) are endemic for lymphatic filariasis (LF) in all ten regions of Cameroon. Mass drug administration (MDA) using ivermectin and albendazole (ALB) was launched in endemic districts to eliminate LF with funding from the USAID. However, due to co-endemicity with *Loa loa*, entire or part of 31 HDs highly endemic for loiasis were not included in the MDA. This hinders the LF elimination process in the country. In view of cross-reactivity of ICT cards with other non-LF filarial antigens and in preparation for implementing a biannual ALB MDA for LF elimination, a confirmation survey was conducted in 2016 in 31 HDs (13 HDs in East, 10 HDs in Center, 4 HDs in South and 4 HDs in the Littoral) using Filariasis Test Strips (FTS) and thick blood films (TBF) were used to detect microfilaria (mf) for the FTS positive cases. The survey was conducted in line with the WHO LF mapping recommendations, but four sites/villages each HD were purposefully selected to give better geographical representation. In total, 14,577 participants were selected and tested with 40% of them between 9-14 years and 60% ≥ 15 years old. The results showed that 5 HDs had <1% FTS positives in all sites and are not considered LF endemic. 26 HDs had at least one site with ≥1% FTS positives (site prevalence range: 0-11%): 12 HDs in East, 9 HDs in Center, 4 HDs in South and 1 HD in Littoral. Among a total of 235 FTS positives, 210 were tested by day TBF and 185 by night TBF. No LF microfilaria (mf) was identified in any FTS positive cases, while *Loa loa* or *Mansonella perstans* mf was found from 135 and 28 cases respectively in day TBF, and from 113 and 30 cases in night TBF. The results suggests that there is no indication of LF endemicity in these 31 HDs, as the FTS positives may have been mainly due to cross-reactivity with *L. loa* or *M. perstans*. There is no need of LF MDA in these HDs. This takes Cameroon a great step forward towards LF elimination by the year 2020.

## PHARMACOKINETICS OF TRIPLE DRUG THERAPY IN PATIENTS WITH AND WITHOUT WUCHERERIA BANCROFTI INFECTION

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Recently we have shown a single co-administered dose of diethylcarbamazine (DEC), albendazole (ABZ) and ivermectin (IVM) is much more effective in sustained elimination of *Wuchereria bancrofti* microfilariae (Mf) compared to DEC+ABZ or IVM+ABZ currently used for

mass drug therapy for lymphatic filariasis (LF). However, the effect of active LF infection on the pharmacokinetics of this triple drug regimen are not known. The primary objective of the present analysis was to evaluate the pharmacokinetics of DEC, IVM and ABZ when administered to patients with and without LF infection. A total of 56 participants (32 infected and 24 non-infected) were enrolled in the study. Twelve plasma samples per subject were collected from 0 - 168 hours post-dose of DEC (6mg/kg), ABZ (400mg) and IVM (0.2mg/kg). Plasma samples of DEC, ABZ, albendazole sulfoxide (ABZ-OX), and albendazole sulfone (ABZ-ON) were measured simultaneously using liquid chromatography-mass spectroscopy. Ivermectin was measured separately using an established liquid chromatography method with fluorescence detection. Pharmacokinetic parameters were determined using standard non-compartmental analysis methods. Statistical analysis was performed using JMP software. The maximum concentration (C<sub>max</sub>) mean (range) values in LF infected patients for DEC, IVM, ABZ, ABZ-OX and ABZ-ON were 2602 (1728-3367), 87 (25-173), 56 (2-263), 407 (118-1256) and 24 (7-114) ng/mL, respectively. The mean (range) values of AUC<sub>0-∞</sub> for DEC, IVM, ABZ, ABZ-OX and ABZ-ON were 41930 (22749-69500), 2395 (693-6469), 250 (12-1196), 5584 (1352-18108), and 346 (80-1343) hr\*ng/ml, respectively. The mean AUC<sub>0-∞</sub> and C<sub>max</sub> for each drug was not different between patients with and without LF infection. Although all patients with parasitemia at diagnosis were clear of infection by 39 hours post treatment there was significant variation in AUC<sub>0-∞</sub>. The relationship between drug levels and clearance of Mf and adult worm one-year post-treatment will be reported. In conclusion, LF infection does not alter the pharmacokinetics of this three drug regimen.

## HYDROCOELE SURGERY FOR LYMPHATIC FILARIASIS: MEASURING THE IMPACT ON PATIENT CAREGIVERS IN MALAWI

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Lymphatic filariasis (LF) is a neglected tropical disease (NTD) targeted for elimination as a public health problem by 2020. Hydrocoele is the most common clinical manifestation, which affects approximately 19.4 million men worldwide and can be cured by surgery. Whilst surgery has been shown to positively impact men's lives by improving their physical and socio-economic outcome; there has been no research on how this may impact the people who care for them (caregivers). To explore this further, the main caregivers of hydrocoele patients in Malawi were surveyed pre- and post-surgery. A semi-structured questionnaire quantified differences in the level and type of assistance required, the number of days off work or school necessary to care for patients, and their own socio-economic wellbeing based on a scoring method (1=no problem, 2=mild, 3=moderate, 4=severe). In total, 40 caregivers were surveyed: mean age 36 years, majority female (75%; predominately wives), and approximately half illiterate (55%). Pre-surgery, 29 caregivers (72.5%) assisted men with at least one activity, which reduced to nine caregivers (22.5%) post-surgery, and most commonly was helping with the farm. Pre-surgery, caregivers were unable to work or attend school on average for 8.2 days per month, which reduced to zero days' post-surgery. Furthermore, positive significant differences were found when comparing caregivers pre- and post-surgery scores (p<0.05) in relation to their own usual living activities (ability to do their own job, household activities and leisure activities), social issues (joining in regular social activities in and outside the home, and romantic relationships), and psychological health (worried about their own health, their own future, feeling neglected from friends or family, and the ability to plan for the future). This study highlights the wider burden of filarial hydrocoele, and that both the patient and their caregivers can significantly benefit physically, economically, educationally, socially and psychologically from the outcome of surgery.

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### LYMPHATIC FILARIASIS TRANSMISSION VARIATION WITHIN AN IMPLEMENTATION UNIT - THE CASE OF THE LIMBE COMMUNE IN THE NORTH DEPARTMENT OF HAITI

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Lymphatic Filariasis (LF) was determined to be endemic in all communes in Haiti in 2000-2001 after an extensive mapping exercise. The Haiti Neglected Tropical Diseases (NTD) Control Program, in line with the global goal, aims to eliminate LF as public health problem by 2020. There has been considerable progress to date; mass drug administration (MDA) has been conducted in all 140 communes, and 85% of communes have stopped MDA and are in the post-MDA surveillance phase. However, LF transmission persists in 21 communes, including Limbé commune in the North Department. Limbé passed a school-based transmission assessment survey (TAS) in 2015 in which 12 of 1,411 6-7 year old children tested were LF-antigen-positive (critical cutoff for antigen positives was 16). Of note, most of the positive individuals were geographically concentrated in schools around downtown Limbé. In a community-based TAS conducted in Limbé in the last quarter of 2016, 4,237 people were tested including 1,412 6-7 year old children from randomly selected households throughout the commune. In total 117 positives were found, including 25 6-7 year old children. The majority (n=93; 79%) of those who tested positive lived within a 5km radius of the downtown Limbé area and more than 50% (n=59) were within 1km of downtown Limbé; although the latter radius represents less than 3% of all the Limbé commune area. This pattern suggests a concentrated transmission area around the urban center. The NTD Control Program strategy of MDA and TAS is uniformly applied throughout implementation units (IU) and MDA coverage is calculated for whole IUs, but TAS results are applied across a widespread area. Our data demonstrate the need for revised strategies for evaluating disease prevalence that address areas with heterogeneous disease patterns, as in Limbé.

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### MODELLING THE ROLE OF LONG LASTING INSECTICIDE-TREATED BEDNETS IN THE REDUCTION OF LYMPHATIC FILARIASIS PREVALENCE ACROSS A RANGE OF SETTINGS

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Since being marked for elimination by 2020 by the World Health Organization (WHO) six countries have been confirmed to have eliminated lymphatic filariasis (LF) as a public health problem; another 13 have completed 4-6 rounds of annual mass treatment and are in surveillance to confirm interrupted transmission. However, 29 of the 54 countries that still need to complete treatment by 2020 will need enhanced strategies. Long lasting insecticide-treated bednets (LLINs) have long been a vital tool in malaria control, but have only recently started being used against LF. Following interrupted transmission in The Gambia, despite no antifilarial distribution, there is evidence to suggest that LLINs could play a significant role in achieving the 2020 goals in areas with anophelene mosquitoes. We combine a standard model of LF infection with methods of modelling LLIN-influenced vector dynamics adapted from the malaria literature. Firstly we demonstrate that low prevalence, as in The Gambia, can plausibly be achieved using bednets over these timescales; secondly we explore LLIN coverage in a variety of settings. Model results predict LLINs to have more impact than previous studies suggest, with coverage of 40-60%

sufficient in low endemicity settings to achieve the 1% microfilaria (mf) prevalence WHO threshold for cessation of drug programs. In the 29 countries requiring additional measures LLINs could play an important role in achieving the 2020 goals and avoid a scale up drug usage. Introducing MDA to the model shows that implementation of LLINs could force mf prevalence to below the targeted 1% in settings where MDA alone would be insufficient. This highlights the need for further data-driven analysis into the impact of LLINs and indicates a scale-up in the worldwide use of LLINs could be vital in tackling LF transmission.

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### ATTENUATED VARIANT OF LEISHMANIA DONOVANI CAUSES CUTANEOUS LEISHMANIASIS IN SRI LANKA

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*Leishmania donovani* is the etiological agent of visceral leishmaniasis in the Indian subcontinent. However, it almost exclusively causes cutaneous leishmaniasis (CL) in Sri Lanka with over 6000 cases reported during the past decade. Visceralization potential of *L. donovani* in Sri Lanka (*L. donovani*-SL) is yet unknown. This study aimed to investigate the disease sequelae of CL patients through long-term follow-up and virulence of parasites through *in vitro* experiment. Patients (n=250) were recruited via passive case detection, after laboratory confirmation of diagnosis and were treated with weekly intra-lesional sodium stibogluconate up to 10 doses (IL-SSG) and followed up weekly for 3 months and bi-annually thereafter, up to 4 years. Patients were examined by a Physician at each follow up, to assess healing and to detect sign(s) of visceralization. Patients' serum samples were tested for anti-*L. donovani* antibodies (rK39 dip stick assay). Macrophage cultures were infected with *L. donovani*-SL(10:1, parasite: macrophage) and with *L. donovani*-1S as a positive control. Measures of infection efficiency, intracellular growth and parasite survival in infected macrophages were recorded. The age range of patients was 1-70 years (median=40); male to female ratio was 3:1 (179:71). There were no signs of systemic infection and no evidence for the presence of anti-*L. donovani* antibodies observed during the follow up period. Treatment success of IL-SSG was 76.4% and the remaining patients (23.6%) were cured following extra 4-9 doses. Recurrence of lesions was observed in 9 patients. In the *in vitro* study, infection efficiency and intra-cellular growth of *L. donovani*-SL was significantly lower at 48 hrs post-infection when compared to *L. donovani*-1S. The survival rate of *L. donovani*-1S was as twice as that of *L. donovani*-SL, at 48 and 72 hr post-infection. Sri Lankan *L. donovani* appears to be essentially dermatotropic. Diminished infection efficiency, intracellular growth and survival of *L. donovani*-SL suggest a natural attenuation of virulence of *L. donovani*-SL strain(s), which is likely to contribute to the resultant atypical clinical picture.

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### FROM DECIPHERING THE SPECIFIC IMMUNE RESPONSE TO A NOVEL BIOMARKER FOR MONITORING CHAGAS DISEASE

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One of the fundamental medical needs for Chagas disease is a qualified test to assess treatment efficacy, and spontaneous cure, in *T. cruzi*-infected patients. The current consensus for parasitological cure is to monitor conversion to negative serology. However, seroreversion by standard tests

can take many years and up to decades. Hence, the current strategy, for investigational settings, is to use PCR to measure parasitemia as primary endpoint. PCR, especially qPCR, is costly and can still fail to detect parasites because parasitemia is extremely low and fluctuating over time in chronic patients. This is an important concern not only for the development of novel drugs but also for evaluation of clinical trials and the clinical management of Chagas disease patients. In this study we devised an innovative multi-parametric screening technology to identify surrogate antibody biomarker(s) that can be instrumental to monitor parasite persistence regardless of PCR results. We analyzed the diversity of antibody response against 15 well-selected *T. cruzi* peptide and protein antigens in a large sample collection from chronic Chagas patients (SaMi-Trop cohort), either BZN-treated or untreated, and revealed reliable changes in these responses that are associated to therapy. Using an interdisciplinary approach, we explored our experimental data with different mathematical models and were able to show that one unique and novel antibody (Ab3) is considerably more meaningful than PCR to monitor *T. cruzi* parasite persistence (information derived from PCR positive data). Importantly, the dynamic decline of the antibody titer, upon drug treatment, also seems to perform well in longitudinal studies (Gates-Slim, McGill-HUG). The performance of Ab3 has, so far, been tested and validated on over 2000 patients and four independent cohorts i.e. SaMi-Trop, REDS II, Gates-Slim, McGill-HUG. A simple immunoassay measurement of Ab3 has not only the potential to act as a treatment efficacy indicator but could also serve as a flag which guides the medical decision prior to therapy.

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#### **PERSISTENCE OF *TRYPANOSOMA CRUZI* DNA COPIES BY QUANTITATIVE REAL TIME PCR 12 MONTHS AFTER TREATMENT WITH BENZNIDAZOLE AMONG CHILDREN AGED 4-15 YEARS OLD IN BOLIVIA**

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Chagas disease is a parasitic infection caused by *Trypanosoma cruzi* that remains endemic in Latin American countries. In Bolivia, the National Program for Chagas has been implemented for 10 years and the natural infection is greatly reduced in number. Therefore, the program moved to initiate a treatment regimen of Benznidazole (5mg/kg/day) for 60 days in seropositive children aged 4-15 years living in areas certified as vector controlled areas. The efficacy and complications of the treatment for chronic Chagas remains controversial. We performed a 12 month follow-up study in collaboration with the departmental program of Chagas Control in Santa Cruz, Bolivia. From 2012 to 2015, 90 seropositive children were recruited from six regional hospitals and performed 60 days treatment. 3 ml of blood samples were collected 7 times as follows: (1) before treatment, (2) 30 days after the initiation of treatment, (3) 60 days, (4) 4 months, (5) 6 months, (6) 8 months and (7) 12 months. The quantitative real-time PCR assay (qPCR) with a dual-labeled TaqMan Probes was performed in each specific time points. The median age was 9 years, and 13 children experienced a side effect of treatment with one case being diagnosed as Stevens-Johnson syndrome. 17 children out of 46 participants from whom samples collected 12 months after initiation of therapy were available showed qPCR positive results though parasitemia levels were lowered when compared with initial sample results collected before start of the treatment. Because natural infection never occurred

in this area, this study indicates 37% of treatment failure 1 year after treatment. However, further follow-up should be done to clarify the meaning of this persistence of DNA copies in the peripheral blood.

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#### **BENZINIDAZOLE TREATMENT IS ASSOCIATED WITH *TRYPANOSOMA CRUZI* BLOOD PCR NEGATIVITY AND LESS CARDIAC LESIONS IN CHAGAS DISEASE: NIH SAMITROP STUDY**

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Chagas Disease (ChD) remains an important cause of cardiomyopathy in Latin America. Although persistence of tissue parasitism seems to play a role in the pathogenesis ChD, the efficacy of anti-parasite treatment with Benznidazole (BZN) in chronic phase remains unclear. The purpose was to evaluate if previous treatment with BZN is associated with less advanced cardiac disease and lower parasitemia positivity in patients with chronic ChD. The study was conducted using baseline data of the NIH-sponsored SaMi-Trop cohort, established in a highly endemic region of Brazil in which the vectorial transmission has been interrupted. Patients with reported ChD were submitted to interview, reporting previous use of BZN, NT-ProBNP measurement and ECG. ChD subjects that received at least one course of treatment with BZN were classified as treated group (TrG) and we used a pairing method (genetic matching) to select a comparison group (CG) of patients with similar characteristics, using gender, age, income, literacy, time of known ChD and hypertension as covariates. Clinical outcomes were: presence of major ECG abnormalities typical for ChD, age-adjusted NT-ProBNP levels suggestive of heart failure or both, and the parasitological outcome was PCR positivity for *Trypanosoma cruzi* in blood. Results of this paired analysis were compared with logistic regression and generalized estimating equations. From 1,959 patients with positive serology (EIA), 1803 with NT-proBNP results were studied (mean age: 59 years, 68% female). A total of 493 patients reported previous use of BZN (TrG) (average time of use: 90 days, 75% treated > 5 years before). Matched CG had frequencies of covariates similar to the TrG. Parasitological and clinical outcomes were worse in CG. A similar effect size was observed for whole sample using conventional multivariate analysis. When compared with a similar control group, patients previously treated with BZN had significantly reduced parasitemia positivity and lower frequency of markers of cardiomyopathy severity in ChD. Use of BZN may be related to better clinical and parasitological outcomes, if used in the early phases of the disease.

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#### **POTENTIAL IMPROVEMENT IN THE DIAGNOSIS OF CANINE VISCERAL LEISHMANIASIS IN BRAZIL BY IMPLEMENTATION OF AN ELISA TEST USING RECOMBINANT PROTEINS OF *LEISHMANIA***

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Visceral Leishmaniasis (VL) caused by *Leishmania infantum*, is a serious neglected tropical disease and a public health problem worldwide distributed, being dogs the main urban reservoir of the disease.

Identification and euthanasia of infected dogs are one of the main strategies for VL control recommended by the Brazilian Ministry of Health. Thereby, make the accurate diagnosis is crucial to correctly identify animals infected with *L. infantum*. Twelve recombinant antigens were selected from a cDNA library of *Leishmania infantum* due to their strong potential as candidates in diagnostic testing. From the 12 recombinant antigens, 1 was selected using the antigen trial techniques of Multiple Antigen Print Immuno Assay (MAPIA) and ELISA immunoassay. The present study aimed to compare the accuracy of the commercial ELISA (EIE Bio-Manguinhos), actual confirmatory test recommended by Minister of Health in Brazil, and an ELISA using the selected antigen (rLci5). The accuracy was evaluated using sera obtained from 153 infected dogs (108 symptomatic and 45 asymptomatic) and from 117 negative dogs. All the samples were selected according to the results of culture and real-time PCR using splenic aspirate of dogs, evaluated in an endemic area of canine VL (CVL) in Brazil. Sensitivity obtained were 80%, 73%, and 64%, respectively for ELISA Lci5, DPP CVL, and EIE Bio-Manguinhos. Specificity was 92% for ELISA Lci5, 96% for DPP CVL and 90% for EIE Bio-Manguinhos. The accuracy was 85%, 83%, and 75%, respectively for ELISA Lci5, DPP CVL, and EIE Bio-Manguinhos. Then we compared the performance of the actual protocol of CVL diagnosis, recommended by Brazilian Minister of Health, using DPP CVL as screening test and EIE Bio-Manguinhos as confirmatory test, and the altered protocol, replacing the EIE Bio-Manguinhos by ELISA Lci5. The actual protocol presented sensitivity of 58%, specificity of 97% and accuracy of 75%, while the altered protocol presented a higher sensitivity (68%), specificity (99%) and accuracy (81%). We concluded that ELISA rLci5 is a promising test that could replace the EIE Bio-Manguinhos and improve the diagnostic of the CVL in Brazil.

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#### PROGNOSTIC MARKERS OF DEATH FOR CHAGAS DISEASE IN REMOTE AREAS OF BRAZIL

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Chagas Disease (ChD) is an important cause of death in Latin America. Impaired left ventricular function is the most consistent predictive marker of death in longitudinal studies. However, in remote areas, where echocardiogram is not available, there is a need for alternative markers to evaluate risk of death. We have established a large cohort (SaMi-Trop) to evaluate markers of disease progression in a highly endemic region of Brazil. Twenty-two small municipalities in which vectorial transmission was interrupted, were included in the study. Inclusion criteria were individuals with confirmed seropositive for ChD and ECG abnormalities at baseline. The participants were submitted to an interview, sample collection, ECG, and followed for two years. Cox proportional-hazard model including sex, age, New York Heart Association (NYHA) class, Nt-ProBNP, number of major abnormalities on ECG and self-reported health state was used to access the risk for death. We included in this analysis 1,584 patients, 537 man (33.9%), and Nt-ProBNP values were  $\geq 300$  pg/mL in 550 (34.7%), and 3 or more ECG major abnormalities were detected in 89 (5.6%) of them. During the two years of follow-up 125 patients (7.9%) died. Age (HR: 1.01[1.002-1.03]), 3 or more major abnormalities in ECG (HR: 2.09[1.001-4.37]) and Nt-Pro-BNP  $\geq 300$ (HR 5.28[3.25-8.58]) were significantly associated with death. Our data suggest that Nt-Pro-BNP could be used as an alternative marker of risk of death when echocardiogram is not available.

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#### MAXIMIZING THE UTILITY OF VL CLINICAL TRIAL DATA WITHIN AN ETHICAL DATA-SHARING FRAMEWORK

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Over the last decades a substantial amount of trials measuring the efficacy of antileishmanial drugs have been carried out, but these data are scattered across geographical regions and published trial reports lack detailed information. Data on more than 35,000 patients enrolled to clinical trials internationally have never been pooled or standardised to allow for meta-analysis. These data could provide the necessary evidence to fill knowledge gaps, optimise treatment and guide future research. A variety of factors should be taken into account when considering the best therapeutic options to cure VL patients, including geographical area, drug resistance, and co-morbidities such as malnutrition or HIV co-infection. It is currently impossible to compare the efficacy of differing drugs, regimens and regions, since only summary statistics of VL clinical trials are available, and trial reports lack standardisation. A central database populated with individual patient data from clinical trials would improve our understanding of the clinical outcomes of VL treatments by enabling the pooling and comparison of the existing, diverse clinical trial datasets. Engaging the VL research community in developing a data platform, identifying relevant scientific questions and analysing the data would be critical to the success of such an enterprise. The involvement of policy-makers would also be critical in setting the research agenda and adapting treatment policy to reflect the resulting evidence. A partnership between the Infectious Diseases Data Observatory and the Drugs for Neglected Diseases initiative has engaged investigators in the planning and design of a VL data repository. An initial pilot project and systematic search of published and unpublished literature has assessed the feasibility of a VL data-sharing platform to facilitate the amalgamation and standardisation of available data. A VL data-sharing platform would maximise the utility of existing resources and enable researchers to address priority questions in VL treatment within a framework that recognises the contributions of data contributors and clinical trial participants.

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#### EFFECTS OF HABITAT PERTURBATION ON RODENT ASSEMBLAGES AND THEIR GEOGRAPHIC DISTRIBUTIONS ALONG THE INTER-OCEANIC HIGHWAY IN MADRE DE DIOS, PERU

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Human development results in migration into previously uninhabited natural environments with consequent habitat perturbation including changes in land use and cover. Migration increases contact between humans and animals with the potential transmission of zoonotic pathogens. As part of a multidisciplinary network of investigators from Peru, Bolivia, Ecuador and the United States, we studied the influence

of human habitat perturbation on the risk of rodent-borne diseases. We studied habitat perturbation caused by the construction of roads through forests in Peru, Bolivia, and Ecuador. We worked along the inter-oceanic highway (IOH) through the southern Peruvian Amazon, in the Madre de Dios Region, bringing growth in human settlements and forest disturbance. To explore longitudinal changes in rodent populations, we trapped small mammals every 4 months for 4 years at 4 sites along the IOH. Permanent trapping grids were set on gradients from unperturbed to highly perturbed habitats. Rodents were caught, marked, and released with exception of terminal captures at the end of each trapping trip. Landscape changes were recorded in-situ using GPS photography and satellite images. We captured 1130 animals (14 species) with a 3% recapture rate. Rodents of the species *Oligoryzomys microtis* (522/46%) were caught in the greatest numbers followed by *Necomys lenguarum* (153/ 14%) and *Euryoryzomys nitidus* (123/11%). Three of the species captured, *O. microtis*, *N. lenguarum*, and *Necomys spinosus* (47/4%) have been shown to be reservoirs of Rio Mamoré and Andes hantaviruses, causative agents of hantavirus pulmonary syndrome. The majority of animals were trapped in dry (479) and dry-to-rainy (378) seasons. Changes in land use and land cover were obvious and happened faster than expected; preliminary analysis of remote sensing data show 26% forest loss from 2001-15, mainly due to forest conversion into cropland, etc. Analyses of associations between rodent assemblages, species distribution and habitat perturbation, as well as pathogen testing on rodents are ongoing and will be presented at the meeting.

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### PARASITES IN THE PARK PART 2: AN EPIDEMIOLOGIC STUDY OF *TOXOCARA SP.* IN NYC PLAYGROUNDS AND USE OF A NOVEL SOIL-TRANSMITTED HELMINTH IDENTIFICATION TOOL

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*Toxocara* species are common pet parasites that can be found in the stool of dogs and cats. Eggs released in the stool, subsequently become infective in soil, where they survive for many years, and can be ingested by children who encounter them on playgrounds. *Toxocarasis* is listed by the CDC as one of five neglected parasitic infections in the US. It is considered a neglected disease of poverty, remaining largely underdiagnosed. Infection in humans can lead to visceral or ocular larva migrans, blindness, and silent brain infection that can diminish neurological cognition. It has been suggested that certain NYC neighborhoods may pose a higher risk for *Toxocara* infection, particularly in lower socioeconomic communities. The specific goals of the study are to: 1) determine the burden of *Toxocara* in parks 2) species determination. Soil is obtained and samples are analyzed by modified soil flotation method and standard microscopy. Speciation is achieved by multi-parallel quantitative real-time PCR (qPCR) and a novel DNA extraction method using specific primers to *T. cati* and *cani*. Preliminary results from 80 sites spanning NYC, identify eggs in 34% of sites, with considerable variation between the different boroughs. For example, 55% of samples from the Bronx were positive, compared to 41% in Staten Island, 31% in Queens, 27% in Brooklyn and only 25% of Manhattan samples. 75% of the *Toxocara* in Bronx samples were noted to be in infective stages, compared to none of the other sites tested thus far. qPCR data shows a Spearman value of 0.900 ( $p=0.08$ ), with a trend toward significance identifying *Toxocara cati* as the only contaminating species. This data strongly indicate that (i) *Toxocara* is common in play areas, (ii) prevalence of eggs is likely secondary to feline contamination, and (iii) a substantial health risk exists, particularly in poorer areas. Other parasites were noted during microscopy and qPCR will be used to identify these species using a novel soil-transmitted helminth identification tool for zoonotic infections especially looking to identify *Ancylostoma sp.* and *Baylisascaris* species.

### INVASIVE *POMACEA* SNAILS AS NEW HOST OF *ANGIOSTRONGYLUS ANTONENSIS* IN LAOS, CAMBODIA AND VIETNAM: IMPLICATION FOR OUTBREAKS OF EOSINOPHILIC MENINGITIS

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Angiostrongyliasis, caused by infection with the rat lungworm, *Angiostrongylus cantonensis*, is a major cause of eosinophilic meningitis in tropical and subtropical regions. While *A. cantonensis* completes its lifecycle between rats and mollusks, humans become incidental hosts through consumption of infected snails. Due to limited diagnostic infrastructure in tropical settings, the geographic distribution of *A. cantonensis* is poorly understood. Although eosinophilic meningitis is commonly reported in Thailand and China, there are fewer reports in the interjacent continent, including Laos, Cambodia and Vietnam. We conducted a multi-country survey to investigate the prevalence *A. cantonensis* infection among freshwater snails in the region. We selected 17 sites along the Mekong river and eastern coast, collected at least 50 snails from each site, and examined them for the presence of *A. cantonensis* by microscopy. We confirmed the species and examined genetic diversity by sequencing the complete cytochrome c oxidase subunit I (*cox1*) gene, and assembling a Bayesian phylogenetic tree incorporating all published *Angiostrongylus cox1* sequences. Among the 17 study site, 15 sites were infested by *Pomacea spp.* snails, a known host for *A. cantonensis*. Among these, we detected *Angiostrongylus* in 7 sites, all in Cambodia and Vietnam. The prevalence in *Pomacea* snails ranged from 1% to 16% between the sites. Further genetic identification confirmed that all specimens were *A. cantonensis* and were closely related genetically. These findings indicate that *A. cantonensis* is widely distributed in Southeast Asia, particularly along the lower Mekong river and the eastern coast of Vietnam. Surveillance studies among snails may represent a powerful tool for risk-mapping of Angiostrongyliasis. This approach could assist targeting of public health campaigns to avert outbreaks of eosinophilic meningitis in this region.

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### RISK FACTORS FOR MIDDLE EAST RESPIRATORY SYNDROME CORONAVIRUS (MERS-COV) SEROPOSITIVITY AMONG ANIMAL MARKET AND SLAUGHTERHOUSE WORKERS IN ABU DHABI, UNITED ARAB EMIRATES (UAE), 2014-2016

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Population seroprevalence of MERS-CoV is low in countries with active MERS-CoV transmission, with higher seroprevalence in workers with regular camel contact. Camel contact has been shown as a risk factor for MERS-CoV infection but exposures associated with MERS-CoV seroprevalence in camel workers are not fully understood. In this study,

seroprevalence was assessed in animal market and slaughterhouse workers, alongside a survey detailing duration, intensity, and types of contact with camels, and other risk factors, e.g. chronic medical conditions. Seroprevalence surveys were performed in 2014, 2015, and 2016, and an epidemiologic survey in 2016. Workers were recruited from two slaughterhouses and one live animal market on a shared site in the Eastern Region of Abu Dhabi Emirate. All facilities worked with sheep, goats, cattle and camels. Human sera were tested for anti-MERS-CoV antibodies using indirect ELISAs for nucleocapsid (N) and spike (S) proteins followed by a confirmatory microneutralization test. Samples were considered positive if either positive on (1) both N and S ELISA, or (2) N or S ELISA and microneutralization. Logistic regression was used to identify factors associated with seropositivity. During 2014-2016, 44/298 (14.8%) workers were seropositive. Seroprevalence was highest in camel salesmen (19/46, 41.3%) and butchers (16/99, 16.2%) relative to other occupations. Of 46 re-tested seronegative workers, two seroconverted. On univariate analysis, handling camels, feeding camels, cleaning camel housing, and contact with ill camels were associated with seropositivity. On multivariate analysis no specific exposures were associated with seropositivity. All significant risk behaviors were performed predominantly by salesmen, the occupation with the highest odds of seropositivity (odds ratio 6.5, confidence interval 1.2-32.9). Workers in this study showed higher seroprevalence than previous studies. Camel salesmen had higher seroprevalence compared to other occupational groups and performed many daily tasks involving direct camel contact. These results are important for communicating infection risk.

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### EMERGING BAT PATHOGENS IN MYANMAR: A ROAD MAP FOR SURVEILLANCE OF POTENTIAL SPILLOVER RELATED TO CAVE UTILIZATION

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Cave-dwelling bats are a potentially significant reservoir of emerging viral threats across Southeast Asia. In Myanmar, cave activities like ecotourism and religious shrine visitation increase the likelihood of bat-human interactions, and, thus, the possibility for zoonotic disease exposure. There are seventy-two identified species of cave-dwelling bats present in Myanmar; however, research on the infection status of these bats is limited. This study attempts to delineate potential high-contact zones, based on spatial analysis of bat habitat, cave utilization, and detection of viruses that pose high risk of morbidity and mortality. With tens of thousands of Buddhist sacred sites across Myanmar, tourism to cave shrines is rapidly increasing, as are the number of commercial guide outfitters that advertise cave-tours and exploration, making these sites more accessible. Guano extraction from caves is a source of revenue for small family operations, while large-scale limestone extraction operations also exist. Published and informal data sources regarding human use of caves were compiled, as well as published detections of filo-, corona-, paramyxo-, or lyssa- viruses presented in proximity to Myanmar. Using bat species range data maintained by the International Union for the Conservation of Nature (IUCN), the range extent for each species was refined with sensitivity to elevation suitability, and then used to map the density of species linked to each viral family of import. Spatial analysis indicated regions with dense presence of bat species identified as possible reservoirs with known high-traffic cave locations. These "high contact potential zones" provide a spatial roadmap for future investigations and priority areas for outbreak surveillance.

## 644

### MEAT AND FISH AS A SOURCE OF EXPOSURE TO ANTIBIOTIC-RESISTANT ENTEROBACTERIACEAE IN PHNOM PENH, CAMBODIA

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Extended spectrum  $\beta$ -lactamase (ESBL) genes encode resistance to penicillins and cephalosporins and can be horizontally transferred among Enterobacteriaceae. More than 60% of healthy humans living in southeast Asia are faecal carriers of ESBL-producing Enterobacteriaceae (ESBL-PE), compared to <10% in Western Europe, suggesting diverse exposure routes. In Cambodia, meat and fish consumption is relatively high, antibiotic use in animal agriculture is unmonitored, and food safety is poorly enforced. We hypothesized that meat and fish could be a reservoir for community exposure to ESBL-PE. From September-November, 2016, we conducted a meat sampling study in Phnom Penh in collaboration with the BIRDY program (<http://www.birdyprogram.org/>), an ongoing study of neonatal health in low-income countries. We evaluated ESBL-PE contamination among pork, fish, and chicken from two markets in the neighbourhood where healthy mothers participating in BIRDY reside. We used logistic regression to evaluate whether stall hygiene, meat storage practices, and reported animal antibiotic use were associated with ESBL-PE contamination of meat and fish. We performed whole genome sequencing on ESBL-PE recovered from meat and fish and from BIRDY mothers who provided faecal swabs less than one year prior. Among 150 pork, fish, and chicken samples, 75% were positive for ESBL-PE. Pork was most commonly contaminated ( $p < 0.01$ ). ESBL-PE from 8/150 samples demonstrated *mcr-1*-mediated colistin resistance, and *E. coli* from 2/150 samples were carbapenem-resistant. Pork and chicken fed medicine even when healthy versus those not were 2.8 times as likely (95% CI: 1.1, 7.3) to be contaminated with multidrug-resistant ESBL-PE. ESBLs belonging to the CTX-M-3 group (e.g. CTX-M-3, M-15, M-55) predominated across all hosts. Genomic comparisons between ESBL-PE from meat and humans are ongoing. A high proportion of meat and fish in Phnom Penh are contaminated with ESBL-PE, and similar ESBL-types can be detected among healthy humans. This work highlights the need for improved regulation of food-animal farming in Southeast Asia in order to protect public health.

## 645

### USING GPS TRACKERS TO EXPLORE FINE-SCALE HUMAN AND LIVESTOCK MOVEMENT IN BUSIA COUNTY, KENYA AND ITS IMPLICATIONS FOR ZOOSES

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Human and livestock mobility are key factors in the transmission of several high-burden zoonoses, yet our knowledge of them is relatively poor due to difficulty in quantifying population-level movement patterns. Significant variation in the movement patterns of individual hosts means it is necessary to capture their fine-scale mobility in order to gain useful knowledge at a population level. Here we explore how the movements of people and their livestock and their trips to various types of land cover correlate with livestock ownership, which could affect individual exposure to zoonoses. The study was conducted in Busia County, western Kenya, where the population are mostly subsistence farmers operating a mixed crop/livestock farming system. GPS trackers were fitted to one human and one cattle or other ruminant from each of the 27 households in the

study for a duration of one week. Households without livestock were also included for comparison. A total of 47 human and livestock individuals were tracked in July/August 2016, and the study was repeated at the end of the same year to compare movement patterns across these seasons. Although lengths of trips taken outside the household did not vary significantly by the type of livestock kept or by season, the maximum distance travelled did. We explored the association between the numbers and types of livestock kept, the relative wealth of the households, and the time spent by the people and livestock living there in different types of land cover. Significant differences were found in the amount of time people and livestock spent in swampy and woodland areas, with those keeping more livestock tending to spend more time in these areas. Finally, we looked at the home ranges of people and their livestock and found that people from less wealthy households had significantly larger home ranges. These results indicate that some individual-level mobility may be predicted by household characteristics such as livestock ownership and household wealth, which could have practical uses for assessing individual risk of exposure to some zoonoses and for future modelling studies of zoonosis transmission in similar rural areas.

## 646

### GENE EDITING OF OMEGA-1 OF SCHISTOSOMA MANSONI BY CRISPR-CAS MODIFIES DENDRITIC CELL INFLAMMATORY RESPONSE

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A key goal of functional genomics for parasitic helminths involves lentiviral based gene editing in schistosomes; delivery by lentivirus can be expected to provide a 'hands-free' approach to enable scaling of gene editing and increase accessibility to less accessible cells. The egg stage of the blood fluke *Schistosoma mansoni* plays central roles in pathogenesis and in disease transmission. The T2 ribonuclease *omega-1* is a powerful Th2-inducing glycoprotein secreted by the eggs of this schistosome. We investigated knockout and tagging of the *omega-1* gene; this gene was targeted both as a representative, model schistosome gene to establish facile gene editing but also because of the pivotal role of this antigen in immunopathogenesis. Approaches including delivery of the editing components, including guide RNA and Cas9 nuclease by electroporation and as delivered by pseudotyped lentiviral virions were investigated, with the latter providing more efficient performance. Furthermore, the target locus was further modified by knocking-in (KI) a six-stop codon tag by providing *in trans* single-stranded oligodeoxynucleotide DNA (ssODN) bearing micro-homology arms to mediate homology directed repair following Cas9 double stranded cleavage at *omega-1* ( $\omega-1$ ). Efficiency of the latter approach targeted at cultured eggs was >11%, while from 45 to 83% reduction in specific transcript levels were seen within 48 hours of treatment. When dendritic cells (JAWSII) were pulsed with soluble egg antigen from gene-edited (KI) eggs or wild type soluble egg antigen, marked differences in expression of anti-inflammatory cytokines were apparent, including marked upregulation of DC-SIGN and IL-10. These findings revealed not only that Crispr-Cas9 based gene editing including donor directed homology directed repair was functional in schistosomes, and that gene editing will facilitate deeper investigation of the physiology and pathogenesis of these major neglected tropical disease pathogens including the function of the ribonuclease *omega-1*. 1

## 647

### DETERMINING THE MECHANISM OF ENDOSYMBIOSIS BETWEEN FILARIAL NEMATODES AND WOLBACHIA

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Filarial nematodes represent one of the leading causes of disability in the developing world. Many filarial worm species, including *Brugia malayi*, one of the causative agents of lymphatic filariasis, have an obligate endosymbiotic relationship with the alpha-proteobacteria *Wolbachia*. To better understand the molecular interplay between these two organisms, we profiled the transcriptomes of *B. malayi* and *Wolbachia* across the life cycle of the parasite using dual RNA-seq. With these data, we built a co-expression network for the two organisms using weighted gene correlation network analysis (WGCNA). WGCNA is a well-established method by which expression data and trait data are integrated to identify co-expressed pathways. This allowed us to pinpoint functional pathways involved in this essential symbiotic relationship provided by the co-expression of nematode and bacterial genes. Using new transcriptional data from both the bacteria and worm, we have identified co-expressed pathways required for molting of the worm from L3 to L4. This molt is of particular importance as it marks the establishment of infection in the human host and is characterized by a 1000X expansion in *Wolbachia* population over the course of the molt. We found *Wolbachia* genes involved in heme, riboflavin, and purine/pyrimidine biosynthesis to be significantly upregulated during molting. These pathways are of interest as they are present in the *Wolbachia* genome but incomplete in the *B. malayi* genome. We also found genes involved in DNA replication, cellular respiration, chaperone function, and cell wall synthesis to be differentially expressed during molting. In parallel efforts, we are using these data to characterize the endosymbiotic relationship at the metabolic level using Flux Balance Analysis, identifying choke points that could be exploited for therapy. By creating a draft metabolic network for *B. malayi* and *Wolbachia*, and using *in silico* knockouts, we will determine the necessary pathways for growth and virulence and how these pathways are influenced by the presence of *Wolbachia*.

## 648

### GLOBAL TRANSCRIPTOME ANALYSIS OF WOLBACHIA STRAIN WOO UNDER ANTIBIOTIC PRESSURE IN VIVO

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The potential use of antibiotics targeting the bacterial endosymbiont, *Wolbachia*, as macrofilaricidal drugs remains a major focus of research in the filarial field. In a previous *in vitro* study using *Wolbachia* strain wMelPop in a mosquito cell line, we reported global changes in symbiont RNA and protein expression following a brief exposure to doxycycline. We showed that *Wolbachia* is capable of regulating expression of transporters, energy metabolism and outer membrane proteins during doxycycline treatment and these changes may contribute to antibiotic tolerance. Here, we extend our analysis to *Wolbachia* strain wOo from *Onchocerca ochengi* during oxytetracycline treatment of cattle. In this system, a short intensive oxytetracycline regimen (SIR) leads to a transient depletion of *Wolbachia* followed by symbiont recrudescence and survival of the nematode host; whereas prolonged intermittent therapy (PIR) suppresses *Wolbachia* densities long-term, resulting in death of most adult worms. During these treatments, bovine intradermal nodules containing *O. ochengi* were removed sequentially, and total RNA was subjected to RNA-Seq. Reads were mapped to the bovine, *O. ochengi*, and wOo genomes

and significant differential expression of wOo genes with a fold-change  $\geq 2$  ( $P \leq 0.01$ ) was calculated. We compared gene expression at the critical time-point of 8 weeks post-treatment, when *Wolbachia* density reaches a minimum in both treatment groups, but symbionts in the SIR group are capable of recrudescence. At this stage, stationary-phase morphogene *boIA*, involved in adaptation to stress and changes in cell morphology, was upregulated in the SIR group relative to the PIR group. Surprisingly, one year after SIR treatment had terminated, >30 genes remained downregulated in wOo from treated animals relative to untreated controls, including several membrane protein genes and other transcripts involved in translation and secretion. These findings highlight the adaptability of *Wolbachia* during antibiotic stress and may help to explain why the identification of short, effective antibiotic regimens for filarial infections has posed special challenges.

## 649

### **SCHISTOSOMA HAEMATOBIIUM IPSE INDUCES CELLULAR PROLIFERATION, CELL CYCLE ALTERATIONS, ANGIOGENESIS, AND TRANSCRIPTIONAL PROFILES CONSISTENT WITH PRO-CARCINOGENIC EFFECTS**

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*Schistosoma haematobium* infection, also known as urogenital schistosomiasis (UGS), affects over 112 million people globally. *S. haematobium* worms live in host pelvic veins and deposit eggs in the bladder [FF1]. The eggs secrete antigens that induce granuloma formation, in turn provoking urothelial hyperplasia and carcinogenesis. The IL-4-inducing principle of *Schistosoma mansoni* eggs (IPSE) is an abundant antigen released by schistosome eggs. IPSE binds immunoglobulins and chemokines, translocates into host cell nuclei and modulates gene transcription, and induces basophils and mast cells to release IL-4, thereby orchestrating a dominant Th2 response. Given that IPSE genes are only found in *Schistosoma*, their protein products may be pro-carcinogenic factors in schistosomal bladder cancer. We hypothesize that the *S. haematobium* homolog of IPSE (H-IPSE) plays a role in driving urothelial proliferation and bladder carcinogenesis in UGS. After cloning H-IPSE through RACE PCR and expressing it in recombinant form using a mammalian expression system, H-IPSE proteins were co-cultured with urothelial bladder cell lines derived from primary and transformed tissues, representing diverse species of origin and stages of carcinogenesis: HTB-9 (Grade II human bladder carcinoma), HCV-29 (normal human bladder urothelium) and MB49 (mouse urothelial carcinoma). Readouts included CFSE proliferation assays, cell cycle analysis, xCELLigence real-time monitoring of cell proliferation, and RNA-Seq. H-IPSE protein was also screened for its ability to bind cytokines and nuclear proteins in a 288-plex array. Across assays, H-IPSE induced proliferation of HCV-29 and MB49, but not HTB-9 cells. H-IPSE also altered cell cycle status of both HCV-29 and MB49 cells. Data also suggest that H-IPSE promotes angiogenesis, and alters transcription of many genes potentially relevant to carcinogenesis. H-IPSE bound multiple cytokines and nuclear proteins previously implicated in oncogenesis. These findings indicate that H-IPSE might contribute to bladder carcinogenesis in the context of UGS. Ongoing work aims to define the associated mechanisms.

## 650

### **INFILTRINS AS A NEW CLASS OF PATHOGEN-SECRETED, HOST NUCLEUS INFILTRATING PROTEINS IN TREMATODES**

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Infiltrins (short for pathogen-secreted host nucleus infiltrating proteins) are proteins secreted by specific stages of trematode parasites, which are in close contact with host tissues. Such infiltrins have the ability to enter host cells by yet to be described mechanisms, and from there rapidly translocate to the nucleus. The archetypal infiltrin is M-IPSE (a.k.a. IPSE/alpha-1), a glycoprotein secreted by *Schistosoma mansoni* eggs, characterised by the simultaneous presence of a classical secretory and a nuclear localisation signal (CSS/NLS) signal. Within minutes following uptake by mammalian host cells, M-IPSE translocates to the nucleus and binds to DNA. This suggests that infiltrins, by acting e.g. as transcription factors, might play a central role in controlling the host-parasite relationship at the molecular level. Together with their secretory status, this role makes infiltrins interesting targets for vaccination. Here, we demonstrate similar properties for H-IPSE, the homologue of M-IPSE in *Schistosoma haematobium*. We first generated a series of truncated constructs fused with AcGFP1, which were transfected into mammalian cells. Nuclear localisation of fluorescence confirmed the existence of a single, monopartite NLS located near the C-term of H-IPSE. The predicted H-IPSE 'SKRRRKY' NLS motif, inserted into Tetra-EGFP, but not an Alanine NLS mutant, redirected the encoded approx. 100 kDa protein entirely to the nucleus. More importantly, wildtype recombinant H-IPSE, added exogenously to HTB-9 bladder carcinoma cells, fully translocated to the nucleus, whereas the Alanine NLS mutant remained in the cytoplasm. Similarly, *Fasciola hepatica* proteins H2A and GST-sigma were able to enter Huh7 hepatocarcinoma cells and translocate to the nucleus. We have scanned the *F. hepatica* genome with a stringent approach designed to detect genes encoding dual CSS/NLS functionality and identified several other potential infiltrins. Overall, the existence of infiltrins in *S. mansoni*, *S. haematobium* and *F. hepatica* suggests that these proteins may represent a more general regulatory principle operating in parasitic trematodes.

## 651

### **IDENTIFICATION OF ANTHELMINTIC DRUGS BY HIGH THROUGHPUT SCREENING OF A SCHISTOSOME SEROTONIN RECEPTOR**

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The need to discover new anti-schistosomal targets and lead compounds is driven by the fact that the current frontline drug, praziquantel, does not kill all stages of parasite infection and there are no broad spectrum schistocidal alternatives. Serotonergic G-protein coupled receptors (GpCRs) are attractive drug targets given that serotonin (5-HT) controls schistosome neuromuscular function. While GpCRs are privileged targets, accounting for >1/3rd of all approved drugs, target based screens have been slowed by the fact that no flatworm G-protein coupled receptor has been functionally expressed in a high throughput assay. Here, we have screened a 5-HT receptor cloned from the predominant *Schistosoma* species responsible for human infection (*S. mansoni*, *S. japonicum*, *S. haematobium*) - advancing hits identified *in vitro* against recombinantly expressed receptors to validation against parasites cultured *ex vivo* and finally *in vivo* animal infections. Libraries of natural products were screened against *Schistosoma* 5-HT receptors stably expressed alongside a cAMP reporter in HEK293 cells. Hit compounds clustered into groups containing either tryptamine or benzyloisoquinoline ring systems, and structure-

activity profiling of these pharmacophores identified potent agonists ( $EC_{50} < 100\text{nM}$ ) and antagonists ( $K_i < 100\text{nM}$ ) active against receptors from all three species. 5-HT receptor agonists stimulated movement in cultured adult schistosomes, with some compounds demonstrating ~1000x higher potency than serotonin. Similarly, 5-HT receptor antagonists blocked parasite movement. Drugs prioritized from *in vitro* screens proved effective *in vivo*. Injection of compounds into infected mice resulted in a rapid shift of parasites from the mesenteries to the liver, and a 65% reduction in worm burden when dosed over one week. Oogram analysis revealed a 97% reduction in schistosome egg burden- a crucial outcome given that schistosomiasis pathology is driven by the immune response to parasite eggs. These data provide the first high throughput screen of a schistosome GPCR and validate serotonergic signaling as an anti-schistosomal target.

## 652

### MINIMIZING THE COST OF CONGENITAL CHAGAS DISEASE IN THE UNITED STATES THROUGH MATERNAL SCREENING

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Chagas disease, caused by *Trypanosoma cruzi*, is transmitted by insect vectors, as well as through blood transfusion, organ transplant, consumption of insect feces in food and water, and from mother to child during gestation. Programs of vector control and screening of blood products and transplant organs have been successful in reducing transmission. Congenital infection, however, could perpetuate Chagas indefinitely, even in countries with no or almost no autochthonous vector transmission. Even mothers who themselves have been infected congenitally and who are not symptomatic can transmit to their babies. About 30% of infected persons will develop lifelong cardiac or digestive complications that can be fatal. Treatment of infants with benznidazole has close to 100% cure rate, and efficacy in adults is estimated between 40% and 70%. This is the first study of the costs of screening and treatment for Chagas in the United States. We constructed a decision-analytic model to find the cost-minimizing option, comparing the costs of testing and treatment, as indicated, for mothers and infants with the lifetime societal costs of no testing and consequent morbidity and mortality due to lack of treatment or late treatment. We find that a protocol of screening and treatment is cost-minimizing for all rates of congenital transmission, even as low as 0.1%, and all levels of maternal prevalence, as low as 0.1%. Lifetime societal savings due to screening and treatment are more than \$1,300 per birth to Hispanic women from endemic areas. There are more than 480,000 births to Hispanic women from endemic areas in the United States per year, resulting in a saving of more than \$600 million in lifetime savings per birth-year cohort. Educating obstetricians to offer prenatal or cord blood serologic screening to Hispanic mothers makes it possible to treat mothers, infants, siblings, and other family members at risk of serious Chagas morbidity.

## 653

### RISK FACTORS FOR ACUTE MESOAMERICAN NEPHROPATHY IN NICARAGUAN SUGAR WORKERS

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An epidemic of kidney disease of unknown etiology is unrelenting among primarily young agricultural workers in Central America. Patients with Mesoamerican nephropathy (MeN) lack traditional risk factors for kidney disease. Until recently, MeN was defined only as a chronic kidney disease (CKD), but we recently documented acute kidney injury (AKI) associated

with MeN. In order to shed light on the most likely etiology and gain a better understanding of factors contributing to acute MeN, we conducted a prospective study at a large sugar estate in Nicaragua, an area heavily affected by MeN. Physicians conducted case surveillance in patients presenting for care, and controls were recruited during health screening visits. Using structured interviews, we documented demographic, medical, occupational and behavioral data. In this univariate case-control analysis, we compare data from workers with acute MeN to that from healthy control workers in order to determine occupational risk factors for acute MeN. Interview data was available on 225 cases and 167 controls, mostly male (90%). Cases were older than controls (median 31 yrs vs 28 yrs;  $p=0.025$ ) and more likely to live in the village adjacent to the sugar fields ( $p=0.012$ ). Case patients were more likely to work in direct contact with the sugar fields ( $p<0.001$ ) but not with water, compared to controls. We also found that controls more often wore protective gear on the job: gloves ( $p<0.001$ ); glasses ( $p<0.001$ ); mouth cover ( $p=0.001$ ); nose cover ( $p=0.041$ ); and boots ( $p<0.001$ ). Cases were more likely to drink well water ( $p=0.002$ ) and less likely to drink from fountains ( $p=0.001$ ). Controls had a higher frequency of handwashing before eating lunch on the job ( $p=0.010$ ). Interestingly, cases reported having a family member with MeN ( $p<0.001$ ) more frequently than controls did. This is the first analysis to explore work-related risk factors of acute MeN. These data suggest an environmental source of MeN to which workers are exposed. Our ongoing analysis and predictive modeling will further elucidate which exposures are the most important and may inform where preventive measures could be used to prevent disease.

## 654

### BARRIERS TO PEDIATRIC INPATIENT CARE GUIDELINE ADHERENCE: A MIXED METHOD ASSESSMENT OF EIGHT HOSPITALS IN ASIA AND AFRICA

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Difficulty adhering to evidence-based guidelines is an important driver of inpatient child mortality. A mixed method assessment was conducted in eight district or national referral hospitals in Bangladesh (2), Kenya (3), Malawi (1), Pakistan (1) and Uganda (1) between July and November 2016. Key informants (administrators, clinicians, nurses, nutritionists, pharmacists) were interviewed and essential hospital infrastructure, equipment and medicines was assessed by direct observation. The clinical notes of 240 children aged 2-23 months were reviewed and indicators of adherence to guidelines for common pediatric conditions, including severe acute malnutrition (SAM), pneumonia, diarrhea, and anemia were abstracted. All facilities had local guidelines adapted from WHO recommendations. Staffing levels varied between 2.4-16.1 nurses, 0.0-11.9 nursing assistants and 2.6-12.4 clinicians per 1000 pediatric admissions. Equipment, medicines and infrastructure were generally adequate for guideline implementation, but the notes review demonstrated variable adherence. Prescribed antibiotics corresponded with recommended regimens for 79% (n: 174) of cases with a documented antimicrobial indication. Universal malnutrition screening was not implemented in four sites. Recommended SAM feeding volumes were followed in 83% (n: 72) of cases but relactation was not offered at four sites. Pulse oximetry was noted in 80% (n: 88) of pneumonia cases and

oxygen therapy was documented for 74% (n: 19) of hypoxemic children. Hemoglobin results were recorded in 70% of reviewed notes, but only 14% (n: 63) with mild or moderate anemia had documented diagnoses of anemia. Key informants reported inadequate human resources, frequent rotation of junior clinicians and difficulty providing senior supervision as key barriers to guideline adherence. Adherence to guidelines remains a challenge across a range of childhood conditions even when access to recommendations and the materials necessary for implementation are in good supply. Facility staff perceive inadequate human resources as the primary constraint to consistent guideline adherence.

## 655

### THE PREVALENCE AND DETERMINANTS OF DISCLOSURE OF SEXUAL PRACTICES TO OTHER FAMILY MEMBERS AMONG MEN WHO HAVE SEX WITH MEN IN LOME AND KARA, TOGO

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Supporting HIV prevention and treatment among people at risk/living with HIV necessitates understanding their specific HIV acquisition/transmission risk. Disclosure of sexual practices facilitates improved service provision. However, in settings of higher HIV prevalence, disclosure of sexual practices may lead to stigma and discrimination. This study aims to characterize the prevalence and determinants of disclosure of sexual practices to a family member (DFM) among MSM in 2 cities in Togo. 683 MSM ≥18 years of age were recruited using respondent driven sampling for a cross-sectional survey (354/683 in Lomé and 329/683 in Kara). Participants completed a structured questionnaire and biological testing for HIV and syphilis. Statistical analyses included t-test, RDS-weighted (RDS-w) proportions, bootstrapped confidence intervals and logistic regression models. Overall median age was 23.9 years. Most participants reported a weekly income of 2001-12,000 CFA (RDS-w Lomé= 46.5% vs. Kara=48.7%,  $p<0.01$ ), and reported their sexual identity as gay/homosexual (RDS-w Lomé=61.2% vs. Kara: 62.6%,  $p=0.59$ ). In total, 62/683 (9.1%) MSM were living with HIV (RDS-w Lomé=10.4% vs. Kara: 0.2%,  $p<0.01$ ). Difficulties accessing health services and blackmail as a result of having sex with men were reported by less than 20% of participants. Overall, 26.8% (183/683) participants had DFM, 24.0% (85/354) in Lomé and 29.8% (98/329) in Kara. MSM living in Kara (aOR=2.05, 95% CI=1.35, 3.14) showed an increased odds of DFM compared to MSM living in Lomé. DFM was associated with income level (aOR=1.68, 95% CI=1.11, 2.56), gender (aOR=2.16, 95% CI=1.12, 4.16), living with HIV (aOR=2.03, 95% CI=1.08, 3.81). MSM who have DFM demonstrated increased odds of experiencing difficulties accessing health services (aOR=1.93, 95% CI=1.15, 3.24) and increased odds of blackmail (aOR=2.43, 95% CI=1.45, 4.07). These results highlight the individual, social, and community level determinants of DFM among MSM in Togo. Moving forward necessitates studying the temporality of disclosure in relation to other factors in order to better inform HIV treatment and prevention programs.

## 656

### SEASONAL FOOD INSECURITY IN HAYDOM, TANZANIA IS ASSOCIATED WITH LOW BIRTH WEIGHT AND ACUTE MALNUTRITION: RESULTS FROM THE MAL-ED STUDY

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In rural agricultural communities, the pre-harvest “hunger” season is a particularly vulnerable period that may cause increased food insecurity and have lasting impact on child growth. We assessed seasonal patterns of food insecurity and related health outcomes among children in the MAL-ED birth cohort study at the rural Haydom, Tanzania site. Children were followed twice-weekly to document food intake and monthly for anthropometry until two years of age. Household food insecurity was reported by caregivers every 6 months. We modelled the seasonality of food insecurity, intake of foods, birth weight, and acute malnutrition to estimate the impact of the high food insecurity season on these outcomes. We assessed severe outcomes from pediatric admissions with acute malnutrition at Haydom Lutheran Hospital in 2010-2015. Of 262 enrolled children, 158 (60.3%) of their caregivers reported food insecurity at least once. Food insecurity was highly seasonal with peak insecurity from December to February. Children born during these 3 months had an average 0.35 z-score (95% CI: 0.12, 0.58) lower enrollment weight (within 17 days of birth) compared to children born in other months. In addition, weight-for-length z-scores measured in these months were on average 0.15 z-scores lower (95% CI: 0.10, 0.20) compared to other months, adjusting for enrollment weight, and this disparity was sustained up to two years of age. These associations corresponded to the seasonal availability of foods. Consumption of animal milk was 9% lower (95% CI: 5, 12) and consumption of root vegetables was 77% lower (95% CI 71, 81) during the high food insecurity months. Correspondingly, the number of admissions with acute malnutrition at the local hospital was highest at this time, with twice as many cases in December-February compared to July-September. We identified seasonal trends of malnutrition in Haydom, Tanzania that correspond to both caregiver reports of food insecurity and child food intake. Targeting of prenatal care and child feeding interventions during high food insecurity months would likely have the greatest impact on reducing child malnutrition in this community.

## 657

### MOVING BEYOND PRIMARY HEALTH BENEFITS: EVALUATING THE IMPACT OF DRAINAGE INFRASTRUCTURE IMPROVEMENT PROJECT ON BUSINESSES AND TRAFFIC FLOW IN LUSAKA, ZAMBIA

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Impact evaluations of health intervention projects often fail to consider secondary potential benefits, possibly undervaluing the true value of the intervention. The Government of Zambia is upgrading drainage infrastructure in Lusaka, with one goal to reduce the impact of infectious diseases associated with seasonal flooding. To assess additional benefits, we collected, as part of a comprehensive evaluation of the drainage improvement project, baseline measurements of potential impact on households, businesses and traffic flow. We conducted business and traffic studies to evaluate the impact of flooding on travel time and business operations. We surveyed 587 randomly selected businesses located in a

main market within the drainage catchment area. The survey collected information on business closings and operations, property damage, revenue generation, and commute time of workers during rainy season. We conducted traffic and pedestrian flow assessments by counting the number of vehicles and pedestrians in 15-minute time intervals at major intersections located in close proximity to the drain and measured round trip travel times along 11 routes passing through the intersections. Eighty-one percent of businesses experienced flooding in the 2015-16 rainy season; 28% of businesses reported shutting down their shops due to flooding for one to two days in the rainy season. Number of goods-containing vehicles being loaded or unloaded in the business area was significantly lower during flooding ( $p < 0.01$ ) and about 50% of businesses reported that flooding also affected punctuality at work; 30% of workers were late to work for an average of 62 minutes. Property damage was reported by 147 businesses and the average value of damage was \$567 (SD: \$139) per business. Mean (SD) business revenues decreased by \$600 (\$1,058) per business due to floods. The number of vehicles passing through intersections increased by 3.6% due to rain or floods. On average, it took 1.66 minutes (3%) longer per round trip during flooding. Business and traffic components are essential components of the financial rationale for this public health intervention.

## 658

### REDUCTION IN CASE FATALITY RATE DUE TO ACUTE ENCEPHALITIS SYNDROME THROUGH INTEGRATED APPROACHES

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Acute encephalitis syndrome (AES) is a major public health problem in India. It is estimated that approximately 375 million people are at risk of developing AES in India, with an incidence rate of 0.46 per 100,000 population. Following a massive outbreak of Japanese encephalitis (JE) in 2005, claiming 1600 lives, the government of India introduced JE vaccination for the age group of 1-15 years in the endemic districts which resulted in significant reduction of JE positivity. Considering the complex etiology of AES, in 2012 the government of India initiated a policy around integrated approach for prevention and control of AES/JE which involved interventions on improving water, sanitation, social justice and empowerment, animal husbandry, rural development, housing and poverty alleviation and education. However, the two most populous states, Uttar Pradesh (UP) and Bihar continue to grapple with AES outbreaks due to unknown etiologies. To address this problem, PATH initiated a project in Bihar and UP to support the governments in AES prevention and control. New sustainable initiatives were introduced which included upgradation of the primary and secondary health facilities to function as Encephalitis Treatment Centers (ETCs). By 2016, 49.5% AES cases were treated at the ETCs with a case fatality rate of 6%; Over 60,000 health personnel were trained on management of AES cases; 2092 AES cases were followed up from onset of symptoms to referral, management, rehabilitation and epidemiological information. For the first time, 19 etiologies of AES were identified. Mass awareness campaigns were conducted through wherein 16.4 million people were sensitized. Over 175,000 people were sensitized through village level community awareness; standardized/innovative tools were introduced for data management; a technical advisory group on AES was introduced at the national and state levels; based on the recommendations, government of UP has decided to pilot evidence based integrated control measures in high risk villages by leveraging on government-funded schemes to improve health, water, sanitation and environmental contributors to combat AES.

## 659

### USING G6PD TESTS TO ENABLE THE SAFE TREATMENT OF *PLASMODIUM VIVAX* INFECTIONS WITH PRIMAQUINE ON THE THAILAND-MYANMAR BORDER: A COST-EFFECTIVENESS ANALYSIS

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Primaquine is the only licensed antimalarial for the radical cure of *Plasmodium vivax* infections. Many countries, however, do not administer primaquine due to fear of hemolysis in those with glucose-6-phosphate dehydrogenase (G6PD) deficiency. In other settings, primaquine is given without G6PD testing, putting patients at risk of hemolysis. New rapid diagnostic tests (RDTs) offer the opportunity to screen for G6PD deficiency prior to treatment with primaquine. We assessed the cost-effectiveness of using G6PD RDTs on the Thailand-Myanmar border and developed an online model to examine the validity of these findings in other settings. Decision tree models for the management of *P. vivax* malaria evaluated the costs and disability-adjusted life-years (DALYs) associated with recurrences and primaquine-induced hemolysis from a health care provider perspective. Screening with G6PD RDTs before primaquine use was compared to (1) giving chloroquine alone and (2) giving primaquine without screening. Data were taken from a recent study on the impact of primaquine on *P. vivax* recurrences and a literature review. Compared to the use of chloroquine alone, the screening strategy had similar costs while averting 0.026 and 0.024 DALYs per primary infection in males and females respectively. Compared to primaquine administered without screening, the screening strategy provided modest cost savings while averting 0.011 and 0.004 DALYs in males and females respectively. The probabilistic sensitivity analyses resulted in a greater than 75% certainty that the screening strategy was cost-effective at a willingness to pay threshold of US\$500, which is well below the common benchmark of per capita gross domestic product for Myanmar. In this setting G6PD RDTs could avert DALYs by reducing recurrences and reducing hemolytic risk in G6PD deficient patients at low costs or cost savings.

## 660

### DEVELOPMENT OF THE WHO INTERNATIONAL EXTERNAL QUALITY ASSURANCE SCHEME FOR MALARIA NUCLEIC ACID AMPLIFICATION TECHNIQUES

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With expansion of molecular methods for malaria detection and renewed emphasis on malaria elimination; in 2014, the WHO recommended establishing an international external quality assessment (EQA) scheme for nucleic acid amplification (NAA) assays to ensure that data generated by these methods is reliable and comparable and can support policy development. WHO and UKNEQAS, with support of FIND, have collaborated to launch the WHO Malaria NAA EQA scheme targeting public health and research laboratories in endemic and non-endemic settings. A repository of EQA materials sufficient to allow for biannual distributions to 60 laboratories over a minimum of two years was

established. The repository was prepared from *in vitro* cultures of *P. falciparum* (Pf) and *P. knowlesi* (Pk) and redundant clinical samples of Pf, *P. vivax* (Pv), *P. ovale* (Po) and *P. malariae* (Pm) and negative blood. Positive samples range in parasitaemia from a maximum of 2x10<sup>6</sup> p/mL to a minimum of 10p/mL. Specimens are stored as 500µL aliquots of lyophilized (LY) samples and as 50µL dried blood spots (DBS). First distribution was sent to 53 participating labs in January, 2017 and 41 labs returned results within the allotted timeframe. Initial analysis of data from 41 labs showed that labs performing assays on LY specimens performed better compared with DBS specimens: (% correct: LY 78.8% vs. DBS 62.5%; % incorrect: LY 15.8% vs. DBS 37.5%). A higher percentage of laboratories correctly identified Pf and Pv (average 66%) compared to Pk (55%). A higher rate of incorrect results was observed when parasitaemia was lower [Pf- 62.5% (50 p/µL) vs 28% (2,000 p/µL)]; Pv- [35.1% (18 p/µL) vs 9.8% (180 p/µL)]. False negatives were observed in all samples: [DBS (45.8%) compared to LY(18.9%)]. False positives were observed in all samples [DBS (17.2%) compared to LY (13.4%)]. Conclusions: WHO has established an EQA Scheme for Malaria NAA assays. Initial results illustrate problems with false positives and false negatives particularly when assaying specimens containing low parasitaemias; specimens containing non *P. falciparum* species (especially *P. knowlesi*) and with DBS compared to LY source material.

## 661

### CLINICAL AND LABORATORY PREDICTORS OF SEVERE KNOWLESI MALARIA: IMPLICATIONS FOR INITIATION OF PARENTERAL ARTESUNATE TREATMENT AND HOSPITAL REFERRAL

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*Plasmodium knowlesi* is increasingly reported in Southeast Asia and can cause severe and fatal disease. Over 3.5 years, we prospectively assessed patients of any age with PCR-confirmed *Plasmodium* mono-infection presenting to 3 district hospitals in Sabah, Malaysia. Clinical and laboratory predictors of severe disease (WHO 2014 criteria) were evaluated using multivariate logistic regression and receiver operating characteristic analysis. 481 *P. knowlesi* (44[9%] children ≤12 years), 172 *P. vivax* (71[41%] children), and 96 *P. falciparum* (31[32%] children) malaria cases were enrolled. In *P. knowlesi*, severe malaria occurred in 6.4% (95%CI:3.9-8.3) of adults but not in children. The commonest *P. knowlesi* severity criteria were acute kidney injury, hyperparasitaemia, jaundice and anaemia. No patients had coma. Independent predictors of severe *knowlesi* malaria included age overall or age ≥45 years, parasite count, chronic comorbidities and abdominal pain. A parasite threshold of 15,000/µL had the best-combined sensitivity (74%) and specificity (87%) for predicting severe *knowlesi* malaria, with an AUC of 0.80 (95%CI 0.71-0.90) and a negative predictive value of 98.5%. Age ≥45 years in addition to parasitaemia >15,000/µL gave an adjusted OR for severe disease of 23.1 (95%CI 7.1-76.1; p<0.001), with a sensitivity of 65%, specificity of 96% (AUC 0.81; 95%CI 0.71-0.91), and a negative predictive value of 98%. Age ≥45 years was the best predictor of hyperparasitaemia (parasite count >100,000/µL) when controlling for other variables (AOR 4.9; 95%CI 1.0-23.9; p=0.048). Adults with *knowlesi* malaria had a higher risk of severe disease compared to adult *vivax* malaria patients (OR 3.4; 95%CI 0.8-14.5; p=0.098), and a comparable risk to *falciparum* malaria. Parasitaemia independently predicted *knowlesi* disease severity: intravenous artesunate is warranted initially for those with parasitaemia >15,000/µL given the potential delay or inability to evaluate other laboratory markers of severe disease in most primary care settings. Age and parasitaemia guide the initial clinical evaluation for hospital admission or tertiary hospital referral.

## 662

### BUILDING AND MAINTAINING HEALTH CARE WORKER PERFORMANCE OF MALARIA RAPID DIAGNOSTIC TESTS IN EIGHT SUB-SAHARAN AFRICAN COUNTRIES

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Relative ease of use and decreasing cost have led to a rapid expansion of rapid diagnostic tests (RDT) for parasitological confirmation of suspected cases of malaria at both facility and community level in countries in sub-Saharan Africa. Errors in key performance steps can reduce the specificity and sensitivity of RDTs. The President's Malaria Initiative (PMI) through the PATH-led MalariaCare partnership has supported National Malaria Control Programs (NMCP) to develop and implement a system of quality assured malaria diagnostics to build and maintain performance of health care workers (HCW) to conduct RDTs. We report here the results of 8,000 observations in 8 countries of HCW RDT performance in field settings at all levels of the health care system. The power of this multi-country monitoring data is in its large numbers measuring changes in performance in the challenges of the work place. MalariaCare works with NMCPs to train laboratory and clinical experts in quality RDT performance, including proper steps, benefits and limitations of the test, and common reasons for false positives and negatives. Best performers are trained as on-site supervisors who work with local laboratory and clinical staff at health facility level to perform on-site outreach training and supportive supervision (OTSS). The OTSS visits focus on observation of RDT conduction using a 13-point checklist and on the spot problem solving. Data from facilities receiving between 2 and 5 OTSS visits have shown steady improvements, averaging 7 percentage points in the performance of RDTs across all cadres of HCWs at all levels of health facilities. Across countries, an average of 96% of steps in the checklist were being performed correctly. Steps most commonly performed incorrectly include labeling of the cassette and checking of the expiry date, but key steps such as waiting the correct amount of time to declare a test negative and using the correct amount of blood and buffer were performed correctly by 89%, 90% and 92% respectively. Findings suggest a system of training and supervision supports strong RDT performance at all levels that improves access to effective case management.

## 663

### USING OUTREACH TRAINING AND SUPPORTIVE SUPERVISION TO MAINTAIN MICROSCOPY COMPETENCY IN SEVEN SUB-SAHARAN AFRICAN COUNTRIES

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Parasitological confirmation of suspected malaria is performed through the use of rapid diagnostic tests (RDT) or microscopy. The President's Malaria Initiative (PMI) through the PATH-led MalariaCare partnership supports National Malaria Control Programs (NMCP) to implement a system of quality assured malaria diagnostics. Relative to RDT use, microscopy requires more supplies, functioning equipment, extensive specialized

training, and requires regular use of skills to maintain competency - opportunities for which are decreasing with the increasing use of RDTs. MalariaCare works with NMCPs and national laboratories to train diagnostics experts in microscopy. Best performers - from central and sub-national reference laboratories - are then trained as on-site supervisors who work with laboratory staff at health facility level to perform on-site outreach training and supportive supervision (OTSS). The OTSS visits focus on skills observation and on the spot problem solving. The primary goal is to improve skills in preparation and accuracy of malaria slide reading guided by a 3-part checklist: preparation, staining and reading. In 966 laboratory observations across 7 sub-Saharan African countries during the most recent OTSS visit, 88% of hospitals, 82% in health centers, and 84% at health posts (in 2 countries where microscopy is performed at this level) are performing microscopy at a minimally acceptable level - measured as obtaining a score of 75% or higher on a competency weighted checklist. MalariaCare has noted an average improvement of 14 percentage points in performance in facilities receiving 4 rounds of OTSS. Change in performance is more variable in facilities receiving 3 or fewer rounds. Additional observed problems include 26% of facilities without functioning microscopes, 42% without electricity, 72% with stock-outs of pH paper, and 29% with stock-outs of other key commodities. Findings indicate training and at least 4 OTSS visits can significantly improve microscopy competency.

## 664

### PERFORMANCE ASSESSMENT OF LABORATORY TECHNICIANS ON MALARIA MICROSCOPY IN FIVE HIGH ENDEMIC DISTRICTS OF RWANDA

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Accurate malaria diagnostics help to establish the true prevalence of each *Plasmodium* species and can ensure appropriate treatment. Light microscopy is the gold standard for malaria diagnosis and sufficient training of laboratory staff is paramount for the correct microscopy diagnosis of malaria. In Rwanda each of about 400 health centers has a laboratory able to perform malaria microscopy, at least 2 trained lab technicians and 1 to 2 functioning microscopes. The objective of the study is to evaluate the performance of laboratory technicians in detecting and quantifying malaria parasites in 81 health centers from 5 highly endemic districts (Huye, Nyanza, Ngoma, Kirehe, Kayanza, Gatsibo). In October 2015 the Rwanda Biomedical Center and partners trained 1 lab technician per health center from these districts in malaria microscopy. The training emphasized determining parasite density and detection of malaria species. From August to September 2016 a follow-up assessment was conducted. Of the 81 technicians trained, 30 were randomly chosen and assessed at their health facilities. A standardized pre-validated slide panel of 5 slides was distributed, a comprehensive checklist used to collect information and conduct visual inspection and maneuvers used in routine malaria diagnosis. During the training a significant increase was found between pre and post tests with median scores improving from 47% to 85%. As part of the assessment 150 lab tech-prepared slides were analyzed to evaluate the quality of thick and thin blood smears. There was a significant increase in quality of both blood smear types. The sensitivity and specificity of participants in detection of malaria parasites were 100% and 86% respectively, while species identification and parasite quantification accuracy were 79% and 75% respectively. The findings of this assessment support the need for continuous capacity building for laboratory staff to ensure accurate malaria diagnosis for appropriate treatment and suggest that District hospitals may benefit from conducting regular malaria microscopy diagnosis quality control/assurance activities at health center laboratories.

## 665

### IMPLEMENTATION OF A QUALITY IMPROVEMENT APPROACH FOR MALARIA SERVICE DELIVERY IN ZAMBEZIA PROVINCE, MOZAMBIQUE

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In Mozambique, malaria in pregnancy (MIP) is one of the leading causes of maternal and newborn morbidity and mortality. Malaria also accounts for over 40% of deaths in children less than five years old. With provincial and facility-level commitment, a simple and comprehensive quality improvement (QI) system has been established in 10 of 16 districts in Zambezia Province. Since 2016, the Mozambique Ministry of Health (MOH) and Zambezia Provincial Health Directorate, in collaboration with partners, have implemented a malaria QI effort based on the Standards-Based Management and Recognition (SBM-R) approach. A standards-based approach to improving quality of malaria care engages both management and service providers to work together to assess the current performance, address gaps to ensure that all patients receive a minimum (standardized / evidence-based) package of care, and ultimately improve patient outcomes and facility performance. Thirty-one performance standards in five content areas (MIP, Case Management, Laboratory, Pharmacy, and Management of Human Resources and Malaria Commodities) were developed and adopted by the MOH in 2016. With support from partners, 40 health workers, including managers, clinicians and lab technicians, received training on SBM-R, and facility QI teams were established. These teams use checklists based on standards to conduct quarterly assessments that identify performance gaps, and then develop action plans to address areas of improvement. The MOH antenatal care and child health registers also contain information on coverage of key malaria interventions, including IPTp, and malaria diagnosis and treatment during pregnancy and for children under five with fever. Average attainment of standards at baseline in 20 health facilities was 30%, and is expected to improve as implementation progresses with quarterly application of the checklist (data will be available before November). Improvements in key malaria indicators for pregnant women and children under five years old are expected as the percentage of standards attained increases.

## 666

### A LARGE NON-HIV OUTBREAK OF AFRICAN HISTOPLASMOSIS IN RURAL COMMUNITY IN KIMPESE CITY, DEMOCRATIC REPUBLIC OF CONGO

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African histoplasmosis due to *Histoplasma capsulatum* var *duboisii* is a rare chronic mycotic disease, endemic in several parts of equatorial Africa. Only incidental cases have been documented in humans. Herein we report the first large outbreak of African-Histoplasmosis in non-HIV patients from Kimpese in the Kongo-Central Western province of DR Congo. An observational study was conducted, based on consecutive routine biopsies done between July 2011 - January 2014 at Kimpese hospital. All biopsies were accessioned and diagnosed as *H. duboisii* by histopathology. Gomori methenamine silver staining was performed in Israel confirming initial observations. Immunohistochemistry (IHC) was done at the Pasteur Institute, Paris. A specific real-time polymerase chain reaction (RT-PCR) assay was done in Madrid, Spain. Finally, a field survey was conducted with

interviews and household visits. Altogether 36 patients were histologically diagnosed with Histoplasmosis. Patients originated from the city of Kimpese and its vicinity. The mean age was  $25 \pm 19$  (range 3- 67) years, with sex ratio F/M 1.7:1. The clinical picture included skin, bone and lymph node disorders. No pertinent laboratory abnormalities were noted. All HIV serologies were negative. Histology and histochemistry disclosed florid granulomatous inflammation with a myriad of thick double-walled yeasts consistent with *H. duboisii*. Ancillary IHC and RT-PCR assays confirmed the findings. The search for a putative natural reservoir of the pathogen pointed to *Chaeperon pumilus* cellar bats and guano fertilizer exploitation. All but two patients received and responded well to Ketoconazole therapy whereas the remaining 2 died from lack of medication and poverty. In conclusion, an unusual outbreak of non-HIV African histoplasmosis was encountered in a community setting in a novel hyperendemic location not hitherto identified in DRC. This observation might indicate a much larger public health threat, yet unrecognized. This opens new research perspectives for a better understanding of this neglected tropical disease.

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### EFFECT OF ANTI-MYCOBACTERIUM TUBERCULOSIS THERAPY ON MORTALITY AMONG HIV-INFECTED PATIENTS ADMITTED WITH SEVERE SEPSIS TO A REGIONAL REFERRAL HOSPITAL IN UGANDA

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Mycobacterium tuberculosis (MTb) is a common cause of severe sepsis among HIV-infected patients in sub-Saharan Africa. However, initial empiric treatment of severe sepsis does not frequently include anti-MTb therapy. We conducted a retrospective study at Mbarara Regional Referral Hospital (MRRH) in Uganda to 1) measure the proportion of severely septic patients who received anti-MTb therapy and 2) determine the relationship between initiation and timing of anti-MTb therapy administration and in-hospital mortality. We defined sepsis as clinical suspicion of infection plus  $\geq 2$  out of 3 systemic inflammatory response syndrome criteria (white blood cell count was excluded due to lack of availability) and severe sepsis as sepsis with systolic blood pressure  $<90$ mmHg, mean arterial pressure  $<60$ mmHg, or Glasgow coma scale score  $<15$ . We used the first clinical observation carried backward to impute missing admission vital signs and logistic regression to determine the association between anti-MTb therapy and in-hospital mortality. Of the 150 patients with HIV infection admitted to MRRH between January 2014 and December 2015 with sepsis, 87 (58.0%) had severe sepsis. There were 29 (19.3%) patients with severe sepsis that received anti-MTb therapy. Patients with severe sepsis were not more likely to receive anti-MTb therapy than patients without severe sepsis (odds ratio [OR]: 0.67, 95% confidence interval [CI]: 0.34-1.30,  $p=0.24$ ). Among patients with severe sepsis, 34 (39.1%) died, and there was no relationship between receiving anti-MTb therapy and in-hospital mortality (OR: 0.59, CI: 0.22-1.50,  $p=0.28$ ). There was also no relationship between the timing of administration of anti-MTb therapy and in-hospital mortality for patients with severe sepsis (OR: 1.14, CI: 0.94-1.45,  $p=0.22$ ). For patients with severe sepsis in a setting with a high prevalence of HIV and MTb in Uganda, the initiation and timing of anti-MTb did not affect in-hospital mortality.

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### IMMUNE RESPONSE FOLLOWING REACTIVE VACCINATION CAMPAIGN USING FRACTIONAL DOSE YELLOW FEVER VACCINE — KINSHASA, DEMOCRATIC REPUBLIC OF CONGO, 2016

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In 2016, several large outbreaks of yellow fever (YF) led to a global YF vaccine shortage. To vaccinate 7.6 million people in Kinshasa in an outbreak response campaign, fractional (1/5; 0.1ml) dose of 17DD YF vaccine was provided to children age  $\geq 2$  years and non-pregnant adults via subcutaneous injection. We assessed the effectiveness of this novel control strategy. Participants were recruited in four age strata at six vaccination sites. We assessed the presence of YF virus-specific neutralizing antibodies from blood samples collected pre-vaccination and 28 days post-vaccination using the plaque reduction neutralization test (PRNT50). Participants with PRNT50 titers  $\geq 10$  were defined as seropositive. The study criteria for immune response were seroconversion (seronegative on pre-vaccination blood to titer  $\geq 10$  at follow-up), or  $\geq 4$ -fold increase in titer. 764 participants were enrolled. Of the 716 participants (94% that completed follow-up, 705 (98%; 95% confidence interval: 98%-99%) participants were seropositive for YF antibody. Of these, 630 (89%) met criteria for immune response and 75 (11%) had preexisting immunity and did not meet criteria. Stratified by age, the proportion seropositive at follow-up was 98% (157/161) in 2-5 year olds; 100% (189/189) in 6-12 year olds; 97% (182/187) in 13-49 year olds; and 99% (177/179) in those 50 or older. For the same age groups, the proportions meeting immune response criteria were 80%, 93%, 92% and 85% respectively. There was a significant association between lower baseline titers and developing an immune response ( $p<0.0001$ ). Among baseline seronegative participants, no significant difference in seroconversion rate (range 95%-100%) was observed by age; there was a small but significant difference by sex (male 99%, female 96%,  $p=0.03$ ). In this cohort, fractional dose YF vaccine was highly effective at eliciting an immune response among those without preexisting immunity. These findings support the use of fractional dose vaccination for outbreak control, an important approach in the context of ongoing YF vaccine shortage. Additional studies are needed to assess the duration of immune response.

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### CEREBROSPINAL FLUID MARKERS TO DISTINGUISH BACTERIAL MENINGITIS FROM CEREBRAL MALARIA IN CHILDREN

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Acute bacterial meningitis (ABM) and cerebral malaria (CM) are common causes of acute non-traumatic coma, an important cause of morbidity and mortality among paediatric hospital admissions in Africa. Clinically distinguishing children with ABM from CM in high malarious areas is challenging, and both may be present. Few hospitals have diagnostic capacity for distinguishing the two syndromes resulting in broader antibiotic cover than necessary. A biochemical marker of ABM would

facilitate precise clinical diagnosis and management of these infections. We used Label-free protein quantification by mass spectrometry to identify cerebrospinal fluid (CSF) markers that distinguish ABM from CM in Kenyan children. Biomarker extraction combined differential protein expression and assignment of biomarker importance using random forests. Receiver operator characteristic (ROC) curves evaluated diagnostic characteristics of the biomarkers. The host CSF proteome response to ABM (*Haemophilus influenzae* and *Streptococcus pneumoniae*) is significantly different to CM. Among the differentially expressed proteins (FDR<0.01, Log FC≥2), Myeloperoxidase and Lactotransferrin were present in 37(100%) and 36(97%) of ABM cases respectively but absent in CM (n=22). Area under the ROC curve (AUC), sensitivity, and specificity were for Myeloperoxidase (1, 1, and 1; 95% CI, 1-1) and Lactotransferrin (0.98, 0.97, and 1; 95% CI, 0.96-1). Myeloperoxidase and Lactotransferrin have high potential to distinguish ABM from CM and thereby improve clinical management. Their validation requires a larger cohort of samples that includes other bacterial aetiologies of ABM.

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### CONTRIBUTING FACTORS FOR ANEMIA IN YOUNG CHILDREN IN COASTAL KENYA

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This study measured prevalence of anemia in conjunction with parasitic infection and nutritional status in a child cohort in rural Kenya. In a project where children have been followed biannually with stool, urine, and blood testing for parasitic infections, this 6-week sub-study in 2016 involved in depth testing for anemia and its risk factors using complete blood counts, nutritional assessment, and linkage to mother's and children's parasitic infection data for 244 children 0.5 to 3 years old. CBC showed 76% of infants were anemic (Hb < 11g/dL) at the 2016 visit. Longitudinal data indicated that one infant was anemia-free for her entire 20-month life and 11 had a lifetime average hemoglobin (Hb) that was non-anemic; however, 95% of children had an average lifetime Hb that was anemic, with younger infants more likely to be anemic at the 2016 visit. Anthropometric analysis showed that 26.5% of the currently anemic group were growth stunted, compared to 18.6% in the currently non-anemic group. Of note, maternal Hb at delivery was significantly lower for the currently anemic group, so analysis of the impact of parasitic infections included both antenatal maternal infections and infant infections during the postnatal period. Multivariable analysis, correcting for age and gender, indicated that antenatal malaria, as well as current infection with hookworm, *Trichuris*, *Strongyloides*, *Ascaris*, or malaria, were significantly associated with decreased current Hb. The nutritional assessment revealed that infants with a decline in Hb ate less vitamin A-rich vegetables and eggs, more milk and bread, and lived in a household that experienced significant food shortage in the past year. Maternal slate chewing during pregnancy and infant pica were also more frequent in the group with a decline in Hb. This study calls attention to the extremely high prevalence of anemia among vulnerable infants in a rural Kenyan population. Given that the anemia in this cohort appears to be multifactorial in origin, interventions need to address both diet and parasitic infections in pregnant women and their infants to effectively combat this priority public health threat.

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### CAUSES OF NON-TRAUMATIC PARAPLEGIA IN MALAWI

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Non-traumatic paraplegia is neglected tropical syndrome. It is not uncommon in Malawi; however there are only scanty reports on its pathogenesis. This study was undertaken to study the most common causes of paraplegia and make recommendations for treatment. We studied 50 sequential patients with paraplegia of < 6 months duration. A full neurological assessment was made using the American Spinal Injury Association (ASIA) scale. An MRI scan and a lumbar puncture was done as clinically indicated. Serum samples and CSF were screened for viral infections, schistosomiasis, tuberculosis and syphilis. Tuberculosis was the most common diagnosis (36%), followed by malignancy (20%) and transverse myelitis (10%); 3 cases were diagnosed as presumed neuroschistosomiasis. The diagnosis of tuberculosis was based on clinical and radiological evidence; in none of the CSF samples *M. tuberculosis* could be demonstrated by PCR. Four patients had evidence of neurosyphilis. A novel cyclovirus was found 10% of 40 CSF samples examined; other viruses found in the CSF included HIV, EBV, HSV and hepatitis B virus. In conclusion, tuberculosis was the most common cause of non-traumatic paraplegia; the contribution of schistosomiasis was lower than expected. The role of cyclovirus needs to be confirmed. The current policy of empirical treatment for schistosomiasis seems still justified; evidence for tuberculosis should be sought in all patients.

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### ACUTE KIDNEY INJURY FOLLOWING MULTIPLE WASP STINGS - A CLINICOPATHOLOGICAL STUDY FROM A MOUNTAINOUS STATE OF INDIA

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Acute kidney injury (AKI) after multiple wasp stings is well known, but still a rare phenomenon. A retrospective study of clinicopathological spectrum AKI following multiple wasp stings patients admitted at Indira Gandhi Medical College Hospital, Shimla over 13 years (July 2003 to June 2016). 32 patients were diagnosed with AKI due to multiple wasp stings. Mean age was 42.7±16.7 years and majority (62.5%) were male. The mean number of stings was 44±23 with a range of 15-100 stings. 26 (81.3%) of the patients had oliguria and 19 (59.4%) of the patients had history of hematuria or having passed cola-colored urine. Hemoglobin was 8.8±2.4 g/dL and white blood cell (WBC) count was 14±7.3 (x103/mm<sup>3</sup>). Peak serum urea and creatinine were 252±113 mg/dL and 11±5.7 mg/dL respectively. The hematological and biochemical laboratory abnormalities were: anemia (96.9%), leucocytosis (53.1%), hyperkalemia (71.9%), severe metabolic acidosis (56.3%), hepatic dysfunction (71.9%), hemolysis (90.6%) and rhabdomyolysis (59.4%). Main complications were acute respiratory distress syndrome (ARDS) and encephalopathy 4 (12.5%) patients each, gastrointestinal bleed, hypertension and eosinophilic panniculitis 2 (6.3%) patients each and one (3.1%) patient each developed intra abdominal bleed, stroke and polyserositis. 29 (91%) patients required dialysis. 10 (31.3%) patients died. The proportion of patients with laboratory abnormalities of leucocytosis (p=0.06), severe metabolic acidosis (p=0.019) and the complications of ARDS and encephalopathy (p=0.006) were significantly higher in patients who died. Kidney biopsy was done in 13 patients. Acute tubular necrosis (ATN) with or without pigmented granular cast was seen in 10 (77%); four (30.8%) severe ATN and other six (46.2%) ATN associated with mild to moderate acute interstitial nephritis (AIN). Three (23%) had only moderate

AIN with overall AIN in nine (69.2%) kidney biopsies. The kidney functions normalized at  $5.6 \pm 2.6$  weeks. AKI due to multiple wasp stings is severe and is associated with high mortality. ATN and AIN are common findings on renal histology.

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### EFFECT OF HUMAN C5A PROTEIN ON MOSQUITO CELLS AND ITS IMPLICATIONS IN ZIKA VIRUS TRANSMISSION

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After ingesting blood from an infected host, the mosquito midgut becomes the first place where the pathogen, the mosquito, and vector factors are in close contact. The interactions occurring at this stage and site have profound implications in pathogen transmission and vector survival. Our previous research suggests that human complement interacts with mosquito proteins, modulating gene expression and defense against pathogens. To test the effect of the human complement on Zika virus (ZIKV) infection of mosquito cells, we treated Aag2 cells with recombinant C5a prior to ZIKV (MR-766 strain) exposure. We found that cells treated with C5a presented lower ZIKV infective particles than the untreated control cells. The effect was abrogated mosquitoes after knock-down of two mosquito trans-membrane proteins suspected to directly interact with C5a. In addition, RNAseq data showed that AAEL009292 (involved in protein-protein interaction) and a CLIPB serine protease protein were significantly up-regulated in cells treated with C5a while AAEL018103 (involved in lipid transport) was significantly downregulated. Our data suggest that human complement may play an important role in modulating the immune response mounted against arboviruses by the arthropod vector.

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### ZIKA-AEDES MOLECULAR INTERACTIONS AND MOSQUITO IMMUNITY-MEDIATED VIRAL SUPPRESSION

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Zika virus (ZIKV) is an emerging arbovirus that recently became a public health threat. ZIKV is transmitted between humans through *Aedes* mosquitoes. However, the molecular interactions between the vector and the virus remain largely unknown. Using RNA sequencing analysis of mosquito's midguts infected with ZIKV or Dengue virus (DENV), we demonstrate that 40% of the mosquito's infection-responsive transcriptome is virus-specific. Regulated genes included key factors of the mosquito's anti-viral immunity. Comparison of ZIKV and DENV infection-responsive transcriptome data to those for Yellow fever virus and West Nile virus identified 26 genes likely to play key roles in arboviral infection of *Aedes* mosquitoes. Through reverse genetic analyses, we show that the Toll and the Jak/Stat innate immune pathways mediate increased resistance to ZIKV infection, and the conserved DENV host factors vATPase and inosine-5'-monophosphate dehydrogenase are also utilized for ZIKV infection. Here, we describe a comprehensive study on the *Ae. aegypti* molecular responses to ZIKV infection, and the implication of mosquito immune system in suppressing virus infection.

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### CHEMICAL DEPLETION OF GRANULOCYTES REVEALS CONTRIBUTIONS OF HEMOCYTES TO ANTI-PLASMODIUM IMMUNITY

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Malaria, caused by *Plasmodium* parasites, is one of the most devastating human diseases, resulting in half a million deaths every year. The interaction between *Anopheles* mosquito vectors and malaria parasites is a key determinant of vector competence. Therefore, an understanding of immune molecules that influence parasite development in the mosquito host can provide opportunities to disrupt disease transmission. Immune cells known as hemocytes play critical roles in killing invading pathogens by phagocytosis, encapsulation and nodulation. However, little is known about hemocyte-mediated immune responses to malaria parasite infection. To address the immune responses mediated by phagocytic granulocytes on bacterial infections and parasite development, we depleted *An. gambiae* granulocyte populations using a chemical-based approach. In subsequent experiments, granulocyte depletion impeded immune defenses to bacterial and malaria parasite infections, causing high mortality and increased parasite survival. Furthermore, we establish that immune responses mediated by granulocytes shortly after parasite infection (<24h) are critical to both early- and late-phase immune responses that limit malaria parasite survival. To our knowledge, this study provides the first definitive insight into the temporal function of cellular immune responses in mosquitoes to malaria parasite infection, and serves as an important advancement to study of phagocytic cells in other insect vectors.

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### LOSS-OF-FUNCTION STUDIES WITH KNOCK OUT AEDES AEGYPTI LINES GENERATED BY CRISPR/CAS9 HIGHLIGHT THE PHYSIOLOGICAL RELEVANCE OF SALIVARY D7 PROTEINS IN BLOOD FEEDING AND PARASITE TRANSMISSION

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*Aedes aegypti* saliva facilitates blood meal acquisition through pharmacologically active compounds that prevent host haemostatic and immune responses. The D7 salivary proteins are one of the most abundant, accounting for 30% of the total salivary protein content. The D7 salivary family of proteins are encoded by a multigene family related to the arthropod odorant-binding protein superfamily and have been shown to act as scavengers of biogenic amines and eicosanoids. Our goal was to generate *Ae. aegypti* D7 knock out (KO) lines by CRISPR/Cas9 system to further characterize the biological function of these proteins by loss-of-function studies. We generated 2 homozygous mosquito lines for the salivary proteins AeD7L1 and AeD7L2. Global transcriptome and proteomics changes were analyzed by RNAseq and LC/MS/MS, confirming the absence of D7 protein expression in the KO mosquitoes. Lack of these proteins was also observed by confocal microscopy of the salivary glands with D7 specific antibodies raised in rabbits. KO mosquitoes took longer to locate blood vessels when feeding on C57BL6 mice and, therefore, had significantly longer probing times (17-300 s,  $P < 0.0001$ ) when compared with parental mosquitoes (18-80 s). These differences in probing time were abolished when B6.129S2-Alox5tm1Fun mutant mice were used instead. Mutant mice do not produce leukotrienes due to the lack of arachidonate 5-lipoxygenase, confirming the role of D7 proteins as leukotrienes scavengers. We also investigated the role of D7 salivary proteins in *Plasmodium gallinaceum* infection and transmission. Our results show that both KO lines carried significantly less oocysts per midgut and a 5-fold reduction in sporozoites number in salivary glands. *Ae. aegypti* KO

lines were successfully produced by CRISPR/Cas9 technology and allowed us to better understand the role of D7 salivary proteins in preventing host hemostatic and immune responses. The information generated by this work highlights the biological functionality of salivary gene products in blood feeding and pathogen transmission.

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### IDENTIFICATION OF A RECEPTOR FOR *PLASMODIUM FALCIPARUM* Pfs47 IN THE *ANOPHELES GAMBIAE* MIDGUT

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We have found that *Plasmodium falciparum*, a human malaria parasite, requires its Pfs47 protein to evade the mosquito immune system in order to infect the vector midgut. We have also obtained evidence that the mosquito immune system can be an important barrier for adaptation of the parasite to evolutionarily distant vectors, through natural selection of Pfs47 haplotypes that are compatible with a given vector. Pfs47 is a member of the 6-cys (s48/47 domain) protein family. It is a polymorphic gene that has signatures of selection with its haplotypes presenting a strong population structure at continental level, consistent with selection by the vector. Here we present evidence for a Pfs47-receptor protein in the *An. gambiae* midgut. The presence of specific binding to Pfs47 by *An. gambiae* midguts was detected by ELISA. Using far-western blotting of midgut proteins probed with recombinant Pfs47, we were able to identify and sequence a specific midgut protein that binds Pfs47. The Pfs47-receptor in *An. gambiae* is a gene of unknown function that has orthologues in other anophelines. dsRNA based knock down of the Pfs47-receptor led to a decrease in *P. falciparum* infection in the midgut, suggesting that it may be a negative regulator of the midgut immune response against the parasite. Binding analysis showed that a recombinant Pfs47 haplotype prevalent in Africa interacts with recombinant Pfs47-receptor from *An. gambiae* (Africa) and *An. dirus* (Asia) while it does not interact with an *An. albimanus* (Americas) Pfs47-receptor. The differences in binding specificity of Pfs47 with its receptors recapitulate the infection compatibility of tested African *P. falciparum* lines with the 3 vectors. This gives more evidence for the "lock and key" model of compatibility between *P. falciparum* and its vectors, in which selection of compatible Pfs47 by the different vectors has determined the adaptation of *P. falciparum* to different vectors around the world.

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### FACTORS TRIGGERING *PLASMODIUM* DEVELOPMENT FOLLOWING *ANOPHELES* SALIVARY GLAND INVASION

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Using an *in vitro* sporozoite development assay mimicking salivary gland invasion we report the results of efforts to identify and characterize insect factors triggering essential changes in *Plasmodium berghei* that are associated with the invasion of mosquito salivary glands and that result in sporozoites competent to infect their mammalian host. Plasmodium parasites undergo a sequential series of developmental changes as they traverse and invade various tissues of both vertebrate and invertebrate hosts. These developmental changes are reflected in the parasites' morphology and/or changes in levels and patterns of gene expression. In *Anopheles* mosquitoes, sporozoites from oocysts and hemolymph differ from sporozoites in salivary glands in that they are less capable of infecting their hosts and are less motility. Following salivary gland invasion sporozoites become competent to infect their mammalian hosts and lose their capacity to reinvade salivary glands. These developmental changes

are reflected in changes in gene expression including up-regulation of some 30 genes. The factors associated with salivary glands that trigger developmental changes during and after salivary gland invasion are largely unknown. Using a UIS4:mCherry-containing line of *P. berghei* (RMgm-1339) that only has visible mCherry expression following salivary gland invasion, we show that UIS4:mCherry up-regulation can occur *in vitro* following exposure to adult female *Anopheles stephensi* salivary gland homogenates and fractions. Treated sporozoites have a transcription profile like that of post-invasion sporozoites. Here we report on results aimed at addressing the nature of the factors associated with salivary glands responsible for triggering this major developmental transition in *Anopheles* mosquitoes.

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### THE ROLE OF TWO FEMALE ATRIAL PROTEASES IN THE REFRACTORINESS OF *ANOPHELES GAMBIAE* MOSQUITOES TO FURTHER MATINGS

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Although the reproductive rate of *Anopheles gambiae* mosquitoes is a key aspect of their capacity to transmit malaria, fertility of these vectors depends on a single copulation event as females become refractory to mating after a first copulation. Interfering with mating is therefore a promising alternative to control mosquito populations in endemic areas, potentially reducing malaria morbidity and mortality. In this study, we show the involvement of two female atrial serine proteases in shaping the mating receptivity of *An. gambiae* females. These two proteolytic enzymes ensure correct processing of the mating plug, a gelatinous structure composed of male seminal secretions that is transferred to the female reproductive tract during mating. We show that correct processing of the plug by these proteases is necessary to trigger a series of events that lead to the reduction of mating receptivity experienced by females after a first copulation. Moreover, these proteases are associated with, and possibly contribute to the formation of a peritrophic matrix-like structure secreted by female atrial cells, which surrounds the plug after mating and may be critical for its function. These results shed new light on the mechanisms regulating female receptivity to mating in *An. gambiae*, with possible implications for reducing the reproductive output of these mosquitoes.

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### EFFECTIVENESS OF HAND SANITIZER WITH HAND AND RESPIRATORY HYGIENE EDUCATION IN REDUCING INFLUENZA-LIKE ILLNESS AND LABORATORY CONFIRMED INFLUENZA AMONG SCHOOL CHILDREN IN BANGLADESH, 2015

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School children are important vectors for transmitting influenza. High infection rates among children and overcrowding at schools in many low-income countries make children more susceptible to exposure and spread of influenza viruses at school. Common measures to prevent transmission include non-pharmaceutical interventions such as handwashing and self-isolation. Handwashing is often a challenge in places like Bangladesh, because of limited availability of soap and water. Evaluations of alternative hygiene options, such as hand sanitizer, in reducing influenza virus

infection are needed. For three months during the 2015 influenza season in Bangladesh (May-September), we conducted a cluster randomized control trial in 24 primary schools in Dhaka city to assess the effectiveness of hand sanitizer with hygiene education to reduce influenza-like illness (ILI) and laboratory confirmed influenza. Twelve randomly selected schools received hand sanitizer with an educational intervention and students were encouraged to use the hand sanitizer at specific times at school. In addition, we taught children to cover their mouth and nose with their upper arm while coughing and sneezing. The 12 control schools received no intervention. We conducted daily follow-up at schools through visits and by phone if a child was absent to identify new ILI cases (cough with fever) and collect nasal swabs to test for influenza viruses. After controlling for cluster level effects and adjusting for classroom and household factors, ILI was 36% lower in the intervention schools compared to the control schools, but this was not statistically significant (Intervention: 22%, Controls: 27%,  $p=0.07$ ). Laboratory confirmed influenza was 55% lower in the intervention schools compared to the control schools after adjusting for gender (Intervention: 3%, Controls: 6%,  $p=0.008$ ). Hand sanitizer with hygiene education was associated with a reduction in ILI and laboratory confirmed influenza among school children. Future influenza prevention strategies in Bangladesh could explore the feasibility of including hand sanitizer and hygiene education in schools.

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### **SPILLOVER EFFECTS OF A COMBINED WATER, SANITATION AND HANDWASHING INTERVENTION IN RURAL BANGLADESH: A RANDOMIZED CONTROLLED TRIAL**

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Water, sanitation, and handwashing (WSH) interventions may confer indirect benefits on neighbors of recipients by interrupting pathogen transmission; prior trials have not measured such "spillover effects". We measured spillovers in WASH Benefits, a large, cluster-randomized trial in rural Bangladesh, by comparing outcomes among neighbors of intervention vs. control participants. WASH Benefits had randomly allocated geographically-defined clusters to a compound-level intervention (chlorinated drinking water, upgraded sanitation, and handwashing promotion) or control and followed children for 2 years. We enrolled neighboring children age-matched to trial participants (within ~ 22 months) that would have been eligible for WASH Benefits had they been conceived slightly earlier or later. After 28 months of intervention, we quantified fecal indicator bacteria in toy rinse and drinking water samples, measured soil-transmitted helminth infections, and recorded caregiver-reported diarrhoea and respiratory illness. Neither fieldworkers nor participants were masked. Analysis was intention-to-treat. Registration: ClinicalTrials.gov #NCT02396407. We enrolled neighbors of the main trial participants in 90 control (N=900) and 90 intervention clusters (N=899). Neighbors' characteristics were balanced across arms. Detectable *E. coli* in tubewell samples was significantly lower among neighbors of intervention compounds (55%) vs. neighbors of control compounds (66%) (prevalence ratio=0.84; 0.73, 0.96). There was no difference in *E. coli* or coliform prevalence between arms for other environmental samples. Neighbors' disease prevalence was similar in intervention vs. control: *Ascaris* (prevalence difference [PD]=0.02; -0.09, 0.05), hookworm (PD=-0.01; -0.01, 0.04), *Trichuris* (PD=-0.01; -0.04, 0.02), diarrhoea (PD=-0.01; -0.03, 0.02), respiratory illness (PD=0.02; -0.01, 0.06). In this randomized trial, we found a compound-level combined WSH intervention improved neighbors' tubewell water quality but did not improve child health outcomes in neighboring compounds.

## 682

### **THE ROLE OF THE ENVIRONMENT IN ROTAVIRUS TRANSMISSION: TEMPERATURE AND HYDROLOGIC FACTORS**

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Rotavirus is known to persist in water sources and its incidence has been shown to vary with temperature. At the same time, studies generally do not assert that its incidence can be reduced by targeting water and sanitation pathways. Here we use compartmental models to examine the potential environmental mechanisms more in detail. We conducted a meta-analysis to estimate the effect of temperature on the rotavirus decay rate in water and examined mechanistic relationships between temperature and incidence using a two-community rotavirus transmission model that accounted for both human-to-human and water-mediated transmission. Pathogen decay rates are significantly related to temperature ( $N=39$ ,  $p<.0001$ ) when modeled with an exponential relationship (fitted equation: ). For temperatures below 20°C, each 1°C increase in temperature results in a 0.004 unit increase in decay rate compared with a 0.016 increase at 25°C. Water acted as an effective disseminator of disease between the upstream and downstream communities for all temperatures, whereas direct transmission was more important for disease amplification within communities. For standing water, this increase in decay led to decreased risk of disease, with every 1°C increase in temperature leading to a 2.5% decrease in risk. Consistent with prior meta-analytic work and time series analyses of climate and rotavirus, our models suggest that water could be an important source of risk for rotavirus that is often overlooked by conventional epidemiologic studies. This environmental transmission of rotavirus is likely to be most important in cooler seasons (resulting in increased persistence) and in larger communities with a standing water source. In these situations, joint interventions that target both direct and indirect pathways are needed to interrupt transmission and the critical coverage to interrupt this pathway is temperature dependent. In contrast, in communities with flowing water systems, targeting direct transmission alone is likely to be sufficient to stop the spread of disease unless the size of the reservoir is small.

## 683

### **IDENTIFICATION OF SPECIFIC ENTEROPATHOGENS AS PREDICTORS OF LINEAR DECLINE IN ENVIRONMENTAL ENTERIC DYSFUNCTION**

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Environmental enteric dysfunction (EED) is an acquired small intestinal inflammatory condition underlying high rates of stunting in children <5yrs in low- and middle-income countries. EED is hypothesized to be due to chronic exposure to enteropathogens, resulting in malabsorption. We aimed to characterize the association between enteropathogenic infection, EED biomarkers and linear growth in a prospective birth cohort in rural Pakistan. Growth was measured monthly for 18 mo. Length-for-age-Z (LAZ) scores were calculated according to WHO 2006 guidelines. Stool and serum samples were collected at 6 and 9 mo for biomarkers of systemic/ enteric inflammation, enterocyte regeneration and bacterial translocation. Molecular screening tests for an array of > 40 enteropathogens were conducted using PCR-based TaqMan cards. Linear regression was used to study the effect of specific enteropathogen infection on change in linear growth  $\Delta LAZ(18mo-birth)$  of  $n=272$  children. Subjects with  $\geq 1$

identified enteropathogens had similar  $\Delta$ LAZ(18mo-birth) scores at six and nine mo, compared to those with no identified pathogens. Increasing numbers of any identified enteropathogen correlated with serum flagellin Ig A (6mo,  $r=0.19$ ,  $p=0.002$ ), fecal Reg1b (6mo,  $r=0.16$ ,  $p=0.01$ ; 9mo,  $r=0.16$ ,  $p=0.008$ ) and serum Reg1b (6mo,  $r=0.26$ ,  $p<0.0001$ ; 9mo,  $r=0.16$ ,  $p=0.008$ ). At 6 mo of age, *Astrovirus* ( $\beta$ (SE)-0.38(0.19),  $p=0.046$ ), *Campylobacter jejuni/coli* ( $\beta$ (SE)-0.36(0.16),  $p=0.03$ ), *Cryptosporidium spp* ( $\beta$  (SE)-0.45(0.20),  $p=0.03$ ) and *Giardia* ( $\beta$ (SE)-0.57(0.23),  $p=0.01$ ) were associated with a trend of negative  $\Delta$ LAZ scores over 18 mo (implying higher LAZ scores at the end of follow-up). At 9 mo this was true of *Norovirus* ( $\beta$  (SE) -0.29 (0.15),  $p=0.049$ ) only. These results showed multiple enteropathogen infections (potential therapeutic targets) occurred early on in life, which is consistent with our hypothesis that EED secondary to enteric infections, is a mediating factor for growth decline in children in low-income countries. The relationship between enteropathogen infection and intestinal pathophysiological changes associated with EED require further study.

## 684

### ANTIBIOTIC RESISTANCE IN DENSE, LOW-INCOME NEIGHBORHOODS: THE ROLE OF SANITATION IN GENE DISPERSION

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Antibiotic resistance (AbR) threatens global public health, yet there has been little focus outside the clinical realm to the role environmental improvements may play in mitigating AbR, particularly in enteric bacterial pathogens. The goal of this study was to understand how sanitation infrastructure may act as a source of or containment strategy against AbR gene dispersion. We aimed to 1) describe the diversity and concentrations of AbR genes circulating in families and young children specifically; and 2) evaluate whether children born into compounds with improved, shared sanitation infrastructure had less diversity and lower prevalence of AbR genes compared to children born into control compounds. We examined the prevalence and diversity of AbR genes in households enrolled in a controlled before-and-after urban sanitation trial. We sampled household latrines (a proxy for family-level measures) and children's stool in intervention and control compounds before and after the implementation of a sanitation intervention with improved containment in Maputo, Mozambique. Samples are being analyzed for AbR genes and gene origins by metagenomic techniques, and tested against a panel of gene targets by qPCR. Subsequently, AbR gene concentrations will be assessed by droplet digital PCR and analyzed for differences by intervention status using regression modelling. Before the intervention, latrine waste had high prevalence and diversity of AbR genes—9/10 latrines were positive for at least one resistance gene target, and 8/10 were positive for 15 or more—and prevalence of enteric infection, measured in children's stool, was high (~90%). Post-intervention latrine waste, and also children's stool specimens, are currently being evaluated for AbR gene content (results by summer). This study will be the first to characterize AbR gene prevalence and diversity in the context of a sanitation intervention, yielding new evidence for environmental approaches to mitigate AbR in dense, highly-contaminated, low-income urban areas with high antibiotic use.

## 685

### EVALUATION OF WATER, SANITATION, AND HYGIENE INFRASTRUCTURE IN RURAL HEALTHCARE FACILITIES — KAMWENGE DISTRICT, UGANDA, 2017

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Lack of access to safe water, sanitation, and hygiene (WASH) in healthcare facilities (HCFs) poses health risks to patients and health workers. Universal WASH coverage in HCFs is included among Sustainable Development Goals (SDGs). We evaluated access to handwashing facilities and safe drinking water, and adequacy of waste management in HCFs in Kamwenge District, Uganda. Our HCF assessments included water sources and treatment, the availability of hand washing and drinking water stations, and tests of water from HCF sources and a sample of drinking and handwashing stations for *Escherichia coli*. Of 62 HCFs surveyed, 30 (48%) were public and 32 (52%) were private. Outpatient services were provided by all HCFs and inpatient services by 42 (67%), including 30 (94%) private HCFs. The main water source was classified as improved for 53 (85%) HCFs; water sources were on premises in 32 (52%) HCFs. Water was unavailable from the main source for  $\geq 3$  months per year in 16 (26%) HCFs. Water was always available in patient care areas in 19 (31%) HCFs. Hand washing stations were observed in 56 (92%) of 62 HCFs. These 56 HCFs had a total of 159 patient care areas, and 99 (62%) usable handwashing stations (defined as having water and soap present, and no broken parts). While 32 (51%) of 62 HCFs reported usually having drinking water for staff and patients, 23 (38%) had drinking water available on interview day. Regular drinking water treatment was reported by 30 (48%) of 62 HCFs, with 16 (53%) reporting chlorination and 14 (47%) boiling. *E. coli* contamination was found in water samples from 17 (39%) of 44 accessible water sources, 2 (13%) of 16 drinking water stations, and 16 of 72 (22%) handwashing stations. Of 62 HCFs, 33 (53%) separated infectious and noninfectious waste, and 10 (16%) disposed of sharps securely. All HCFs burned at least some waste, with 2% using a lined pit for disposal. Most HCFs lacked access to reliable supplies of water for handwashing and drinking, and had inadequate waste management practices. Achievement of the SDG for HCFs in Kamwenge District will require substantial WASH investments.

## 686

### EVALUATION OF A LARGE-SCALE DISTRIBUTION OF WATER FILTERS IN WESTERN PROVINCE, RWANDA

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Unsafe drinking water is a major cause of death and disease in low-income countries, and interventions to improve water quality within the household can reduce the risks associated with high exposures. However, little is known about the effectiveness of these interventions delivered programmatically at scale. In late 2014, a public-private partnership between the Rwanda Ministry of Health and DelAgua Health, a private social enterprise, distributed and promoted the use of tabletop Lifestraw Family 2.0 water filters in Western Province. Filters were delivered free to the poorest 25% of households (N=101,000) within randomly allocated sectors (administrative units), with the remaining sectors serving as controls. Using two-stage random sampling, we selected 1582 households from 199 villages to assess household-level filter uptake and impacts on water quality and diarrhea in children under 5 years of age. Households

were visited every 4 months over a 12-month period; 94.9% were followed up at visit 1, 91.6% at visit 2, and 89.1% at visit 3. At visits 1, 2, and 3, 94.0%, 93.8%, and 91.7% of households had the filter, and of these, 69.8%, 66.9%, and 58.7% had water respectively. Using a random intercept mixed effects negative binomial model accounting for clustering within villages and households, we found a 77% reduction in thermotolerant coliform contamination in the intervention arm (IRR .23, 95% CI 0.15-0.36,  $p < .001$ ). Using a random intercept mixed effects logistic model adjusting for clustering at village, household, and child levels, caregiver-reported child diarrhea within the previous 7 days was also significantly reduced in the intervention arm (OR 0.60, 95% CI 0.47- 0.76,  $p < .001$ ,  $n = 2345$  children contributing 5925 observations). The results of this trial, the first of a large-scale programmatic household water filter intervention, demonstrate positive household environmental and child health improvements as delivered. However, declining filter usage over the study period suggests continued programmatic engagement and behavior change is necessary to maintain and maximize health benefits.

## 687

### THE EFFECT OF CHRONIC HELMINTH INFECTION ON IGE-MEDIATED ANAPHYLAXIS IN SENSITIZED MICE

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Numerous animal studies have reported that helminths can prevent allergic sensitization, yet few have evaluated the effects of infection on pre-existing allergy. We hypothesized that chronic infection would protect against the clinical symptoms of IgE-mediated anaphylaxis in previously sensitized mice. Mice were sensitized by weekly intraperitoneal (IP) injection of either ovalbumin (OVA)/alum or PBS/alum for 3 weeks. At 4 weeks, mice were infected with *Litomosoides sigmodontis* (*L.s.*), a rodent filarial parasite, or mock infected. Mice were challenged IP with OVA during the twelfth week of infection. Following challenge, serum levels of murine mast cell protease 1 (mMCP-1) were significantly lower in sensitized mice that were *L.s.*-infected as compared to mock infected. Additionally, *L.s.*-infected mice that had been previously sensitized exhibited an average drop in core body temperature of 3.5 °C after 30 min, which was significantly less than the 5.0 °C drop observed in sensitized controls. Anaphylaxis was IgE and mast-cell dependent, as mice deficient in FcεRI or mast cells released little to no mMCP-1 and exhibited minimal drops in core body temperature in response to challenge. Experiments suggest that changes in mast cell functionality were central to the protective effect. First, serum levels of OVA-IgE were not altered by *L. sigmodontis* infection. Second, purified mast cells from OVA-sensitized and infected mice released less histamine than OVA-sensitized and mock-infected mice in response to stimulation with anti-IgE. These results suggest that chronic helminth infection protects against anaphylaxis by decreasing allergen-driven mast cell degranulation. Interestingly, 7 weeks of infection with *L. sigmodontis* did not protect against clinical symptoms of anaphylaxis in sensitized mice. Studies are underway to determine the effect of chronicity of infection on anaphylaxis. Last, IL-10 was investigated as a potential mechanism for protection against anaphylaxis at 12 weeks post-infection. IL-10 was not required for protection as IL-10 knockout mice were protected against hypothermia.

## 688

### INTERLEUKIN 13 AND HEDGEHOG SIGNALING PATHWAYS REGULATE FIBROSIS COLLABORATIVELY IN SCHISTOSOMIASIS MANSONI

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IL13 and Hedgehog (Hh) signaling pathways have both been implicated in the pathogenesis of schistosomiasis. In this study, we investigated if there is cross-talk between IL13 and Hh pathways in schistosomiasis liver fibrosis. Hh and IL13 signaling were investigated in uninfected healthy transplant donors, hepatointestinal and hepatosplenic patients by qRT-PCR, immunohistochemistry and ELISA. To confirm the association between Hh and IL13 pathways, we investigate the levels of Hh pathway activation in *Schistosoma mansoni* infected wild-type mice, IL13Rα1-/- mice lacking IL13 signaling and TKO (IL-10-/-, IL12p40-/-, IL13Rα2-/-) mice exhibiting enhanced IL13 activity. To investigate the role of IL13 in Hh pathway, primary murine and human Kupffer cells and human hepatic stellate cells were stimulated with recombinant IL13 *in vitro*. To elucidate the role of IL-13 in Hh pathway activation *in vivo*, wild-type mice were injected with GFP or IL13 overexpressing plasmid. Infected mice treated with isotype control or anti-IL13 neutralizing antibody were included to confirm if Hh ligands/transcription factors are dependent on IL13. Patients with severe schistosomiasis had increased IL13 and Hh pathway activity and both pathways correlate with each other and with collagen deposition, fibrosis staging by ultrasound and severity of portal hypertension. Infected TKO mice had more collagen, myofibroblasts, M2 macrophages and Hh pathway activity than infected wild-type mice, infected IL13Rα1-/- mice, and non-infected controls. Infected IL13Rα1-/- mice had lower expression of Ihh, Shh, Dhh, Gli1 and Gli2 than uninfected controls, suggesting that IL13 promotes Hh signaling. IL13 directly induced Hh ligand production by Kupffer cells and induced Gli2 expression in hepatic stellate cells. Overexpression of IL13 *in vivo* demonstrates that Ihh and Dhh are target genes of IL13 signaling. Neutralization of IL13 abrogates Hh ligand production/ activation of Hh pathway and reduced fibrogenesis. IL13-mediated activation of Hedgehog pathway promotes liver fibrosis in schistosomiasis and could be a novel therapeutic target.

## 689

### TRANSCRIPTOMIC-BASED FUNCTIONAL CHARACTERIZATION OF HOST SYSTEMIC ADVERSE EVENTS FOLLOWING LYMPHATIC FILARIASIS TREATMENT

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Lymphatic filariasis (LF) is a neglected tropical disease caused by the nematode parasite *Wuchereria bancrofti*. The primary tool used by the Global Program to Eliminate LF is mass drug administration (MDA), and some 500 million people take the medications each year. Mild to moderate adverse events (AEs) are common after LF treatment, and these pose a challenge for the LF elimination program. To better understand the pathogenesis of AEs, we studied patients from a LF treatment trial in Côte d'Ivoire. Total RNA was extracted from peripheral blood leukocytes collected before and 24h after treatment (when AEs peak). Global RNA sequencing was performed for 9 individuals with systemic AEs and for 9 matched controls without AEs. Differential gene expression analysis (DESeq) identified transcriptional signatures (TS) associated with post-treatment AEs. Out of the 36 sequenced samples, the 9 post-treatment samples from subjects with AEs had a distinct TS ( $P = 0.006$  by clustering analysis); 744 genes were significantly upregulated in this group (post vs pre-treatment, paired). These genes were enriched for many biological pathways that included pro-inflammatory pathways such as TLR and NF-kappa B signaling. Genes upregulated in AEs were also significantly enriched for having STAT1/2/3 transcription factor binding sites indicating

the importance for interferons in the AEs pathogenesis. A computational method to infer leucocyte proportions (CIBERSORT) identified that neutrophil numbers increase while lymphocytes decrease more post-treatment in individuals with AEs compared to people with no AEs, suggesting an AEs stress response. Additionally, a method to compare transcriptional signatures to existing datasets (GeneQuery) matched the AE TS to endotoxin exposure signatures, indicating a possibility for *Wolbachia* lipoprotein to be involved in AE development. This was supported by significant post-treatment increases in plasma LPS Binding Protein (LBP) in individuals with AEs. Improved understanding of the pathogenesis of AEs may lead to improved management or prevention that could increase MDA compliance and hasten LF elimination.

## 690

### PROTECTIVE IMMUNITY WITH HUMANS IS CONSISTENT WITH A DEFINED IMMUNE RESPONSE AGAINST THE TWO LEAD *ONCHOCERCA VOLVULUS* VACCINE CANDIDATES, OV-103 AND OV-RAL-2

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*Onchocerca volvulus*, a filarial nematode is an etiologic agent of river blindness that infects approximately 17 million people, mostly in Africa. The current strategy for elimination of *O. volvulus* focuses on controlling transmission through ivermectin-based mass drug administration (MDA) programs. Due to potential ivermectin resistance, the lack of macrofilaricidal activity by ivermectin and the prolonged time line (>20 years) needed for transmission interruption, additional tools are critically needed including the need for a vaccine against onchocerciasis. Ov-103 and Ov-RAL-2 are presently the most promising vaccine candidates for a prophylactic vaccine. The mechanism of protective immunity induced in mice by the adjuvanted Ov-103 and Ov-RAL-2 vaccines appear to be multifactorial with roles for cytokines, chemokines, antibody and specific effector cells. However, the involvement of immune responses against these two larval proteins in the development of protective immunity in humans has not been yet studied. The role of Ov-103 and Ov-RAL2 specific antibodies was determined in two human populations in Cameroon; the putatively immune (PI: Mf- over 5 years) and the infected (INF; Mf+). Both populations have significant cytophilic antibody (IgG1, IgG3) responses to both antigens as measured by ELISA. Moreover, the IgG3 response to both antigens was significantly increased with age (concomitant immunity). Secondly, we analyzed the systemic levels of cytokines/chemokines in plasma of both populations using milliplex magnetic bead assay. We found that cytokines and chemokines associated with monocyte recruitment such as TNF- $\alpha$ , IL-10, IP-10, MCP-1 and MIP-1 $\beta$  were elevated in the plasma of both INF and PI individuals. Finally, we have shown that human mono-specific Ov-103 and Ov-RAL-2 antibodies inhibited 70-80% of molting of L3 larvae *in vitro* (but did not kill) when cultured in the presence of naive human monocytes, suggesting that monocytes and their soluble factors may be associated with protective immunity also in humans.

## 691

### A NOVEL MECHANISM FOR IMMUNE EVASION BY A HUMAN FILARIAL PARASITE

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A hallmark of chronic infection with many different species of human parasitic helminths is the development of a relative degree of immunosuppression in the infected host. This phenomenon is believed to represent an evolutionary adaptation of the parasite to promote its survival. Cytokine orthologues have been reported in many different

helminths however until recently, there were no examples of a parasite-derived chemotactic factor that had structural similarity to human chemokines. Herein we report an overview of our research on the first reported example of a parasite "physiocrine" an asparaginyl-tRNA synthetase (BmAsnRS) that has one function intracellularly (aminoacylation of tRNA) and a second novel role in signal transduction when it is transported extracellularly via interaction with interleukin-8 receptors. We have developed a hypothesis of how this enzyme can exert two diverse biological activities based on comparative structural studies of the filarial BmAsnRS. This hypothesis suggests that the mechanism of action of BmAsnRS is different from the physiocrine activities reported in human tRNA synthetase splice variants that are known to exhibit diverse and novel biological activities with the ability to specifically bind to CCR5, CCR3 or IL-8 chemokine receptors.

## 692

### CORD BLOOD ANTI-PARASITE IL-10 AS RISK MARKER FOR COMPROMISED VACCINE IMMUNOGENICITY IN EARLY CHILDHOOD

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Previous studies have established a significant impact of maternal parasitic infections during pregnancy on infants' subsequent responses to vaccination. The present study investigated how prenatal infections and lymphocyte cytokine profiles at birth relate to antibody responses to standard vaccination against *Haemophilus influenzae* (Hib), diphtheria toxoid (DT), hepatitis B (Hep B) and tetanus toxoid (TT) during infancy. 450 Kenyan women were tested for malaria, schistosomiasis, lymphatic filariasis (LF), and intestinal helminths during pregnancy. Their newborns' responses to malaria blood-stage antigens, soluble *Schistosoma haematobium* worm antigen, and filaria antigens were assessed in cord blood (CB) lymphocytes collected at delivery. The cohort children were then followed biannually to age 36 months after receiving three rounds of standard vaccination given at 6, 10 and 14 weeks of age. Cohort children were tested for absolute levels of IgG against Hib, DT, Hep B, and TT at each visit. In extended analysis of the time series of post-vaccination IgG levels, we noted 4 types of anti-vaccine response profiles among children: Dimension reduction by functional principal component analysis defined two quantitative components that could be used to quantify each child's pattern: PC1, reflecting height of response over time (high versus low), and PC2, reflecting the tendency to cross over from high to low or from low to high. In linking our observed CB cytokine responses to parasite antigens at birth, a highly significant association ( $P < 0.001$ ) was found between augmented anti-helminth IL-10 response to *Schistosoma* and filaria and downward shifts in antibody response, particularly to DT. Overall diminished response (PC1) was seen for DT ( $P = 0.04$  for prenatal *Schistosoma* exposure) and Hep B ( $P = 0.03$  for prenatal filarial exposure), whereas a late decline (PC2) was seen for anti-Hib responses after prenatal helminth exposure ( $P = 0.02$  for LF and  $P = 0.09$  for *Schistosoma*). Further studies are warranted to document whether treatment of these infections during pregnancy can alleviate prenatal parasitic exposure impact on vaccine responses.

## 693

**THE IMPORTANCE OF US FOREIGN AID FOR GLOBAL MALARIA CONTROL AND ELIMINATION**

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The President's Malaria Initiative is a leading global funder of malaria control interventions. In the period 2005-2015 PMI contributed over \$4.4 billion dollars to malaria control in 21 countries. This constitutes a significant proportion of the global malaria budgetary envelope. We present a case study detailing the past impact and future projected impact that PMI has had on malaria control and the potential synergies that have arisen due to PMI investment. Using an established malaria transmission model we have estimated the past and future global trajectories for malaria under different funding scenarios. We estimate that, since its inception in 2005 PMI has prevented 150 million malaria cases and saved 838 thousand lives. Ongoing investment could avert an extra 131 million cases saving a further 614 thousand lives over the next four years. PMI investments are highly cost effective. Our estimate of \$124 (\$69 - \$775 range across African countries) per DALY averted is significantly below the WHO threshold for cost-effectiveness in those countries. We highlight the impact that any reduction in future PMI funding could have both in terms of the reduction in direct prevention of malaria, but also on the capacity of health systems to respond to increased caseloads. Reduced caseloads due to interventions funded by PMI has knock-on beneficial effects for country health systems, saving an estimated further \$145 million over the period 2017-2020.

## 694

**ESTIMATING THE EFFECT OF HEALTH SYSTEMS ON CHILDHOOD MORTALITY IN SUB-SAHARAN AFRICA FROM 1996-2013**

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Approximately 6 million children die each year before their fifth birthday. The majority of post-neonatal deaths are due to infectious causes such as diarrhea, malaria, and pneumonia, all of which can be effectively prevented or treated with cost-effective interventions delivered through even very basic health facilities. We hypothesize that access to these life-saving interventions may be limited by the health systems that deliver them and therefore sought to understand the role of supply-side factors on childhood mortality. First, we examine the relationship between survival beyond 59 months of age in Kenya and three domains of health services; geographic distribution of health facilities, quality of services, and cost of services. Using birth history data from nearly 2 decades, we assembled a retrospective cohort of 81,000 children and describe the health systems context of each child using Service Provision Assessments representative of the province at the time of a child's birth. We find significant geographic heterogeneity in survival; children in Nyanza experience double the average risk of death while residence in Central and Nairobi conferred a survival advantage. These differences can be partially explained by differences in distribution of health facilities and fees for sick child visits. Higher per capita density of health facilities resulted in a 25% reduction in the risk of death (HRR=0.73, 95%CI:0.58 to 0.91) and accounted for 30% of the between-province heterogeneity in survival. User fees for sick-child visits increased risk by 30% (HRR=1.30, 95%CI:1.11 to 1.53). We did not find an effect of the number and training of clinical staff on survival, perhaps indicating that personnel are not the limiting factor in delivering adequate care. We extend this investigation to describe the relationship between health services and child survival in seven countries in sub-Saharan Africa. Our preliminary results implicate health systems

constraints in child mortality, quantify the contribution of specific domains of health services, and suggest priority areas for improvement to accelerate reductions in child mortality.

## 695

**INFLUENZA VACCINE KNOWLEDGE AND ACCEPTABILITY**

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Vaccination against seasonal influenza has been associated with a decrease in the risk of adverse pregnancy outcomes. Vaccination of pregnant women offers secondary protection to infants under 6 months of age from influenza illness. In 2012, the WHO Strategic Group of Experts recommended influenza vaccination to pregnant women. We administered a knowledge, attitudes, beliefs, and behaviors (KABB) questionnaire to assess the acceptability of this recommendation to a cohort of pregnant women from 2 peri-urban health centers in Panama and El Salvador. From October 2014 - March 2017, 1708 participants completed the questionnaire at the time of survey administration, 75% (n=1281; 95%CI: 0.73 - 0.77) of participants reported they had received the influenza vaccine for the current season. Interview takes place usually at the beginning of enrolment. Coverage was 81% (n=1391 95%CI: 0.80-0.83) at the end of pregnancy. More than half (60%; n=1026; 95%CI: 0.58-0.62) of participants had heard of influenza; 60% (n= 1035; 95% CI: 0.58-0.63) believed they were at risk for influenza; and, 90% (n=1545; 95% CI: 0.89 - 0.92) agreed pregnant women should be vaccinated against influenza. The majority considered vaccines to be safe (81%, n=1389 95% CI: 0.79-0.83) and without harmful long-term effects (58%, n=993; 95% CI: 0.56-0.60). When asked, "During your pregnancy, what would be the main reason why you would not get vaccinated against influenza" participants who did not receive the influenza vaccine before giving birth responded: lack of doctor or nurse recommendation (48%, n=148), concern about the safety and side effects of the vaccine (12%; n=39), "don't know" (8%; n=26), not knowing where to get the vaccine (5%, n=16), never getting sick (4%, n=14), and other reasons (23%, n=74). Influenza vaccination coverage among health center attendees and KABB results suggest the acceptability among pregnant women in Panama and El Salvador. Public health officials may be able to further protect pregnant women and their babies by ensuring physicians consistently recommend and educate about influenza vaccine, and building-on the existing culture of vaccination.

## 696

**ARE ORAL CHOLERA VACCINES COST-EFFECTIVE AND AFFORDABLE IN DHAKA, BANGLADESH? COST-EFFECTIVENESS OF ORAL CHOLERA VACCINE INTRODUCTION IN DHAKA, BANGLADESH**

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Cholera remains a serious public health problem in developing countries, and can be either endemic or epidemic. Cholera epidemics are often associated with humanitarian crises such as floods, other epidemics, population displacement or armed conflict. Vaccination with oral cholera

vaccine (OCV) has been shown to be an effective intervention to prevent cholera in areas with endemic and epidemic cholera in the short- and medium term until improvements in access to clean water and sanitation can be ensured. The cost-effectiveness of introducing oral cholera vaccine in populations at high-risk of cholera in Dhaka in Bangladesh from 2016-2025 is estimated in this presentation. The effectiveness of introducing oral cholera vaccination in Dhaka City was estimated through modeling the number of cases of cholera that would be averted with and without vaccination. To identify areas in Dhaka at high risk of cholera, we analyzed data from the laboratory surveillance of 2% of hospitalized ICDDR,B diarrhea patients in Dhaka from 2011 to 2015. To estimate the impact of different vaccination targeting strategies on the incidence of cholera in this population over ten years, we used a mathematical model that simulates the dynamics of cholera transmission. To estimate the cost of vaccination, we used data from the pilot ICDDR,B OCV introduction in Mirpur, Dhaka. We estimated the net cost of introducing the vaccine through subtracting the cost of treating the disease from the cost of implementation for three age groups: 1-4 years, 1-14 years and all persons greater than 1 year. We estimated the cost per case averted, cost per death averted and cost per DALY averted for each age group. We also conducted univariate sensitivity analysis. In Bangladesh, the results of dividing the net vaccination costs by DALYs averted for each vaccination strategy and vaccine vial size was estimated. The option of vaccinating 1-14 year olds is the most cost-effective, with a cost per DALY averted of \$479 and \$674 for one and ten-dose vial sizes, respectively. The study shows that introduction of oral cholera vaccine is cost-effective and affordable in high risk areas of Dhaka.

## 697

#### VALIDITY OF A MINIMALLY INVASIVE AUTOPSY TOOL FOR CAUSE OF DEATH DETERMINATION IN PEDIATRIC DEATHS FROM SUB-SAHARAN AFRICA

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In recent decades, the world has witnessed unprecedented progresses in child survival. However, our knowledge of what is killing nearly six million children annually in low and middle income countries remains poor, partly because of the inadequacy and reduced precision of the methods currently utilized in these settings to investigate causes of death. The minimally invasive autopsy (MIA) approach has been proposed as an adequate and more acceptable substitute to the complete diagnostic autopsy (CDA) for cause of death investigation in poor settings. The validity of the MIA approach in determining the cause of death was assessed in 54 post-neonatal pediatric deaths in a referral hospital of Mozambique by comparing the results of the MIA with those of the CDA. Concordance between the categories of diseases obtained by the two methods was evaluated by the Kappa statistic and the sensitivity, specificity, positive and negative predictive values of the MIA diagnoses were calculated. A cause of death was identified in all cases in the CDA and in 52/54 (96%) of the cases in the MIA, with infections and malignant tumors accounting for the majority of diagnoses. The MIA categorization of disease showed a substantial concordance with the CDA categorization (Kappa = 0.70, p-value < 0.0001) and sensitivity, specificity and overall accuracy were high. The ICD-10 diagnoses were coincident in up to 75% (36/48) of the cases. The MIA allowed the identification of the specific pathogens deemed responsible for the death in 25/32 (78%) of all deaths of infectious origin. Discrepancies between the MIA and the CDA regarding

individual diagnosis could be minimized with the addition of some basic clinical information such as those ascertainable through a verbal autopsy or clinical record. The MIA showed a substantial performance for cause of death identification in this series of pediatric deaths in Mozambique. This minimally invasive approach, simpler and more readily acceptable than the disfiguring CDA could provide robust data for CoD surveillance specially in resource-limited settings, which can be helpful to guide child survival strategies in the future.

## 698

#### EMPIRICAL ESTIMATES OF DISABILITY BURDEN OF A SYMPTOMATIC DENGUE EPISODE

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Disability weights are critical to assess the cost-effectiveness of dengue control interventions, but existing estimates are few and variable. To generate robust estimates, we combined a meta-analysis and original data from Puerto Rico for acute episodes, and a model for chronic (persistent) dengue, which leads to depression, fatigue and other symptoms. The meta-analysis searched major data bases for empirical studies through 2016 with "dengue" combined with "quality of life," "disability," or similar terms in 4 languages. The Puerto Rico study was based on detailed retrospective interviews with 101 (69 hospitalized and 32 ambulatory) patients with laboratory-confirmed dengue across the island. To compute the disability-adjusted life year (DALY) burden for acute episodes, we fit the extent of disability by day of illness (where 0 denoted no disability and 1 disability equivalent to death) to reported data and computed the area under this disability curve. The model for chronic dengue was based on a prevalence regression (C Tiga et al, AJTMH, 2016) and the disability weight for "major depressive disorder, mild episode" (J Salomon et al, Lancet, 2015). The meta-analysis found 5 published studies. Results are presented as means ± standard errors. The Puerto Rico study found: durations (in days) 15.31±0.99 (all cases), 12.13±1.26 (ambulatory cases), 16.80±1.30 (hospitalized cases); DALY per episode 0.0174±0.0012 (all cases), 0.0150±0.0017 (ambulatory cases), 0.0185±0.0016 (hospitalized cases); disability weights per day 0.4105±0.0145 (all cases), 0.4292±0.0275 (ambulatory cases), 0.4018±0.0170 (hospitalized cases). The model for persistent dengue found 0.092 person years and a DALY burden of 0.0133±0.0026 per dengue episode. Combining all studies, ambulatory and hospitalized episodes have average DALY burdens per episode of 0.0116±0.0026 and 0.0148±0.0053, respectively (acute phase) and 0.0249 ±0.0037 and 0.0281±0.0034, respectively (both phases). These results are within the broad range of previous estimates, but offer more systematic estimates of burden for cost-effectiveness analyses and burden of disease estimates.

## 699

#### SATELLITE AND IN SITU CLIMATE DATA MEASUREMENTS AT CHIKUNGUNYA AND DENGUE STUDY SITES IN KENYA

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Understanding climate and ecological conditions associated with the emergence and propagation of vectors of chikungunya and dengue fever is critical to managing and diminishing the burden of these diseases to

populations at risk. As part of the study of the burden of chikungunya and dengue in Kenya, we have been monitoring various climate variables (including rainfall and temperature) by satellite remote sensing and *in situ* measurements at four study locations in Kenya: inland near Lake Victoria and at the south coast of Kenya. The *in situ* measurements are to inform proximate conditions at the study locations while satellite measurements provide a broader regional view of the conditions. We compare the two sets of measurements and find a good temporal correspondence in the evolution of monthly and daily time series especially over the coastal locations. However, we find that in some instances satellite measurements may overestimate rainfall amounts at inland locations and “underestimate” over the coastal locations. This can be attributed to (a) the different rainfall types: orographic vs convective, (b) area (satellite) versus *in situ* (point) measurements and (c) the random nature of daily rainfall. When aggregated at the monthly time scale, we find a high correlation ( $r = \sim 0.9$ ) overall for between satellite estimates of rainfall and *in situ* rainfall measurements at all sites. We also compare these measurements to vector collections and find a good agreement between rainfall and vector population peaks at all locations; however, there is a time lag ( $\sim 1$  month) between peak rainfall and the peak in vectors numbers collected. We conclude for large area surveillance, satellite derived rainfall measurements can be used to monitor conditions for vector emergence especially areas without surface observations.

## 700

### TIME COURSE OF *PLASMODIUM FALCIPARUM* GAMETOCYTE DEVELOPMENT 1 (PFGDV1) EXPRESSION AND ACTIVITY

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The development of malaria parasite sexual stages is critical for transmission, yet much remains unknown about how this process is regulated. Recently, epigenetic repression and expression of the transcription factor AP2-G have been shown to play important roles in regulating sexual commitment. However, the mechanisms that release repression and consequently allow gametocyte production are still a mystery. Our previous work demonstrated that *Pfgdv1* is required for gametocyte production. Here, we investigate the temporal relationship between PFGDV1 protein expression and gametocyte production by tagging the 3' end of the endogenous *Pfgdv1* gene with green fluorescent protein (GFP) and a ligand (Shield-1)-stabilized degradation domain (DD). Growing tightly synchronized PFGDV1-GFP-DD parasite in the presence of the stabilizing ligand, GFP expression was observed in a majority of parasites from  $\sim 33$ -42 hours post invasion, while in the ligand's absence no GFP expression was observed along with a drastic reduction in gametocyte production and lower transcript levels for AP2-G. To determine when PFGDV1 expression is required for sexual commitment and gametocyte production, PFGDV1-GFP-DD expression was stabilized for discrete time periods and gametocyte production evaluated. Expression was only required during schizogony for optimal gametocyte production following the next cycle of RBC invasion. To evaluate PFGDV1-conditional transcriptional responses during sexual commitment, single cell RNA-seq of developing late asexual blood-stages in the presence or absence of Shield-1 ligand was performed. Using single cell resolution, we identified transcript levels of 27 genes that were significantly up-regulated upon PFGDV1 stabilization. Only two, AP2-G and MSP7-like protein (MSRP1) have previously been associated with gametocytogenesis, while five are predicted to localize to the nucleus, including ALBA1 and DNA-binding chaperone, PF3D7\_1216900. Together these data suggest that PFGDV1 acts upstream of the regulation of AP2-G, prior to the release of gametocyte-committed merozoites.

## 701

### LONG-TERM *IN VITRO* CULTURE OF *PLASMODIUM VIVAX* ISOLATES FROM MADAGASCAR MAINTAINED IN *SAIMIRI BOLIVIENSIS* BLOOD

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*Plasmodium vivax* is the most prevalent human malaria parasite and is likely to increase proportionally as malaria control efforts reduce the impact and prevalence of *P. falciparum*. Despite the prominence of *P. vivax* as a major human pathogen, vivax malaria qualifies as a neglected and under-studied tropical disease. Significant challenges bringing *P. vivax* into the laboratory have limited the study of this parasite's red blood cell (RBC) invasion mechanism, blood stage development, gene expression and genetic manipulation. Patient isolates of *P. vivax* have been collected and cryopreserved in the rural community of Ampasimpotsy, located in the Tsiroanomandidy Health District of Madagascar. Periodic, these cryopreserved isolates are transported to the country's National Malaria Control Program laboratory in Antananarivo preceded onward sample transfer to laboratories at Case Western Reserve University, USA. There, the *P. vivax* isolates have been cultured through propagation in RBCs of *Saimiri boliviensis*. For the four patient isolates studied to-date the average time interval between sample collection and *in vitro* culture has been 509 days (range 166 to 961 days). The average time in culture, continuously documented by light microscopy, has been 111 days; Pv AMP2014.01 was continuously propagated for 233 days. Further studies show that the *P. vivax* parasites propagated in Saimiri RBC retain their ability to invade human RBCs. Long-term culture of *P. vivax* is possible in RBCs of *Saimiri boliviensis*. These studies provide an alternative to propagation of *P. vivax* in live animals that are becoming more restricted. *In vitro* culture of *P. vivax* in Saimiri RBC provide new strategies for investigating the molecular and cellular biology of this important malaria parasite.

## 702

### AN EX VIVO GAMETOCYTE CULTURE METHOD TO DETERMINE *PLASMODIUM FALCIPARUM* GAMETOCYTE COMMITMENT IN THE PATIENT'S PERIPHERAL BLOOD

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Malaria transmission via mosquito is vital to spread the disease, however the factors influencing the production of the sexual stage parasites required have been challenging to define. *In vitro*, commitment to sexual differentiation in *P. falciparum* begins during the trophozoite to schizont transition and leads to the production of gametocyte-committed merozoites. However, distinct gametocyte-specific morphological differences are not apparent for 4-5 days after red blood cell (RBC) invasion. By this time in the human host the early gametocyte stages have already sequestered and cannot be observed in the peripheral blood. Consequently, currently there is no way to monitor gametocyte production until 10-12 days after invasion when mature stage V gametocytes are released into the circulation. To begin to investigate this hidden stage and identify early gametocyte specific biomarkers, we developed an ex

vivo gametocyte culture method to quantify gametocyte-committed parasites circulating in malaria patients' blood. Blood samples were obtained from uncomplicated malaria patients in Ghana (n=170) just prior to treatment and cultured for 8 days in the presence or absence of N-acetyl glucosamine (NAG) to track gametocyte development by counting Giemsa-stained slides (~20,000 RBCs/slide). As seen *in vitro* with strain NF54 parasites, stage II-III gametocytes were observed by Day(D) 4 and continued to mature to stages III-V by D8 in 66% of the samples (n=112). There was good correlation between the D4 and D8 gametocytemias as well as D8 gametocytemia and D8 transcript levels of mature gametocyte marker, Pfs25. Based on the D0 parasitemia, the gametocyte conversion rate ranged from (0.02% to 88%) with a median of 1.72%. Preliminary data suggest that conversion is not related to age, hematocrit or WBC cell count. The gametocyte densities detected were all 100 times higher than the theoretical limit of ~2 gametocyte/ $\mu$ l needed to infect mosquitoes indicating the robust infectious production of the parasite. This assay provides an important tool to directly evaluate both the host and parasite factors that contribute to gametocyte production.

### 703

#### NOT1-G IS A NOVEL MEMBER OF THE CAF1/CCR4/NOT COMPLEX THAT IS ESSENTIAL FOR HOST TO VECTOR MALARIAL TRANSMISSION

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The transmission of the malaria parasite between mosquitoes and mammals requires translational repression to ensure that only the proper proteins are expressed at the right time, while still allowing the parasite to prepare the mRNAs it will need for the next developmental stage. With relatively few known specific transcription factors (ApiAP2 family) that may specifically initiate gene transcription, *Plasmodium* parasites also regulate the stability and turnover of transcripts to provide more comprehensive gene regulation. We and others have demonstrated that members of the CAF1/CCR4/NOT complex have specific and important functions in both the development of the parasite (CAF1) and in its host-to-vector transmission (CCR4-1). In model eukaryotes, this large multi-functional complex uses its largest component, NOT1, as a scaffold for its effector proteins, such as CAF1 and CCR4-1. Here, in stark contrast to most other eukaryotes, we have bioinformatically identified that *Plasmodium* species encode two putative NOT1 proteins. These are only detectable in *Plasmodium* and in the related *Theileria* parasite genome, but are absent in all others, indicating that specific functions may be restricted to this clade of apicomplexans. We find that in *Plasmodium* NOT1 is essential as it is in model organisms, but that *not1-g* parasites produce male gametocytes that cannot activate nor be transmitted to mosquitoes. Interestingly, both NOT1 proteins are expressed throughout the life cycle and yet do not appear to have redundant functions. We hypothesize that NOT1 and NOT1-G associate with distinct accessory factors that determine their function and are now determining this using a proximity based biotinylation approach. Ultimately the NOT1-G complex likely affects mRNAs important to gametocytogenesis and/or gametocyte activation, and we are currently investigating this using comparative transcriptomics. Thus, *Plasmodium* has evolved a paralog of NOT1 that is essential for transmission and plays an important role in the parasites utilization of RNA regulation for this critical process.

### 704

#### PB102, A NOVEL GENE ESSENTIAL FOR FEMALE FERTILITY OR OOKINETE MATURATION OF MURINE MALARIA PARASITE, PLASMODIUM BERGHEI

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A portion of blood stage malaria parasites (~10%) differentiates into male and female gametocytes. Both sex gametocytes fertilize and then

differentiate into ookinetes in the *Anopheles* mosquito midgut. However, the mechanisms of these processes have only been partially clarified. The aim of our present study is to elucidate the molecular mechanisms of *Plasmodium* sexual reproduction. We first screened a set of genes expressed in gametocytes using microarray data of the rodent malaria parasite *P. berghei*. We then produced a series of knockout (KO) parasites for each gene candidate and found a potential candidate gene, *Pb102*, for the regulation of sexual reproduction. Database search revealed that *Pb102* is a novel gene and its orthologue is exclusively conserved in *Plasmodium*, suggesting an indispensable function in the genus. *Pb102*-KO parasites were able to grow and differentiate into morphologically normal gametocytes. However, no mature ookinete was produced *in vitro* fertilization assay, very likely to be involved in gamete fusion or ookinete maturation. To test sex-specific function of *Pb102*, we performed cross-fertilization assay between *Pb102*-KO and *Nek4*-KO (female sterile) or *CDPK4*-KO (male sterile). *Pb102*-KO gametes were able to fertilize with the male sterile gametes and develop into ookinetes. In contrast, no ookinete was produced in cross-fertilization assay with the female sterile gametes. These findings indicate that *Pb102* has female-specific function related to gamete fusion or ookinete maturation. Since *Pb102* contains several potential functional domains, we are producing *P. berghei* parasites expressing a series of truncated *Pb102*s to clarify the role of these domains. We are also generating a transgenic parasite having a *Pb102* with C-terminal GFP to further elucidate subcellular localization. These results will be presented at the Annual meeting.

### 705

#### A LONGITUDINAL COHORT STUDY OF MALARIA EXPOSURE AND CHANGING SEROSTATUS IN A MALARIA ENDEMIC AREA OF RURAL TANZANIA

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Measurements of antimalarial antibodies are increasingly used as a proxy for transmission intensity. Most serological surveys are based on the use of cross-sectional data that, when age-stratified, approximates historical patterns of transmission within a population. Comparatively few studies leverage longitudinal data to explicitly relate individual infection events with subsequent antibody responses. We examined the incidence of seroconversion and seroreversion events for two *Plasmodium falciparum* asexual stage antigens (MSP-1, AMA-1), using 3 annual measurements of 691 individuals from a cohort in a malaria endemic area of rural east-central Tanzania, in a mixed-effects logistic regression model. We also modeled the respective antibody titer distributions directly using mixed-effects linear regression models. While we found the expected population-level relationship between seroprevalence and infection incidence, on an individual level the relationship between detected infections and the antibody response was complex. Changes in serostatus for each antigen were correlated, but a seropositive status for one does not increase the odds of converting for the other. There was no evidence of an age effect on the odds of a conversion or reversion event. In the titer models, MSP-1 antibody responses were more dynamic in response to the occurrence and resolution of infection events than AMA-1, while the latter was more correlated with consecutive infections. The MSP-1 antibody response to an observed infection seemed to decay faster over time than the corresponding AMA-1 response. While our population-level results concur with previously published seroepidemiological surveys, our individual-level results highlight the more complex relationship between detected infections and antibody dynamics than can be observed using cross-sectional data alone. The longitudinal analysis of serological data may

provide a powerful tool for examining the relationship between infection events and the corresponding immune response, thereby improving our ability to rapidly assess the success or failure of malaria control programs.

## 706

### SEASONALITY AND TRENDS OF MALARIA EPIDEMIC IN UNDER-FIVE-YEAR CHILDREN IN LAKESHORE COMPARED TO HIGHLAND AREAS IN ZOMBA DISTRICT, MALAWI

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Malaria infection was seasonal and varies according to geographic location in Malawi, this may influence the number of reported malaria cases among children in different hospitals and areas. This study assessed the seasonality, trends and risk factors of malaria in under-five-year-children originating from highlands compared to those from lakeshore in Zomba district, Malawi. A cross section study was done and secondary monthly data on outpatient hospital visitation for n=550206 under five children were extracted from Zomba health management information system for the period of 2010 to 2015. Meteorological data on Temperature, Rainfall and Humidity was collected for the same period. Risk factor variables for malaria were drawn from exit interviews. Guardians for eligible under five children from 4 randomly selected health facilities, 2 from highland and lakeshore respectively were interviewed. Grouped data logistic regression test was done to come up with malaria risk factors. STATA 13 was used for data analysis. Outpatient malaria cases accounted for 61.4% prevalence and 88% morbidity for Zomba district. Malaria transmission was observed throughout the year with minimal difference between the seasons in both lakeshore and highlands. Malaria diagnosis in the under-five children was less likely if caregiver had knowledge on anti-malaria prescription [adjusted odds ratio (AOR) =0.71, 95% CI 0.64 to 0.80]; if caregiver owned a bed net AOR=0.33 95% CI 0.15 to 0.72; and if the caregiver attended health talks on malaria prevention AOR= 0.45, 95% CI 0.25 to 0.79. People who did not clear weeds regularly, were more likely to have their children diagnosed with malaria, AOR= 1.78, 95% CI 1.20 to 2.63. Annually, malaria was more common in February, (which is spring) in Zomba district, while lowest infections were in the month of August. Intensifying health education on malaria preventive measures will reduce the malarial burden in Malawi. This study affirms that malaria in Zomba district is perennial and does not follow rainfall seasonality both in highlands and lakeshore areas.

## 707

### ONE HUNDRED YEARS OF MALARIA IN PREGNANCY SURVEYS: A SYSTEMATIC REVIEW OF SURVEYS CONDUCTED BETWEEN 1915 AND 2015

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In 1915, Clark published the first survey of malaria (placental) in Panama and the first articles to report on differences in malaria prevalence by gravidity appeared in the 1950s. To examine changes in epidemiology of malaria during pregnancy, we extracted survey data on malaria infection during pregnancy collected by blood smear published over the last century. We used random effects meta-analysis to examine pooled prevalence (PP) during pregnancy by region, over time, by trimester, and gravidity where data were available. Malaria prevalence during pregnancy varied widely and was lowest in South and Central America (PP 0%, 95% CI 0-1%, N=3 studies, 1973-2013), and highest in Nigeria, the country with the highest number of surveys (PP 42%, 36-49, N=112, 1957-2015). In some locations with more than one survey a decrease in malaria prevalence was seen over time (e.g. Lagos, Nigeria: 73% in 1997 to 6% in 2013; Madang Province, Papua New Guinea: 30% in 1986 to 7% in 2011; Mangochi

district, Malawi: 44% in 1988 to 11% in 2012). The prevalence among pregnant women was 1.76 times higher (95% 1.54-2.01, N=29 studies) than among non-pregnant women, 1.60 times (1.51 -1.70) higher among primigravidae compared to multigravidae (N=146) and 1.35 times (1.26-1.45) higher compared to secundigravidae (N=82). Factors associated with the increased risk among primigravidae using meta-regression included study region (less in Asia and the Pacific), and time period (highest in studies before 1990). The prevalence was similar in the first and second trimester (prevalence ratio 0.98, 0.91-1.06, N=76), and higher in the second compared to third trimester (prevalence ratio 1.16, 1.09-1.23, N=80). Further analyses will be presented at the meeting.

## 708

### PREDICTORS OF DETECTING ANTIMALARIALS DRUGS IN THE BLOOD IN COMMUNITY SURVEYS IN TANZANIA

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Measuring antimalarials in the blood can provide accurate estimate of overall levels of circulating drugs, an important driver of emergence of parasite resistance. The aim of the study was to estimate the prevalence of individuals with antimalarials in their blood and to determine the predictors of detecting antimalarials in the blood. Community-based x-sectional surveys were conducted in 2015 in three regions of Tanzania with different levels of malaria endemicity. Information on demographics, history of fever and drug use were collected as well as dried blood spot samples for further antimalarial analysis by LC-MS/MS. Besides, RDT and treatment availability were investigated through health facility and drug stores surveys. Multilevel mixed effects logistic regression models were used to estimate odds ratios for having lumefantrine (LF), sulfadoxine-pyrimethamine (SP) or any antimalarial in the blood. The survey included 6485 individuals. Prevalence of antimalarials in the blood was 21% (1344/6485). In multivariate analysis, the significant predictors for having any antimalarials in the blood were living in places where a high proportion of drug shops had antimalarials in stock (OR=2.8), followed by living in highly endemic regions (OR=2.9 for Mwanza and 2.0 for Mtwara compared to Mbeya), having had a febrile episode in the previous 2 weeks (OR=2.1), living close to a health facility (p<0.01) and being of young age (p<0.05). The predictors for having LF in the blood were the same plus living in a rural district (OR=5.9) and sleeping under a bednet (OR=1.3). The predictors for having SP in the blood were being pregnant (OR =3.9), being of old age (p<0.001) and having had a febrile episode in the previous two weeks (OR=1.6). These data show that the amount of antimalarial drug pressure is high in the population. The main predictors of antimalarial detection in the blood were expected. Measuring drug concentrations in the blood could provide a new tool to monitor drug use, which should help better targeting specific interventions, and hence decrease drug pressure.

## 709

### EVALUATING THE IMPACT OF THE EXPANSION OF MALARIA CONTROL INTERVENTIONS IN KENYA, 2003-2015

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With more than \$700 million for malaria interventions in the past decade, Kenya has expanded key malaria interventions. Measuring the impact of these investments will help inform the policies, strategies and activities of Kenya's National Malaria Control Program and its partners. We assessed trends in all-cause childhood mortality (ACCM) against trends in coverage of key malaria interventions and trends in other factors likely to affect child survival. The evaluation included data from nationally representative household surveys conducted between 2003 and 2015 to estimate intervention coverage and malaria morbidity and mortality, stratified by malaria epidemiologic zones. Household ownership of at least one insecticide-treated net (ITN) increased from 6% in 2003 to 63% in 2015. In malaria-endemic and highland epidemic-prone areas, where ITN distribution has been concentrated, ownership over this period increased in the coast-endemic (13% to 73%), highland epidemic-prone (8% to 73%), and lake-endemic (12% to 87%) zones. Use of ITNs increased in the general population (21% to 61%), pregnant women (28% to 73%), and children under five (26% to 69%). The proportion of pregnant women receiving at least two doses of sulfadoxine-pyrimethamine for prevention of malaria in the malaria-endemic zones increased from 22% in 2010 to 56% in 2015. During the evaluation period, ACCM declined from 114 to 52 (54%) deaths per 1,000 live births. The decline was greatest among children living in the malaria-endemic zones: 213 to 64 (70%) deaths per 1,000 live births. Coverage and use of several non-malaria health interventions and socioeconomic factors also improved during the evaluation period. Given these patterns of improvement in key malaria interventions and declines in ACCM in the malaria epidemiological zones, malaria control interventions are likely to have contributed substantially to the decline in ACCM in Kenya.

## 710

### M-HEALTH INNOVATIONS < THEIR CHALLENGES TO MANAGE MALARIA SENTINEL SURVEILLANCE NETWORK IN MADAGASCAR

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Madagascar is a predominantly rural country ranked among the lowest income countries. Access to community health care infrastructure is difficult. Since 2007, a Sentinel Surveillance Network (SSN) has been set up using mobile technology to transmit country-level health data (M-Health). The SSN collects data from community- to hospital structure (108 Community Health Workers, 54 primary health centers and 18 hospitals) to identify malaria and other epidemics. Daily, the integrated SSN system is analyzing reported febrile syndromes including malaria, influenza like illness, dengue like syndrome and diarrhea. In 2015, an Android platform was developed by Malagasy engineers. Short Message System-based applications were used to transmit data from sentinel sites to the Institut Pasteur in Madagascar. In-app text, graphs, and maps are weekly updated to provide feedback to the community- and hospital-based sentinel sites. In addition, a malaria early warning system is available through an interactive web-based system. A standardized multi-criteria alert threshold is used to promptly identify abnormal situations using semi-automatic analysis based on specific algorithms. When an abnormal situation is detected, a check is done by the SSN team (mostly by phone). The epidemic alert is promptly communicated from central (Ministry of Public Health) to district level to ensure a timely response. The SSN performance indicator analysis is regularly performed to ensure system improvement.

However, technological challenges are inherent to the system efficiency to maintain data quality: wireless network disturbance, slow internet speed, health care provider turn-over and need to replace materials. All efforts are made to ensure SSN data completeness and timeliness. From 2015 to 2016, 122/128 (95%) of alerts have been managed by the health district teams. This system is efficient and it will be complementary to the routine epidemiological electronic surveillance which is progressively deployed by the Malagasy Ministry of Health. Technical details on the sentinel surveillance information and communication system will be presented.

## 711

### MAPPING THE TRAVEL PATTERNS OF PEOPLE WITH MALARIA IN BANGLADESH

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Elimination of malaria in Bangladesh is crucially dependent on effective surveillance, ongoing efficacy of available treatment and a detailed understanding of disease epidemiology. Being adjacent to the Greater Mekong Subregion, Bangladesh is high on the list of possible countries to which artemisinin resistance may spread next but there is little information on international and local movement of people with malaria. We hypothesize that dissemination of malaria and antimalarial resistance could occur through cross border movement and migration of individuals within Bangladesh between highly populated coastal areas around Chittagong and rural Hill Tracts where 90% of the cases are recorded. 2100 unselected patients with malaria of any species were recruited at 109 study sites in 2015-2016 and interviewed including about their travel patterns in the preceding 2 months. This was compared and combined with surveillance data from National Malaria Control Programme (NMCP) and census data. Blood spots were taken to study malaria parasite DNA. Forested hill tract areas in Bandarban District had the highest numbers of malaria cases. *Plasmodium falciparum*, *vivax* and mixed infection were at 74, 16 and 10% respectively. Median age was 20, with 8% children under 5 and 26% aged 5 to 15 years. Males were overrepresented (67%) compared to the census. Farmers (21%) and students (19%) were the top two occupations recorded, with 66 and 47% reporting travel to the forest respectively. 1811 patients reported having travelled, 17% to another district, 1% to another division and 1% to another country. Males aged 25-49 years accounted for 46% of cases visiting forests but only 14% of the study population. Travel patterns were mapped and analysed to identify population groups with high mobility, find nodes of high traffic and ultimately predict possible sources and routes of spread of malaria for targeting by NMCP. Later analyses will include parasite genetic data. Through working in close partnership with NMCP, this improved understanding of the impact of population movement on the spread of malaria and will directly inform malaria elimination strategy in Bangladesh.

712

### BAKER'S YEAST-BASED INTERFERING RNA LARVICIDES TARGETING *Aedes aegypti*

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Novel strategies are needed to control *Aedes aegypti*, a mosquito vector of infectious diseases including dengue, yellow fever, chikungunya, and Zika. RNA interference (RNAi) allows for silencing of gene expression in the target species, but not of genes in non-target species that lack the interfering RNA target sequence. This specificity allows for development of new biorational larvicides to address the increase of insecticide resistance and rising concern for the negative effects of current chemical larvicides on non-target organisms. Baker's yeast, *Saccharomyces cerevisiae*, was engineered to create several strains of yeast that produce short hairpin RNAs corresponding to larval lethal genes. Short hairpin RNAs were inexpensively propagated through yeast cultivation. Two strains that target nervous system development genes were characterized in detail. Feeding *A. aegypti* larvae with the engineered yeasts resulted in silenced target gene expression, disrupted neural development, and up to 100% larval mortality in laboratory trials. Heat inactivated yeasts that were dried into a granular formulation retained larvicidal activity and attracted gravid females to oviposit in lab assays. Field evaluation of these interfering RNA yeast larvicides will soon commence. The low cost of production, lure-and-kill species-specificity, and ease of use of inactivated granular tablet formulations supports the potential use of yeast-based larvicides in integrated mosquito control programs.

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### FUNCTIONAL DIVERSITY OF *ANOPHELES ALBIMANUS* MICROBIOTA PROVIDES NEW INSIGHTS INTO INSECTICIDE RESISTANCE MECHANISMS

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An understanding of the selective forces leading to evolution of insecticide resistance (IR) is needed to mitigate its threat to malaria vector control. *Anopheles albimanus*, the main coastal malaria vector in Latin America, shows resistance to various insecticides, including organophosphates (OPs). Following evidence of endosymbiont-mediated IR in agricultural pests, and the identification of OP resistance in *An. albimanus*, we hypothesized that bacteria may be contributing to insecticide degradation in resistant mosquitoes. To test this, whole metagenome sequencing was used to characterize the microbiota and its functions in Peruvian *An. albimanus* with differing fenitrothion resistance profiles (resistant, FEN\_Res; susceptible, FEN\_Sus). Results showed higher ( $p < 0.01$ ) proportions of OP-degrading bacteria in FEN\_Res compared to FEN\_Sus, and nine enriched ( $p < 0.05$ ) microbial xenobiotic degradation pathways in FEN\_Res. Predominant bacterial species associated with these pathways in FEN\_Res were; *Klebsiella pneumoniae* (81.2%), *Acinetobacter baumannii* (4.2%), *A. pittii* (1.6%), and *Enterobacter cloacae* (0.7%), all known to degrade OPs. The proportion (%) of bacterial enzyme classes involved in these pathways in FEN\_Res & FEN\_Sus were, hydrolases: 20.1 & 16.3, transferases: 17.8 & 17.9, oxidoreductases: 19.6 & 22.9, lyases: 6.9 & 6.7, isomerases: 0.4 & 0.7, and others: 12.6 & 11.6. At  $p < 0.05$  and a difference in proportion between FEN\_Res & FEN\_Sus (Diff)  $\geq 0.2\%$ , hydrolases, the most enriched enzyme class in FEN\_Res, comprised: carboxymethylenebutenolidase (Diff 1.70%), gluconolactonase (Diff 1.46%), alkaline phosphatase (Diff 0.51%), and acid phosphatase (Diff 0.41%) in comparison to FEN\_Sus. These results show differential microbial functional profiles between FEN\_Res & FEN\_Sus, with significant enrichment of hydrolases - the

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principal class of enzymes involved in biodegradation of OPs - in FEN\_Res. Our findings provide novel insights into IR mechanisms that should be considered in the development of new IR monitoring and management tools.

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### EFFECT OF KNOCKDOWN RESISTANCE ON *PLASMODIUM FALCIPARUM* SPOROZOITE RATES IN MALARIA VECTORS SAMPLED FROM WESTERN KENYA

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Genetic mutations associated with knockdown resistance to pyrethroid insecticides have been well described. In western Kenya, both L1014S and L1014F mutations have been reported. We sought to determine whether these two mutations were correlated with increased sporozoite rates in *Anopheles* vectors of malaria in western Kenya. We sampled wild adult *Anopheles* resting indoors and exposed them to 0.05% deltamethrin. We then determined their species, kdr genotypes and sporozoite rates. Of 4,360 wild *Anopheles* sampled, 32.8% were *An. funestus* and the rest *An. gambiae s.l.* Independent t-test showed that *An. funestus* was more resistant than *An. gambiae s.l.* ( $P < 0.001$ ). *An. funestus* had higher sporozoite rate than *An. gambiae s.l.* ( $P < 0.001$ ). *Anopheles gambiae* mosquitoes that had higher resistance showed higher sporozoite rates than their susceptible counterparts ( $X_2 = 7.14$ ,  $P = 0.01$ ) but did not see the same trend in *An. funestus* ( $X_2 = 0.05$ ,  $P = 0.89$ ). The result also indicated that *An. gambiae s.s.* having L1014S mutation have higher sporozoite rate than those that do not have the mutation ( $X_2 = 5.49$ ,  $P = 0.032$ ). *An. arabiensis* that were phenotypically resistant had more sporozoite rate because they are likely to get more blood meal. The significant association between L1014S and sporozoite rate in *An. gambiae s.s.* which confirmed a previous study suggest the epidemiological importance of that marker; it could contribute to malaria transmission.

715

### THE *ANOPHELES GAMBIAE* 1000 GENOMES PROJECT PHASE 2: INSECTICIDE RESISTANCE, GENE DRIVE AND GENOME VARIATION IN 1,142 MALARIA MOSQUITOES

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The *Anopheles gambiae* 1000 Genomes Project (Ag1000G) is conducting whole-genome sequencing of wild-caught malaria vectors from populations across Africa with three core objectives: discover genetic variation, describe population structure/history, and to connect these with malaria epidemiology and vector control. Here we describe results from phase 2 of the Ag1000G project, which includes data on nucleotide variation from the whole genome sequencing of 1,142 individual mosquitoes collected from 13 African countries. The phase 2 cohort includes several new mainland populations representing the incipient species *An. gambiae* and *An. coluzzii* and introduces two new island populations, providing a rich resource for investigating speciation processes, patterns of gene flow, and genome regions under strong selective pressures. Within the cohort, we have discovered over 57 million SNPs, corresponding to a variant allele at every 1.9 accessible bases of the genome on average. We will present the results from analyses of genetic diversity, population structure and demographic history and also show the latest results from haplotype-based analyses of recent selection at genes

involved in insecticide resistance, including new information on the spread of insecticide resistance between different regions and new candidate resistance *loci*. We will discuss the applications of our data to the design of new vector control tools using genetic modification. Advances in CRISPR/Cas9 genome editing may allow efficient gene drive to suppress or replace populations, reducing malaria transmission, however, high genetic diversity could lead to rapid gene drive resistance. Of 12,502 genes in the *An. gambiae* genome, we found 4,303 genes that could be targeted for CRISPR/Cas9 gene drive after excluding target sites with natural variation, and 571 genes that contained multiple conserved targets, including 11 putative sterility genes that could be targeted for population suppression. The Ag1000G data resource has been publicly released and provides unique opportunities for the malaria research community to develop new approaches to vector control.

## 716

### SCIENCE AND NATURE: SUSCEPTIBILITY OF WILD CAUGHT ADULT ANOPHELES GAMBIAE S.S. TO INSECTICIDES MAY NOT DECREASE WITH AGE

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Insecticide resistance increases adult vector longevity and may increase intensity of malaria transmission as a larger proportion of individuals survive the extrinsic incubation period. Previous studies have shown that mosquitoes become more susceptible to insecticide with age, but this has not been studied in wild caught adults harbouring target site resistance. This study investigated the relationship between age and insecticide resistance in wild *Anopheles gambiae s.l.* populations from The Gambia where until 2014, DDT was used for Indoor Residual Spraying (IRS). Female adult mosquitoes were collected using Human Landing Catches; tested for DDT and bendiocarb susceptibility using WHO tube tests and then parity status, as a proxy of physiological age, determined. An alternative age related outcome, infection with *Plasmodium falciparum*, together with known resistance-associated molecular markers were also assessed. A total of 251 (167 exposed to DDT; 84 to bendiocarb) were successfully identified to species (*Anopheles gambiae s.s.* -69.3%; *An. coluzzii*-7.6%; *An. arabiensis*-23.1%). In contrast to earlier lab studies, there was not a significant increase in DDT susceptibility between nulliparous and parous mosquitoes  $\chi^2_{df=1} = 0$ ,  $p = 1$ . The proportion of mosquitoes carrying the *Vgsc*-1014F allele was higher in parous *An. gambiae s.s.* than in nulliparous ( $\chi^2_{df=1} = 4.1$ ,  $p = 0.04$ ). Irrespective of parity status, target site mutations conferred a strong survival advantage to DDT, from *Vgsc*-1014F (OR 18, 95% CI 2-557,  $p < 0.01$ ), and to bendiocarb, from *Ace-1*-119S (OR 41, 95% CI 4-1394,  $p < 0.01$ ). In conclusion, the *kdr Vgsc*-1014F mutation was associated with DDT resistance in mixed age populations of *An. gambiae s.s.* The significantly higher frequency of *Vgsc*-1014F in parous mosquitoes explains the lack of association between insecticide susceptibility and physiological age. This observation suggests that resistance mechanisms may partially counteract the senescence phenomenon commonly observed in laboratory reared mosquitoes which normally become more susceptible to insecticides with age.

## 717

### THE EVOLUTION OF METABOLIC INSECTICIDE RESISTANCE IN AFRICAN MALARIA VECTORS VIA COPY NUMBER VARIATION

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Metabolic resistance via increased expression of detoxification genes underlies much of the insecticide resistance observed in mosquito populations, yet causal genetic variants remain poorly characterised, meaning that logistically-challenging measurements of expression are needed to monitor changes. One way in which changes in gene expression levels can be achieved is via copy number variation (CNV), with recent gene duplications of particular relevance to insecticide resistance. Using 1,142 genomes of *Anopheles gambiae s.s.* and *An. coluzzi* from 16 populations spanning continental Africa, sequenced in phases 1 and 2 of the *Anopheles gambiae* 1000 genomes (*Ag1000g*) project, we have identified CNVs across the genome and characterised duplications in three metabolic genes or gene clusters with known associations to insecticide resistance: *GSTE1-8*, *CYP9K1* and *CYP6P1-5*. All three regions showed multiple independent duplications of varying size, distribution and frequency. Most duplications were rare, being found in only a handful of individuals, and no duplications were detected that spanned all populations. No duplication was fixed in any population, but some were detected at local frequencies up to 50%. Interestingly, all ten of the duplications identified in the *GSTE* cluster encompassed *GSTE2*, a gene with known links to insecticide resistance. Our results point to a varied mosaic of duplications in key detoxification genes across Africa, and identify candidate duplications that have reached high local frequencies. Reads spanning the duplication breakpoints revealed the start and end points for each duplication, which were used to design duplication-diagnostic PCR assays to test the association of individual duplications with insecticide resistance in phenotyped mosquito collections.

## 718

### OXIDATIVE DEFENSE CAPACITY IS CRITICAL FOR FECUNDITY AND XENOBIOTIC METABOLISM IN ANOPHELES GAMBIAE

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Blood digestion in hematophagous insects results in elevated oxidative stress. Capacity for defense against oxidative stress is limited, and if unabated can lead to oxidation of cellular material resulting in a loss of function. Reducing power is often found in the form of NADPH which can be used to regenerate antioxidant molecules. We examined the dynamics of oxidative stress via induction by paraquat (PQ) and the inhibition of the NADPH regeneration by 6-aminonicotinamide (6AN). Important vector associated traits such as fecundity, longevity, and insecticide susceptibility were measured. Both PQ and 6AN feeding increased oxidative stress as measured by the ratio of reduced form vs. oxidized form of glutathione (GSH:GSSG ratio). Both pro-oxidants lowered fecundity. The basal level of reducing power varies in individuals, as measured by GSH:GSSG ratio. Low basal reducing capacity was positively correlated with low fecundity. Co-feeding with the antioxidant, lycopene, attenuated adverse effects on fecundity implying that oxidative stress was the cause of this phenotype. Low dosage (0.5 mM) PQ decreased longevity and pre-feeding with 6AN increased insecticide susceptibility. Both PQ and 6AN resulted in higher expression of gene *nrf2* (mediating oxidative response signaling) and repressed vitellogenin (a fecundity marker). These results indicate that redox homeostasis is critical for fecundity and xenobiotic detoxification. This knowledge can be used to design novel and effective vector control strategies which influence insecticide sensitivity, infection susceptibility, fecundity, and longevity.

## 719

### SAFETY/IMMUNOGENICITY OF A SINGLE INTRAMUSCULAR DOSE OF THE INVESTIGATIONAL RECOMBINANT CHIMPANZEE ADENOVIRUS TYPE 3-VECTORED EBOLA ZAIRE VACCINE (CHAD3-EBO-Z) IN CHILDREN IN AFRICA: A PHASE 2, RANDOMIZED, CONTROLLED STUDY

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The West-African Zaire Ebola virus (EBOV) outbreak in 2014 accelerated vaccine development. This study assessed safety/immunogenicity of ChAd3-EBO-Z (GSK) in children. In this phase 2, multi-country study (NCT02548078) in Africa, 600 children (age strata: 13-17 years [Y], 6-12Y, 1-5Y) were 1:1 randomized to receive ChAd3-EBO-Z (1x10<sup>11</sup> particle units; EBO-Z group) or meningococcal conjugate vaccine (MenACWY-TT, GSK; Control group) at day 0 (D0), with crossover at month 6. 13-17Y were vaccinated first, younger strata followed after independent D0-6 safety data review for ≥50 older children. Solicited/unsolicited adverse events (AEs), serious AEs (SAEs), hematologic/biochemical outcomes (primary objectives) and immune responses were assessed. Results up to D30 are shown. The most frequent solicited AEs (D0-6) were injection site pain (13-17Y: 31% [EBO-Z, N=100] and 14% [Control, N=100]; 6-12Y: 41% [EBO-Z, N=99] and 24% [Control, N=101]; 1-5Y: 55% [EBO-Z, N=101] and 23% [Control, N=99]), headache (13-17Y: 36% and 11%; 6-12Y: 26% and 6%, 1-5Y: not collected per protocol) and fever (13-17Y: 20% and 4%; 6-12Y: 25% and 1%; 1-5Y: 50% and 23%); 8 children had severe solicited AE (all in EBO-Z groups). Unsolicited AEs (D0-29) were reported for 41/300 (14%) EBO-Z vaccinees and 24/300 (8%) controls; most were mild, none severe. SAEs were reported for a 4-year-old (EBO-Z, malaria) and 14-year-old (Control, hepatitis B and D); none were vaccine-related. No clinical impact on platelet function was observed. In the per-protocol cohort for immunogenicity, 17% of all children were seropositive for EBOV at D0. At D30, all EBO-Z vaccinees (except 2 of 1-5Y) were seropositive for anti-EBOV glycoprotein (GP); geometric mean concentrations of anti-GP EBOV antibodies increased to 1575 (95% CI: 1346-1843) in 13-17Y (N=98), 1379 (1165-1632) in 6-12Y (N=99) and 2372 (1915-2939) in 1-5Y (N=97). Polyfunctional ChAd3-EBOV-specific CD4+/CD8+ T-cell responses were observed at D30 in a subcohort of EBO-Z vaccinees of the 6-12Y and 1-5Y strata (13-17Y: data not available). ChAd3-EBO-Z was well tolerated and immunogenic 30 days after a single dose in African children.

## 720

### SAFETY AND IMMUNOGENICITY OF MONOVALENT AD26.ZEBOV AND MULTIVALENT MVA-BN-FILO HETEROLOGOUS PRIME-BOOST VACCINE REGIMENS AGAINST EBOLA IN AFRICAN HEALTHY ADULT VOLUNTEERS

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Ebola virus disease outbreaks with high fatality rates have been reported since the 1970s; by far the most deadly outbreak occurred in 2013–16 in West Africa. Safe and effective Ebola vaccines and prevention measures are needed to help manage and prevent future outbreaks. The safety and immunogenicity of various sequences and schedules of the Ad26.ZEBOV/

MVA-BN-Filo regimen (28-day and 56-day prime-boost interval) were assessed in two separate Phase 1 clinical studies in Africa (EBL1003: Kenya; EBL1004: Uganda & Tanzania). 144 participants (EBL1003: n=72; EBL1004: n=72) were randomised 5:1 (active:placebo). Adverse events (AEs) and serious adverse events (SAEs) were recorded until 21 days post-boost and 1 year post-prime, respectively. Humoral and cellular immune responses were evaluated over one year following vaccination. Post-prime and post-boost rates of solicited local AEs (pooled data) (Ad26: 66%; MVA: 77%) and systemic AEs (Ad26: 75%; MVA: 73%) were similar in both studies; most were mild-to-moderate and short-lived, with injection site pain and fatigue as most frequent local and systemic AEs. At 21 days post-boost, almost all Ad26/MVA recipients from both studies displayed robust binding and neutralizing antibody responses (100% and ≥93%, respectively), which were mostly sustained to Day 360 (EBL1003: 97% and 60% of recipients; EBL1004: 100% and 63%). Cellular responses to Ad26/MVA varied widely across individuals in both studies. In EBL1003, responses were observed 21 days post-boost for CD4+, CD8+ (ICS), and IFN-γ (ELISpot) in 60%, 27% and 62% of recipients, respectively. In EBL1004, responses were observed post-boost in 37%, 54% and 47% of recipients. Responses were sustained to Day 360 in 17%, 17% and 27% of recipients in EBL1003 and 11%, 31% and 24% in EBL1004. Consistent with previously reported UK data, Ad26.ZEBOV/MVA-BN-Filo vaccine regimens were well tolerated and immunogenic among healthy African adults, with antibody responses elicited early on and persisting to Day 360. The regimen may therefore be well-suited for vaccination strategies to counter future Ebola outbreaks and is currently under Phase 2/3 evaluation.

## 721

### SEROLOGIC PROFILING OF THE HUMORAL IMMUNE RESPONSE TO EBOLA VIRUS MINIMALLY OR ASYMPTOMATICALLY INFECTED SUBJECTS

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Evaluation of antibody profiles can provide exposure history to human pathogens. Analyses of humoral responses against different infectious agents are critical for infectious disease diagnostics, understanding pathogenic mechanisms, and the development and monitoring of vaccines and therapeutics. Profiling antibody response in minimally or asymptomatic Ebola virus (EBOV) infection is limited. To better understand optimal antibodies and their targets, which might be important for protection from Ebola virus infection, we characterized the serologic profile of antibodies against multiple EBOV viral proteins in health care workers in the Tshuapa district, the site of the 2014 Ebola outbreak in the Democratic Republic of Congo (DRC). Serum samples from consenting health care workers (HCW) in Boende, DRC were collected one year post outbreak in November 2014. Samples were screened for the presence of EBOV antibodies. An enzyme-linked immunosorbent assay specific for viral proteins NP, GP1–649; VP40 was measured using a fluorescent immunoprecipitation assay and plaque reduction neutralization test (PRNT) was realized. In total, 616 HCW were enrolled. The largest proportion of HCW were seropositive for NP (31, 5%), followed by GP1–649 (27.2%), then VP40 (9%). Comparison of positive immunoreactivity between the viral proteins NP, GP, VP40 and PRNT demonstrated that 9 (1.4%) individual's immuno reactive to both GP and NP were more likely to be positive for PRNT. These findings suggest that different serological methods are needed

to generate complete antibody profiles for EBOV. Developing a better understanding of pathogenic mechanism utilizing multiple assays may aid in the development of vaccines and therapeutics.

## 722

### PREVALENCE AND PREDICTORS OF ROTAVIRUS SHEDDING AMONG A COHORT OF POST-VACCINATED INFANTS IN EL ALTO, BOLIVIA 2013 - 2015

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Historically, rotavirus (RV) associated mortality rates from diarrheal illness disproportionately affect children in low-income countries. Despite the global introduction of oral RV vaccines, reduced efficacy in low-income countries that are the most vulnerable to this disease has been observed. Further understanding barriers to vaccine performance in low-income settings is needed. We characterized the prevalence of RV shedding post-vaccination and identified factors associated with reduced viral shedding. During 2013 to 2015, 355 infants received both doses of the Rotarix® vaccine and were followed for 6 (n=325) and 18 (n=169) months. Of these, a subset (n=158) had stool shedding samples 5 or 7 days post-vaccination that were assayed by a real-time PCR that targets the NSP3 region (Ct ≤ 36 was considered positive). The following data was collected during the study: infant RV-specific IgA (2, 6 months), nutritional markers and anthropometrics (2 months) and demographics; maternal IgG (1 month). Infants with missing seroconversion or inconclusive shedding data (n=4) were excluded from analysis. Of the 154 infants, 38% were positive for RV viral shedding and 4% were positive by EIA (one discordant positive). RV shedding status was not significantly associated with seroconversion: (seroconverters [40% shedding] vs. non-seroconverters [36% shedding]), OR: 1.34, 95% CI: 0.68, 2.70 p = 0.20). RV shedding status was not significantly associated with age at vaccination, vaccine seasonality, preterm status, stunting, wasting, vitamin A or zinc deficiency. RV shedding status was significantly associated with maternal baseline IgG (OR: 0.29, 95% CI: 0.13, 0.62 p = 0.001). High maternal IgG titers were associated with the absence of viral shedding suggesting maternal IgG may have an inhibitory effect on RV vaccine uptake. Our study highlights the importance of utilizing a more sensitive method such as real-time PCR rather than EIA for detecting viral shedding. The low prevalence of RV shedding and the association with high levels of maternal IgG warrant further exploration as causes of vaccine underperformance.

## 723

### LEWIS ANTIGEN AND SECRETOR STATUS MEDIATE SUSCEPTIBILITY TO P-GENOTYPE SPECIFIC ROTAVIRUS INFECTIONS BUT DO NOT AFFECT ROTAVIRUS VACCINE PERFORMANCE AMONG INFANTS IN BANGLADESH

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Rotavirus (RV) is the leading cause of diarrhea among children worldwide. Limited data suggest Lewis (Le) histoblood group antigen or secretor (Se) negative children may be resistant to genotype P[8] RV infection. Both globally-licensed RV vaccines contain P[8] RV, potentially rendering

Le- or Se- infants resistant to vaccination and thus unprotected against heterotypic RV strains. To investigate this, we performed a sub-study within PROVIDE, a randomized, controlled trial of Rotarix vaccine (RV1, GlaxoSmithKline) performed in urban Dhaka, Bangladesh. Infants were enrolled, randomized 1:1 to receive RV1 or no RV1 at 10 and 17 weeks, and underwent active diarrheal surveillance until 1 year of life. Saliva was collected for Le and Se typing by dot-blot assay and ELISA, respectively. RV diarrhea (RVD) was confirmed by stool ELISA and the P-type of each infecting strain was determined by sequencing. Among 562 eligible infants, 82 (14.6%) were Le- and 180 (32.2%) were Se-; 167 (29.7%) had ≥1 episode of RVD, of whom 21 (12.6%) were Le- and 37 (22.2%) were Se-. Among infected infants, Le- infants were less likely than Le+ infants to be infected with P[8] RV (OR 0.095, 95%CI 0.030-0.30) but more likely to be infected with P[6] RV (OR 306, 95%CI 52-1797). However, the overall risk of any RVD did not differ between the groups and Le status did not impact vaccine effect. In contrast, Se- infants were significantly protected from RVD compared to Se+ infants (OR 0.51, 95%CI 0.33-0.77), chiefly due to a protective effect of Se- status compared to Se+ status among unvaccinated infants (N=274, OR 0.355, 95% CI 0.20-0.64) that was not seen in vaccinated infants. Of note, Se- infants were not resistant to P[8] RV infection, with 28 (75.7%) infections in Se- infants due to P[8] RV. This finding suggests susceptibility to RV1 among Se- infants in this cohort. Surprisingly, Se- infants were completely protected against P[4] RV, with all 40 P[4] infections found only in Se+ infants (P<0.0001). In this Bangladeshi cohort, Le and Se status had distinct effects on susceptibility to P-genotype specific RV infections, but did not directly impact vaccine performance.

## 724

### IMPACT OF ROTAVIRUS VACCINE PERFORMANCE ON INFANT UNDERNUTRITION IN EL ALTO, BOLIVIA

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Rotavirus (RV) vaccination has the potential to reduce the burden of undernutrition - stunting and micronutrient deficiencies - caused by RV diarrhea, but in developing countries, oral RV vaccines have lower efficacy. This study sought to determine if impaired RV-specific immunity after vaccination is associated with stunting and micronutrient deficiency in Bolivian infants. In a prospective cohort study of 166 mother-infant pairs from El Alto, Bolivia, infants were followed from birth to 12-18 months of age. Data collected at each visit (2, 6, and 12-18 months) included: sociodemographics, self-reported morbidities (e.g. diarrheal episodes), anthropometry, micronutrients (hemoglobin, iron, vitamin A, zinc), and RV-specific IgA titers. RV-specific seroconversion was defined as a four-fold increase in RV-specific IgA titer between pre-immunization sera and 2 months post-second vaccine dose, stunting as per WHO Child Growth Standards, anemia as hemoglobin <13.7 g/dL (corrected for altitude), vitamin A deficiency (VAD) as retinol binding protein < 0.7 µmol/L, iron deficiency (ID) as ferritin < 12 µg/L, and zinc deficiency as < 65 or < 57 µg/dL, depending on sampling time. Logistic regression models, adjusted for sex, infant age, and inflammation status at the 12-18 month visit, assessed the impact of RV-specific seroconversion on infant nutrition. Only 63% of the 166 infants seroconverted, compared to high-income countries (96%). At 12-18 months, 20% of the infants were stunted, 82% were anemic, 66% were iron deficient, 15% had VAD, 57% were zinc deficient, and 19% of mothers reported infant diarrhea in the past 2 weeks. RV-specific seroconversion was not significantly associated with 12-18 month stunting, anemia, VAD, or self-reported diarrhea, but was significantly associated with zinc deficiency (OR: 2.4, 95% CI: 1.2, 4.7 p =

0.02). Among infants with RV-specific seroconversion, there was a trend towards protection from ID at 12-18 months (OR: 0.5, 95% CI: 0.23, 1.0  $p = 0.06$ ). In conclusion, these data suggest that RV-vaccine performance has a selective impact on certain infant nutritional markers.

## 725

### TRENDS IN CIRCULATING ROTAVIRUS STRAINS IN INDIA FROM 2012-2016: A MULTI-CENTER SURVEILLANCE DATA AMONG UNDER FIVE CHILDREN

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Rotavirus is a leading cause of under-five diarrhea globally and accounts for about 39% of hospitalizations due to diarrhea in children <5 years of age in India. We conducted a multicenter hospital-based surveillance in seven sites, viz. Vellore and Trichy (Tamil Nadu), Kolenchery (Kerala), Tirupati (Andhra Pradesh), Hyderabad (Telangana), Ludhiana (Punjab), and New Delhi from July 2012 to June 2016 to generate information on rotavirus disease among Indian children, with the use of standardized protocols for enrollment and evaluation. All children aged less than 60 months who presented to a study hospital with acute gastroenteritis and required hospitalization with rehydration for at least 6 hours were enrolled in the study after obtaining informed consent from parent/guardian. A single stool specimen was collected from each child enrolled in the study along with clinical information. All samples were screened for by enzyme immunoassay (EIA) at a central laboratory. For the EIA positive samples, genotyping was performed for VP7 and VP4 by reverse-transcription polymerase chain reaction (RT-PCR). During the 4 years of surveillance, a total of 6935 eligible children were enrolled with diarrhea. Rotavirus was detected in 34.9% samples (2291 children). A total of 2207 rotavirus strains were genotyped. The most common types were G1P[8] (in 50.3% of infections), followed by G2P[4] (in 9.6%), G9P[4] (in 8.4%), and G12P[6] (in 5.3%). Mixed infections were approximately 8%. The G and P types could not be identified in 1.8% of infections. The G1P[8] strain increased from 37.6% in 2012 to 69.7% in 2015, and decreased to 24.3% by 2016. G9P[4] strains increased in prevalence from 6.4% in 2012 to 21.5% in 2016. Emergence of G3P[8] was noticed in 2015 and by 2016, the prevalence was 12%. There is a significant burden of rotavirus diarrhea among under five Indian children with a predominance of G1P[8] and G2P[4] strains, but circulation of strains varies in time and space.

## 726

### INTEGRATING A GEOGRAPHIC INFORMATION SYSTEM TO EXPLORE THE EFFECT OF WATER, SANITATION, AND HYGIENE ON TRACHOMA AT AGGREGATE SPATIAL SCALES

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Trachoma is an infectious disease responsible for a large proportion of the global burden of preventable blindness. The WHO endorsed SAFE strategy (Surgery, Antibiotics, Facial cleanliness, and Environmental improvement) has been implemented at scale throughout Amhara National Regional State of Ethiopia since 2007. The effect of F&E on the prevalence of trachoma tends to be contextually dependent, exhibiting variability in prior studies. The aim of this research was to elucidate the relationships between water, sanitation, and hygiene (WASH) indicators and the spatial distribution of trachoma throughout Amhara. Through multi-stage cluster random impact surveys conducted in all districts from 2011 to 2016. Trachoma clinical outcomes were collected using the WHO simplified

grading scale, and WASH indicators were collected through household and individual level assessments. In the analysis, spatial methods corrected biases that arise from geographic relationships. Disease hotspots, defined as evaluation units with high trachomatous inflammation-follicular among children aged 1 to 9 (TF) in relation to a neighborhood, were identified using TF prevalence estimates and the Getis-Ord  $G_i^*(d)$  statistic. Socio-demographic, community, and environmental factors thought to promote the clustering of the disease were modeled with logistic regression. The district-level TF prevalence in Amhara ranged from 1.2% to 73.9%. Twelve districts and 325 villages were identified as statistically significant hotspots with 90% confidence. Percentage of children with clean face and household access to a water source in less than 30 minutes were significantly associated with a reduced odds of residence in a hotspot. The aforementioned variables as well as household access to a latrine were significant predictors in a spatial lag model of the prevalence of TF. This study demonstrated that water and hygiene are important factors in the clustering of trachoma within a hyperendemic area. Intensified promotion of structural and behavioral interventions to increase WASH coverage may be necessary to eliminate trachoma as a public health problem in Amhara.

## 727

### TRACHOMA PREVALENCE AFTER THREE ROUNDS OF MASS DRUG ADMINISTRATION IN KANKAN, MANDIANA AND SIGUIRI, THREE HEALTH DISTRICTS IN GUINEA

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Kankan region in Guinea is highly endemic with trachoma and mass drug administration (MDA) with azithromycin and tetracycline in the region started in 2014 targeting all the at-risk population, with financial support from the United States Agency for International Development (USAID)'s ENVISION Project. In 2016, Kankan, Mandiana and Siguiiri districts completed three annual rounds of MDA and three annual surgery campaigns for trachomatous trichiasis (TT) were also conducted in Kanakna and Siguiiri districts. An impact survey was conducted six months after the last MDA from December 2016 to January 2017. A cross-sectional survey using a cluster sampling method of 20 villages and 30 households randomly selected in each of the three districts were conducted to assess the prevalence of Trachomatous Inflammation - Follicular (TF) in children aged 1-9 years old and the prevalence of TT in adults aged 15 years and above. TF and TT were diagnosed by clinical examination using the World Health Organization (WHO) simplified grading system. Results of the impact survey were then compared to the baseline prevalence. A total of 5,790 children, and 4,624 adults from 1,877 households were screened for clinical signs of TF and TT respectively. Results showed a significant reduction from the baseline in TF prevalence: from 25.1% to 2.3% in Kankan, from 15.0% to 1.4% in Mandiana and from 16.8% to 1.2% in Siguiiri. Reductions were also found in TT prevalence in Kankan (1.5% to 0.6%) and in Siguiiri (5.1% to 1.0%) after three annual TT surgery campaigns, while TT prevalence increased in Mandiana from 0.6% to 0.9% without a TT surgery campaign, however this increase was not statistically significant. Three rounds of annual trachoma MDA with sufficient program coverage have significantly reduced TF prevalence in the three districts to below the threshold of 5%, reaching the criteria to stop MDA in these districts. The persistently elevated TT prevalence highlights the need of systematic TT surgical intervention in Guinea.

## LONGITUDINAL TRENDS IN TRACHOMA OVER EIGHT YEARS IN A HYPERENDEMIC SETTING UNDER THE SAFE STRATEGY: RESULTS FROM SERIAL IMPACT SURVEYS IN WEST GOJJAM ZONE, ETHIOPIA

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As part of the WHO recommended SAFE (Surgery, Antibiotics, Facial cleanliness, and Environmental improvement) strategy to control trachoma, annual community-wide mass drug administration (MDA) with antibiotics is warranted until district level prevalence of trachomatous inflammation-follicular (TF) among children ages 1-9 years falls below the elimination threshold of 5%. Impact surveys are conducted after 1-7 rounds of MDA depending on baseline prevalence, and are used to monitor progress towards the elimination threshold. West Gojjam zone in Amhara region, Ethiopia, is made up of 16 administrative districts with a population of about 2.7 million people, and had a baseline zonal level TF prevalence of 33.1% in 2006. Multi-stage cluster surveys were conducted in all districts throughout the zone first in 2012/2013 after 5 rounds of MDA and again in 2015/2016 after an additional 3 to 5 rounds to monitor the impact of the SAFE strategy. The 2012/2013 impact surveys (n=17,124) demonstrated a TF prevalence of 18.1% (95% confidence interval (CI): 15.8-20.7%), a 45.3% reduction from baseline. The prevalence of trachomatous inflammation-intense (TI) was 3.5% (95%CI:2.8-4.2%) and the prevalence of chlamydial infection in children ages 1-5 years was 2.5% (95%CI:0.0-5.0%) suggesting ongoing transmission. One district in this zone achieved the elimination threshold. Based on the first impact survey results, 15/16 districts in West Gojjam received 3 to 5 more rounds of MDA. Second impact surveys (n=20,134) in 2015/2016 demonstrated a TF prevalence of 13.9% (95%CI: 11.7-16.5%), and an additional district had reached the elimination threshold. The prevalence of TI and chlamydial infection, although lower than in the earlier impact survey, were still present in the zone. Data from 3 population-based surveys across 8 years in West Gojjam zone demonstrated that under the SAFE strategy, TF decreased by 58%, and that 2 districts no longer required MDA. Despite these successes, TF remained above 10% in 14 districts, requiring 3 to 5 additional rounds of MDA. Trachoma hyperendemic areas may require 10 or more rounds of MDA under current treatment guidelines.

## INSIGHTS AND COMPLEXITIES MODELLING SEROLOGICAL DATA FOR TRACHOMA SURVEILLANCE

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Antibodies to the antigens PGP3 and CT694 are reported to have high sensitivity and specificity to detect infection with *Chlamydia trachomatis* (Ct). The seropositivity rates for these responses amongst children increase with age in trachoma-endemic communities, suggesting that age seroprevalence may be an indicator of cumulative exposure to ocular *C. trachomatis* infection within a population. Ct-specific antibody seroprevalence is therefore being investigated for a role in post-mass-drug administration surveillance in trachoma elimination programmes. Before suitable thresholds can be established, the antibody half-life and/or sero-reversion rate (SRR) needs to be estimated in order to understand how long individuals in the population remain sero-positive following elimination of trachoma. Here we analyse data from 5,368 participants aged between 1 and 90 years, collected from 6 different trachoma endemic countries: Nepal, The Gambia, Tanzania, Fiji, Solomon Islands and Kiribati. We use sero-catalytic and antibody acquisition models to investigate age-dependent sero-prevalence and to understand patterns

of transmission within each country. We highlight the challenge of cross-reactivity with urogenital chlamydia infection in different populations, and demonstrate how this can be accounted for to quantify the sero-conversion rate (SCR) for trachoma. We find a significant correlation—follicular (TF) within the population. Using data from regions for which 2 cross-sections are available, we estimate the median population-level half-life for sero-reversion to be 22 years (95% CrI: 17-30 years). Serology can be informative for assessing current and historical trachoma transmission intensity, but accurate understanding of the SRR is needed if estimates of the SCR are to be informative. Furthermore, work is needed to better understand the contribution of urogenital infection to age-specific sero-prevalence.

## TRACHOMATOUS TRICHIASIS SCREENING AND ACTIVE CASE FINDING, AN OPPORTUNITY FOR EYE HEALTH PROGRAMS: CASE STUDY OF THE MMDP PROJECT IN BURKINA FASO

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Trachoma is an ocular bacterial infection whose complications can lead to blindness. Trachoma elimination through the SAFE strategy is recommended to interrupt the transmission of infection. Burkina Faso is a trachoma-endemic country that recently, employed a fixed strategy for Trachomatous Trichiasis (TT) case finding. However, with new resources provided through the USAID-funded MMDP project, we have been able to evaluate another strategy: door-to-door screening. The goal of this research was to examine whether this new strategy improved case identification and surgery uptake and to characterize the prevalence of TT and other ocular pathologies in the region. We conducted two ten-day TT screening and surgery campaigns in two health districts (DS) in the Center North Region in June and October 2016. During the campaigns, we screened 18,902 persons and identified 439 TT cases. Of the persons examined, 4,408 (23.3%) had an ocular pathology. Among the diagnosed eye conditions, TT accounted for 10.1% of the cases (439 cases); pterygium for 28.6% (1259 cases); cataract for 15.18% (669 cases), corneal affections 10.3% (455 cases). To date, 295 of the 439 TT cases were operated, 14 were provided with epilation counseling and 8 were referred for surgery due to lower lid trichiasis. The door-to-door screening process resulted in screening of more people and finding more cases than the fixed strategy, in which 1,751 people were screened and 68 TT cases identified. Through the TT case-finding campaigns we are creating a record of the conditions found and contact information for the affected patients, which might contribute to increasing the likelihood that these patients will receive care for their non-TT ocular problems. Moving forward, we need to share the records with partners to ensure effective collaborations between eye health programs and eye care facilities in order to improve the eye health of patients. This project provides important opportunities to piggyback with other eye health programs to provide much needed services for patients being screened, while also reaching the country's targets for eliminating blinding trachoma.

## 731

### INFLUENCE OF INDIVIDUAL AND ENVIRONMENTAL FACTORS ON THE PREVALENCE OF TRACHOMA IN THE HEALTH DISTRICT OF MOKOLO, CAMEROON AFTER THREE YEARS OF MASS TREATMENT WITH ZITHROMAX AND TETRACYCLINE

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It is commonly believed that personal hygiene and the state of cleanliness of the environment are significant factors in the spread of trachoma. The strategy for control is through SAFE (Surgery, Antibiotic treatment, Facial cleanliness and Environmental improvement). The health district of Mokolo in the Far-North Region of Cameroon, recognized as endemic with trachoma in 2010, benefited from the azithromycin and tetracycline mass distribution during three consecutive years, supported by HKI with funding from USAID's ENVISION Project, managed by RTI International. Rarely actions in F and E components were registered. We aimed at evaluating the influence of facial cleanliness and the environmental factors on the residual infection of trachoma after the treatment. We carried out a descriptive cross-sectional study based on a stratified random sampling in Mokolo in 2015. Selected were 20 communities representing clusters; with 25 households in each. After an interview with the households and ophthalmic assessments by trained trachoma graders, data were collected using the numerical tablets transferred to a central base, before being analyzed using Software SPSS. Among the 827 children aged 1 to 9 years examined, the prevalence of active trachoma (TF&TI) was 1.7% in 2015 against 18.1% in 2010. The proportion of children having dirty faces was 14.85%. A strong association was found between the facial uncleanness and active trachoma infections (OR = 14.79;  $p < 0.01$ ). Out of 91.6% of houses with cattle, 94.7% cohabited with the animals inside the homes. However 60.8% of the households were located within less than 30 min from a source of water. Despite drastic reduction of the disease prevalence to the threshold of the stopping MDA, the individual and environmental factors remain a strong influence. This could compromise the sustainable elimination of trachoma in this health district. More efforts on the F and E components of the SAFE strategy is needed.

## 732

### THE TRACHOMA END-GAME IN VIETNAM: SURVEYING TRACHOMA HOTSPOTS IN HÀ GIANG PROVINCE

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The Vietnam National Institute of Ophthalmology (VNIO) was established over 60 years ago as the National Trachoma Institute. Following launch of the World Health Organization (WHO) Alliance for Global Elimination of Trachoma by 2020 in 1996, the government of Vietnam and partners implemented the SAFE strategy. In 2000-2001, 67 district-based surveys

were undertaken in 27 trachoma-suspect provinces to identify need for SAFE. Impact surveys were done at four time periods in: 2003, 2004, 2005, and 2009-2011. Surveillance surveys done in 2014 revealed the prevalence of trachomatous inflammation—follicular (TF) in children aged 1-9 years was <5% in all 15 evaluation units. However, in one village in Yen Minh district of Hà Giang, the proportion of children with TF was 10.9%. In January 2015, VNIO investigated if trachoma was confined to that village or if it was more widespread by examining 388 children in the village with the highest proportion of children with TF and six surrounding villages. The proportion of children with TF was 8% and they were all offered antibiotic treatment. A follow-up investigation, in August 2015, examined children in the same seven villages, revealing the proportion of children with TF had doubled to 16%. In May 2016, a Trachoma Master Grader from the WHO investigated and confirmed the findings, and recommended population-based prevalence surveys in the entire Hà Giang province. Surveys are underway, with results expected in June 2017. The objectives of the surveys are to: 1) estimate the prevalence of TF among 1-9-year-olds; and 2) estimate the prevalence of trachomatous trichiasis (TT) among people aged ≥15 years. The surveys will be carried out across all 10 districts of Hà Giang combined into five evaluation units, powered to estimate prevalence of TF in 1-9-year-olds of 10% with an absolute precision of 3%, 95% confidence level, design effect of 2.65 and a non-response rate of 20%. Survey results will: 1) inform the next steps in the trachoma elimination process in Vietnam; and 2) help guide decision makers on investigation of trachoma hotspots in other areas.

## 733

### EXPERIMENTAL MALARIA IN PREGNANCY IS ASSOCIATED WITH ALTERED FETAL NEUROGENESIS AND NEUROPSYCHIATRIC DISORDERS IN OFFSPRING

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Each year ~125 million pregnant women are at risk for malaria infection. Malaria in pregnancy (MiP) has a profound impact on mother-child health, including delivery of low birth weight (LBW) infants. Even in the absence of LBW, epidemiological studies show a link between maternal infections during pregnancy and increased susceptibility of offspring to neuropsychiatric disorders later in life. Maternal immune activation can cause disruptions in embryonic neurodevelopment that could mechanistically underlie psychiatric outcomes. The impact of malaria exposure *in utero* on neurodevelopment and long-term vulnerability to psychiatric diseases has not been reported. We hypothesize that prenatal exposure to MiP will interfere with neurodevelopment via a host-mediated immune response and prime offspring to an increased risk of neuropsychiatric disorders. We used the established experimental mouse model of MiP (EMIP) with *Plasmodium berghei* ANKA (PbA). Uninfected adult offspring of dams infected with an inoculum of PbA that does not induce a LBW phenotype were subjected to a battery of standardized behavioural tests. Exposure to EMIP *in utero* induced increased anxiety-like behavior and hypersensitivity to amphetamine, and deficits in prepulse inhibition compared to unexposed offspring ( $p < 0.05$ ). The behavioural outcomes were associated with EMIP-mediated perturbations in embryonic neurogenesis including an altered distribution of embryonic cortical cells and a premature neurogenesis phenotype ( $p < 0.05$ ). Recent work by our lab showed that interrupting pathways of complement activation (i.e. C5a) improved fetal outcome in the EMIP model. Our future work will therefore compare the psychiatric and neurogenesis outcomes in malaria-infected  $\alpha$ -C5a treated and untreated offspring as a putative intervention. Our data implicate MiP as a modifiable risk factor for neurological injury and psychiatric disorders in exposed offspring. This concept represents a

paradigm shift in our understanding of risk factors for mental illness in malaria-endemic settings and may shift global health priorities from costly rehabilitation to prevention.

## 734

### TCR COMBINATORIAL IMMUNORECEPTOR EXPRESSION BY NEUTROPHILS CORRELATES WITH PARASITE BURDEN AND ENHANCED PHAGOCYTOSIS DURING A *PLASMODIUM BERGHEI* ANKA MALARIA INFECTION

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Neutrophils are the first responders of the innate immune response to invading pathogens. During malaria, neutrophils play an important role in the regulation of parasite burden and the immunopathogenesis of disease. In this study, we report the presence and expansion of a novel population of TCR $\beta$ -expressing CD11b+Ly6G<sup>+</sup> neutrophils in the spleen during a *Plasmodium berghei* ANKA infection in mice. Measurement of TCR $\beta$  transcript and protein levels in neutrophils in wildtype versus nude (which lack T cells) and *Rag1* KO (which lack B and T cells) mice showed that the observed TCR expression is not a consequence of nonspecific antibody staining or passive receptor expression due to phagocytosis or trogocytosis of peripheral T cells. Remarkably, on day 3 post-infection, we observed a highly significant correlation between the proportion of neutrophils that express TCR and peripheral blood parasite burden. In addition, we demonstrate that TCR $\beta$ <sup>+</sup> neutrophils phagocytose parasitized erythrocytes four fold more efficiently than TCR $\beta$ <sup>-</sup> neutrophils. Together these results signify that TCR expression by the neutrophil may play an important role in the regulation of parasite burden by enhancing the phagocytic capacity of the neutrophil.

## 735

### SUBMICROSCOPIC MALARIA INFECTIONS ARE NOT ASSOCIATED WITH NEGATIVE BIRTH OUTCOMES IN PREGNANT WOMEN FROM COLOMBIA

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Most of our knowledge regarding malaria in pregnancy stems from research based in Africa, where *Plasmodium falciparum* is the predominant species. However, little is known about the effects of *Plasmodium falciparum* or *P. vivax* infection in pregnancy in other regions, particularly submicroscopic infections (SMI) that are common in areas with lower transmission. Here, we present our findings from the first longitudinal study of malaria in pregnancy in Colombia, with complete molecular diagnostic and clinical outcomes data. The study included 180 women recruited early in pregnancy (median=19 weeks) and followed during antenatal visits (median=3 visits) through to delivery. One quarter of women (n=45) had a SMI at least once during pregnancy. Twenty-one (47%) were caused by *P. falciparum*, 16 (36%) by *P. vivax*, and 8 (17%) by mixed spp. Low birth weight babies (n=10; 5.3%) and pre-term births (n=21; 11.2%) were observed in our cohort but were not associated with SMI during pregnancy. To test whether the positive birth outcomes were

due to protective antibodies, we measured anti-*VAR2CSA* antibodies at enrolment and delivery. Over 60% of women had antibodies to *VAR2CSA* at enrolment, regardless of parity. Antibody levels decreased during pregnancy and we did not observe boosting following *P.* exposure. Using the inhibition of binding assay, 38% of women with an SMI infection had functional antibodies at enrolment that inhibited binding of infected erythrocytes to chondroitin sulphate A, an *in vitro* correlate of protection from *P. falciparum* placental malaria. Among the primigravid women, 33% had inhibitory antibodies at enrolment. These results reveal a high frequency of SMI in this region. However, contrary to the findings from studies in Africa, these infections were not associated with negative birth outcomes. These findings suggest that SMI in pregnancy does not pose a significant risk to the mother and the fetus, and anti-*VAR2CSA* antibodies acquired outside of pregnancy may mediate protection from negative birth outcomes in this setting.

## 736

### PROBABILITY OF TRANSMISSION OF MALARIA FROM MOSQUITO TO HUMAN IS REGULATED BY PARASITE DENSITY IN NAIVE AND VACCINATED HOSTS

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Over a century since Ronald Ross discovered that malaria is caused by the bite of an infectious mosquito it is still unclear how the number of parasites injected influences disease transmission. Currently it is assumed that all mosquitoes with salivary gland sporozoites are equally infectious irrespective of the number of parasites they harbour, though this has never been rigorously tested. Here we analyse >1000 experimental infections of humans and mice and demonstrate a dose-dependency for probability of infection and the length of the host pre-patent period. Mosquitoes with a higher numbers of sporozoites in their salivary glands following blood-feeding are more likely to have caused infection (and have done so quicker) than mosquitoes with fewer parasites. A similar dose response for the probability of infection was seen for humans given a pre-erythrocytic vaccine candidate targeting circumsporozoite protein (CSP), and in mice with and without transfusion of anti-CSP antibodies. These interventions prevented infection more efficiently from bites made by mosquitoes with fewer parasites. The importance of parasite number has widespread implications across malariology, ranging from our basic understanding of the parasite, how vaccines are evaluated and the way in which transmission should be measured in the field. It also provides direct evidence for why the only registered malaria vaccine RTS,S was partially effective in recent clinical trials.

## 737

### CHARACTERIZING THE ROLE OF A UNIQUE PHISTB PROTEIN IN *VAR2CSA* ADHESION, PLACENTAL MALARIA PATHOGENESIS AND IMMUNITY

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*VAR2CSA* is a PfEMP1 variant surface protein that binds chondroitin sulfate A (CSA), allowing *Plasmodium falciparum* to sequester in the placenta, leading to severe pathologies. Antibodies against *VAR2CSA* increase with gravidity as women become resistant to placental malaria (PM), making *VAR2CSA* the leading PM vaccine candidate. Additional

parasite proteins are upregulated in CSA-binding parasites, but their specific contributions to PM pathogenesis and immunity are unknown. One of these, PFI1785w, is a member of the PHISTb family, several of which have been implicated in PM and other severe pathologies. Multiple studies on clinical samples from East and West Africa have demonstrated that transcript and protein expression of PFI1785w are upregulated in parasites from PM patients versus other populations, indicating that it may play a distinct role in PM pathogenesis. PFI1785w is part of a chromosome deletion found in several lab strains, including model strains for PM that retain strong expression of VAR2CSA and CSA binding. Further, additional lab strains contain a polymorphism encoding a nonsense mutation not present in most clinical isolates. While this indicates that PFI1785w is not essential for *in vitro* adhesion to CSA, the *in vivo* role of PFI1785w in PM is undefined. To better characterize this protein, we have epitope-tagged PFI1785w in several clinical isolates to define its expression and localization. Gene knockout parasites are currently being generated with CRISPR/Cas9, and the resulting phenotypes will be described, as will the patterns of antigenicity using immune sera from gravidae compared to other populations. Finally, recent studies have shown that PHIST proteins interact with the acidic terminus segment of PfEMP1 with differential affinities, suggesting that specific PHIST proteins may interact with specific PfEMP1 variants. Co-immunoprecipitation experiments to evaluate whether VAR2CSA interacts with PFI1785w or alternate PHIST proteins in null lines are in progress and will be reported.

### 738

#### TRAFFICKING AND TOPOLOGY IDENTIFICATION OF *PLASMODIUM FALCIPARUM* MAURER'S CLEFT TWO TRANSMEMBRANE PROTEIN

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*Plasmodium falciparum* Maurer's clefts (MC) proteins are potential vaccine and drug candidates. In this study, we investigated the trafficking and biochemical properties of *P. falciparum* Maurer's cleft two transmembrane protein (PfMC-2TM). Previous studies, showed PfMC-2TM is an integral membrane protein encoded by a 13-member multigene family. PfMC-2TM is associated with the parasitophorous vacuole (PV), MC and infected erythrocyte (IE) membranes. PfMC-2TM is transcriptionally upregulated in the trophozoite stage of *P. falciparum*. Based on these observations, we hypothesized that the protein is trafficked to the IE cytosol through the classical secretory pathway after which it associates with MC and IE membranes. To understand the stage specific expression and trafficking of PfMC-2TM, we treated tightly synchronized parasites with 10µg/ml brefeldin A. To detect the synthesis and export, PfMC-2TM was co-localized with the nascent MC resident protein ring export protein 1 (REX1) in all intraerythrocytic stages of the parasite starting from 4-hour post invasion (hpi) by immunofluorescence analysis. The results show that the protein PfMC-2TM is synthesized 4 hpi, co-localizes with REX1 and is actively trafficked by the classical secretory pathway in ring and trophozoite stages of the parasite. Further, to identify the solubility of PfMC-2TM associated with MC and IE membranes, differential carbonate and detergent extraction with protease digestion was performed on IE membrane ghosts. We show that PfMC-2TM retains integral membrane properties upon association with the IE membrane and is accessible to proteases. Additionally, permeabilization studies with streptolysin-O in combination with saponin and trypsin shown that PfMC-2TM associates with the MC with both N and C termini exposed to the erythrocyte cytosol. In conclusion, PfMC-2TM is an additional marker of the nascent MCs. PfMC-2TM is trafficked through the classical secretory pathway into the IE cytosol, associates with the MC membrane with both N and C termini facing the IE cytosol, and also associates with the IE membrane.

### 739

#### INTRA AND INTER-INDIVIDUAL RED BLOOD CELL VARIABILITY IN BIND TO *PLASMODIUM VIVAX* DUFFY BINDING PROTEIN

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*Plasmodium vivax* invasion of the red blood cell (RBC) is considered to require interaction between the Duffy Binding Protein (PvDBP) at the merozoite's apical end and the RBC Duffy blood group protein (Fy). A SNP in the Fy gene promoter underlies the "Fy erythrocyte silent" (FyES) phenotype. However, studies report *P. vivax* infections in FyES individuals from ≥8 malarious African countries where FyES approaches genetic fixation. At least two hypotheses arise to explain these observations. First, low level or periodic Fy expression may occur in FyES people. Second, PvDBP may not interact with Fy, exclusively. We studied Fy and PvDBP interaction with RBCs among Fy-positive (Fy+; n=2) and Fy-negative (Fy-; n=2) individuals over 4-months (6 time-points) to assess intra-individual variability. The relative range of recombinant PvDBP binding to RBC measured by flow cytometry (fold-increase over background) was 4.9-47.8 (mean=18.1) for Fy\*A/\*A, 11.0-110.9 (mean=34.3) for Fy\*B/\*B, 0.7-5.9 (mean=2.0) for one Fy\*B<sup>ES</sup>/\*B<sup>ES</sup> donor and 0.5-4.0 (mean=1.8) for a second Fy\*B<sup>ES</sup>/\*B<sup>ES</sup> donor. Interaction of unfolded PvDBP was 0.6-2.3 (mean=1.1) with Fy+ RBC; 0.5-1.4 (mean=0.9) with Fy- RBC. PvDBP binding to Fy RBCs was chymotrypsin sensitive. To further demonstrate binding specificity, PvDBP interaction with RBC was evaluated in inhibition experiments using the CA111, single domain camelid antibody that recognizes an epitope (Fy6) in the binding domain of Fy to PvDBP. Compared to unchallenged RBC binding, CA111 (60 µg/mL) inhibited PvDBP-RBC interaction by 91.83% for Fy\*A/\*A, 87.93% for Fy\*B/\*B, 69.23% for Fy\*B<sup>ES</sup>/\*B<sup>ES</sup> donor#1 and 65.55% for Fy\*B<sup>ES</sup>/\*B<sup>ES</sup> donor#2. These data show that PvDBP interacts specifically with Fy+ and Fy- RBCs; Fy+ > Fy-. PvDBP-specific binding varied within individuals across the 6 different study time points. Interestingly, PvDBP-specific binding for one Fy- individual was comparable to low-level binding by the Fy\*A/\*A donor at 3 of 6 study time points. It is unclear whether constitutive low level or periodic RBC expression of Fy in Fy\*B<sup>ES</sup>/\*B<sup>ES</sup>, or that PvDBP interacts with more than Fy could trigger *P. vivax* invasion of Fy- RBCs.

### 740

#### PREVALENCE AND INCIDENCE OF SCHISTOSOME INFECTION AND MORBIDITY IN PRE-SCHOOL CHILDREN AGED 6 MONTHS TO 5 YEARS

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For a long time, pre-school children have been considered low-risk for schistosomiasis, received lower research focus and have been excluded from mass drug administration. Here, we describe the prevalence and incidence of first schistosome infection and morbidity in children aged 6 months to 5 years. This is part of a larger longitudinal study of schistosome infections in selected villages in Zimbabwe. A total of 1502 children were recruited in February 2016. Demographic and anthropometric measurements was gathered. Stool and urine samples were taken from each child for parasitological diagnosis of *Schistosoma haematobium* (urine-filtration) and to rule out *S. mansoni* and soil-transmitted helminths (Kato-Katz) respectively. Markers of morbidity including haematuria (macro, micro) and faecal occult blood (FOB) were assessed using reagent dipsticks and rapid tests. *S. haematobium* negative children were split into two groups, followed up quarterly and

annually. At baseline, prevalence of *S. haematobium* infection was 8.5% and infection prevalence/intensity increased significantly with age. FOB was positive in 2.5%, macrohaematuria in 0.7% and microhaematuria in 8.6% of children. Microhaematuria (Attributable fraction in infected–AFI:92.0%; Attributable fraction in population–AFP:40%), macrohaematuria (AFI:71.0%; AFP:1.2%), stunting–HAZ (AFI:38.0%; AFP:10.3%), malnutrition–MUACZ (AFI:34.0%; AFP:3.6%), Underweight–WAZ (AFI:29.0%; AFP:3.9%), and malnutrition–WHZ (AFI:9.0%; AFP:0.8%), showed significant associations with infection. Age (AOR: 1.4) and microhaematuria (AOR: 23.0) were independent predictors of *S. haematobium* infection. Mean quarterly incidence of *S. haematobium* infection was 4.7%, with an associated 5.6% microhaematuria. Annual incidence of *S. haematobium* was 1.8% with an associated 3.6% microhaematuria. We demonstrate for the first time, the incidence of schistosome infections with significant morbidity in pre-school children. Parasitology and haematuria are successful diagnostic and morbidity tools in this age group. Increased awareness, monitoring and treatment in this cohort is essential.

## 741

### DETECTION OF MULTI SCHISTOSOME PARASITES FROM SINGLE FILTERED URINE SAMPLES FROM SCHOOL CHILDREN AFTER MDA IN ZAMBIA

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Schistosomiasis is one of the most important Neglected Tropical Diseases (NTDs). In sub-Saharan Africa two major human schistosomes namely *Schistosoma mansoni* and *S. haematobium* often occur sympatrically largely affecting children. In the age group of 6 - 15 year's infection prevalence and intensity peaks, which associated with growth delays, delayed cognition, poor school performance and a negative effect on the overall growth and quality of a child's life. World Health Organization (WHO) is urging member states to regularly treat at least 75% and up to 100%, of all school-aged children at risk of morbidity. We have detected *S. mansoni* and *S. haematobium* parasite specific small repeat DNA fragment from filtered urine on filter paper by Polymerase Chain Reaction (PCR). In this study we are testing urine samples obtained from Zambian school children after Mass Drug Administration (MDA) to compare between existing diagnostic tests and PCR amplification of parasite DNA from urine. We have assessed 111 samples for the above-mentioned schistosome species by amplifying species-specific DNA fragment from single urine sample. Our approach detected eight times more positive cases (total 77) than by Kato-Katz (KK: 9) for *S. mansoni* and six times more (total 72) than by hematuria (11) for *S. haematobium* and even more than urine filtration (77 compared to only 6). All of the samples were detected with 100% sensitivity and specificity devoid of any cross amplification. The same pattern was observed when stratified for age group and sex specific analysis. In addition, 69 individuals (62%) were co-infected by both parasites. We have demonstrated a significantly higher prevalence of both species than indicated by the classical examination of urine or stool and also the maintenance of infection reservoir after MDA. Our approach of detecting low prevalence multi schistosome infection is an effective means to detect low intensity infection and would enhance the effectiveness of surveillance and MDA control programs of schistosomiasis.

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### ASSESSMENT OF MORBIDITY DUE TO *SCHISTOSOMA MANSONI* IN SCHOOL-AGED-CHILDREN IN MADAGASCAR

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*Schistosoma mansoni* is a significant problem for children in eastern Madagascar. Chronic infection causes intestinal and hepatic disease, contributing to an enormous health burden. Madagascar Medical Expeditions (MADEX) is a student initiative involving schistosomiasis research in the Marolambo District of Madagascar. Across two research expeditions in 2015 and 2016 we have investigated schistosomiasis prevalence and morbidity in school-aged children and carried out educational and treatment programs for children in the district. Prevalence of *S. mansoni* was determined by Kato-Katz stool microscopy and urine circulating cathodic antigen tests. Children were interviewed using structured questionnaires on symptoms and water contact behaviour and the Pediatric Quality of Life Inventory (PedsQL). Anaemia and malaria were assessed using point-of-care tests and periportal fibrosis was investigated ultrasonographically. Preliminary results reveal 97% prevalence of schistosomiasis; nearly two-thirds of children complained of symptoms of infection; 78% children had regular interaction with the river, most commonly fetching water and washing clothes; 57% were anaemic. There were lower than average PedsQL scores in children within medium and high infection intensity subgroups. Results reveal alarming schistosomiasis prevalence and disease burden. A longitudinal approach via annual research, treatment and education based expeditions will provide information into any improvements on disease prevalence and morbidity. Qualitative behavioural studies will be a particular focus for 2017, alongside expansion of education efforts.

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### IMPACT OF THREE YEARS' INTENSIVE, COMMUNITY-WIDE ANTHELMINTHIC TREATMENT ON ALLERGY-RELATED OUTCOMES, HELMINTH PREVALENCE AND HELMINTH-ASSOCIATED PATHOLOGY AMONG HIGH *SCHISTOSOMA MANSONI* TRANSMISSION ISLAND COMMUNITIES OF LAKE VICTORIA, UGANDA: RESULTS OF A CLUSTER-RANDOMIZED TRIAL

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Parasitic helminths co-evolved with mammals. Helminth antigens and allergens have common structures and atopic (IgE-mediated) immune responses probably evolved to protect against helminths and ectoparasites, while helminths evolved strategies to down-regulate atopic processes. While strong inverse associations have been described between helminths (especially *Schistosoma*) and atopy, helminth infections are associated with some serious, and much subtle morbidity. We hypothesised that intensive anthelmintic mass drug administration (MDA) would increase atopic (skin prick test) reactions and allergy-related disease prevalence, while reducing prevalence of helminths and their associated pathology. We conducted a cluster-randomised trial of community-wide intensive MDA (quarterly single-dose praziquantel 40mg/kg plus albendazole 400mg for three days) versus standard, Uganda government intervention (annual praziquantel 40mg/kg; 6-monthly single-dose albendazole); 26 island fishing villages

were randomised, 13 per trial arm, for three years; outcomes were determined in a household survey of 3350 individuals. Intensive (compared to standard) MDA had no effect on wheeze, atopy or allergen-specific IgE levels. Intensive (compared to standard) MDA reduced *S. mansoni* infection intensity: by Kato Katz examination of 1 stool sample, prevalence fell to 23% vs 39% among all participants ( $p=0.003$ ); 36% vs 77% among 10-14 year-olds, but urine CCA remained positive in 85% of participants in both trial arms. Hookworm prevalence was reduced (8% vs 11%,  $p=0.05$ , by PCR) but there was no effect on *Trichuris* or *Strongyloides*. There were no differences in anaemia, growth, hepatosplenomegaly or abdominal ultrasound findings between trial arms. Thus, helminth infections, especially *S. mansoni*, remained almost universal in these high-transmission communities despite sustained, intensive MDA. Intensive MDA produced no clinical harm or benefit compared to standard MDA. While reassuring regarding allergy-related conditions, these results accentuate concern regarding the limitations of what can be achieved by anthelmintic MDA.

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### A HIGH FIELD GRADIENT MAGNETIC PROBE FOR THE ISOLATION OF SCHISTOSOME EGGS FROM FECAL MATTER BASED ON THEIR INTERACTION WITH MAGNETIC PARTICLES

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Schistosomiasis is a chronic parasitic infection caused by helminths of the genus *Schistosoma*. Most diagnostic methods, including the most widely used Kato Katz (KK) method, lack sensitivity when the egg burden in feces is low. The aim of this study was to test the sensitivity of a new method, a magnetic probe, for detecting schistosome eggs in fecal samples. A case-control study was carried out in 40 samples (20 KK +ve and 20 KK -ve) from a field study in an endemic area for *Schistosoma mansoni* in the Northeast of Brazil. Samples were processed according to a highly sensitive method called Helmintex, which starts with 30 g of feces and finishes with approximately 1 mL of sediment in an Eppendorf tube. The fecal samples in the tubes are then mixed with magnetic particles and submitted to a magnetic field, where the eggs interacted and attached to the particles, and were isolated when the magnetic field was applied. The suspension was stirred with the tip of a probe that produces a source of high magnetic field gradient for 20 seconds. Two 40- $\mu$ L droplets were extracted from the sediment by withdrawal of the probe. Each droplet was deposited on a microscope slide during demagnetization of the probe to enable inspection by optical microscopy. The remainder of the sediment was screened carefully for confirmatory results. For analysis of sensitivity, a composite reference standard was defined as follows: if any of KK, probe, or standard Helmintex screening identified an egg, the case is disease positive – otherwise negative. Eggs were detected in all 20 KK positive samples, and in the KK negative samples, the probe yielded positive results for 3 samples. Time needed to read the samples was around 3-5 minutes. Against the composite reference standard, following sensitivities with 95% CIs were observed: KK 83% (63-95%), POC CCA 79% (58-93%), probe 92% (73-99%), standard Helmintex 96% (79-100%). In conclusion, the high field gradient magnetic probe in conjunction with the Helmintex method can provide rapid and sensitive detection of *Schistosoma* eggs.

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### POINT OF CARE DIAGNOSIS FOR MULTIPLE SCHISTOSOME PARASITES: SPECIES-SPECIFIC DNA DETECTION FROM SINGLE URINE SAMPLE BY LAMP AND PCR

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Schistosomes are easily transmitted and multiply considerably so if control strategies based on targeted mass drug administration (MDA) are to succeed it is essential to have a test that is sensitive, accurate and simple to use. It is regularly demonstrated that praziquantel does not always eliminate an infection so in spite of the successes of control programs a residual of the reservoir will survive to re-infect snails. The issue of diagnostic sensitivity will become more critical in the assessment of program effectiveness. While serology or antigen capture tests might improve sensitivity, it has been shown that the presence of species-specific DNA fragment by amplification from urine residue captured on Whatman # 3 filter paper that is dried after filtration improves sensitivity significantly. It is simple to handle, transport and can be stored for several months without freezing. In the current study done in a low to moderate transmission area in Ghana, we assessed the efficacy of detection of either or both *Schistosoma mansoni* and *S. haematobium* specific DNA from 86 urine residues both by PCR and loop mediated isothermal amplification (LAMP). We also compared the DNA extraction techniques by standard extraction kit and field usable LAMP PURE kit and have evaluated these different extractions for species-specific DNA detection. With *S. haematobium* all three methods showed similar sensitivity and specificity when compared with PCR amplification (100%). For *S. mansoni* sensitivity was highest for LAMP amplification (100%) than PCR and LAMP PURE (99% and 94%). The LAMP PURE extraction produced false negatives, which require further investigation for this field usable extraction kit. Overall high positive and negative predictive values (90% - 100%) for both species demonstrated a highly robust approach. The LAMP approach is close to point of care use and more sensitive and equally specific to detection of parasite eggs in urine or stool showing that LAMP can be an effective means to detect low intensity infection and its simplicity and ease of sampling would enhance the effectiveness of surveillance and MDA control programs of schistosomiasis.

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### TREATMENT EFFECTS ON EGG AND ANTIGEN DIAGNOSTICS OF SCHISTOSOMA MANSONI INFECTIONS

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Schistosomiasis is a major socio-economic and public-health problem in many sub-Saharan African countries. After large mass drug administration (MDA) campaigns, prevalence of infection rapidly returns to pre-treatment levels. The traditional egg-based diagnostic for schistosome infections, Kato-Katz, is being substituted in many settings by circulating antigen recognition based diagnostics, such as the point-of-care circulating cathodic antigen test (CCA). The relationship between these two diagnostics, particularly after treatment in drug-efficacy studies, is poorly understood. We created an inference model of schistosome infections to better understand and quantify the relationship between these two egg and adult worm antigen based diagnostics. Due to the semi-quantitative nature of CCA, we focused on the current major challenges of interpreting "trace" CCA results. Our analyses suggest that CCA is generally a better

predictor of prevalence, particularly after treatment, and that trace CCA results are typically associated with truly infected individuals. Even though prevalence rises to pre-treatment levels only a few months after MDAs, our model suggests that the average infection level is much lower, and is probably due to a smaller burden of surviving juveniles from when the treatment occurred. This work helps to better understand CCA diagnostics and the interpretation of post-treatment prevalence estimations.

## 747

### **BREAST MILK EPIDERMAL GROWTH FACTOR IS ASSOCIATED WITH GROWTH AND DIARRHEA IN BANGLADESHI CHILDREN**

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Environmental enteropathy, an intestinal inflammatory condition, has been associated with poor linear growth in children from low-income countries. Diarrhea in this setting represents the second leading cause of mortality and a significant cause of morbidity in young children. Epidermal growth factor (EGF) is 6kDa protein that is known to have cellular proliferative and anti-inflammatory properties. EGF is known to be present in human breast milk. Thus, we decided to measure EGF levels in breast milk of new Bangladeshi mothers and investigate if EGF level was associated with growth and diarrheal disease. Breast milk was collected at 6 weeks postpartum. EGF was measured via commercial ELISA kit (Eagle Biosciences, Nashua, NH USA). Children were followed through 1 year of age with continuous diarrheal surveillance via twice weekly home visits. Anthropometrics data was also collected. Data was first analyzed via Pearson correlation for anthropometrics and univariate poisson regression for diarrhea. Multivariable linear regression models were constructed for change in length-for-age and weight-for-age Z score with EGF, income, postpartum day of milk collection, and maternal height as predictors. EGF was log transformed for analysis. In univariate analysis EGF was associated with improved linear growth at 18 weeks ( $r = 0.13$ ,  $p = 0.004$ ), 24 weeks ( $r = 0.11$ ,  $p = 0.02$ ), and 52 weeks of age ( $r = 0.12$ ,  $p = 0.007$ ). EGF was not associated with improved weight gain. This relationship remained significant when covariates were corrected for at 18 weeks (coefficient [95% CI]: 0.38 [0.12, 0.65],  $p = 0.004$ ), 24 weeks (0.37 [0.085, 0.66],  $p = 0.01$ ), and 52 weeks (0.48 [0.15, 0.83],  $p = 0.005$ ). EGF was also associated with decreased number of diarrheal episodes at 3 months (coefficient [95% CI]: -0.70 [-1.15, -0.25],  $p = 0.002$ ) and 6 months (-0.39 [-0.71, -0.08],  $p = 0.015$ ). This association was not significant at 1 year of age. Our analysis showed that EGF levels in breast milk are associated with improved linear growth through 1 year of age. Additionally, EGF levels are associated with decreased diarrheal episodes through 6 months of age but this effect wanes by 1 year.

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### **VACCINE-INDUCED MUCOSAL IMMUNITY FROM IPV-BOPV AND IPV-ONLY IMMUNIZATION SCHEDULES: ANALYSIS OF AN OPEN-LABEL, RANDOMIZED CONTROLLED TRIAL IN CHILEAN INFANTS**

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A globally synchronized replacement of trivalent oral polio vaccine (OPV) with bivalent OPV (bOPV) lacking a type 2 poliovirus component was successfully implemented in early 2016. To mitigate risks of re-emergent type 2 poliomyelitis following the switch, the World Health Organization recommended the concomitant introduction of at least one dose of trivalent inactivated polio vaccine (IPV). Nevertheless, the capacity of IPV to elicit robust intestinal mucosal immunity against polio - and thereby block poliovirus replication and shedding - remains uncertain. In a Chilean clinical trial (NCT01841671), infants were randomized to receive IPV-bOPV-bOPV, IPV-IPV-bOPV, or IPV-IPV-IPV at 8, 16, and 24 weeks of age and then challenged with monovalent OPV type 2 (mOPV2) at 28 weeks. Using fecal samples collected at 28, 29, 30, 31, and 32 weeks, we investigated the extent to which the immunization schedules induced polio type-specific neutralization and immunoglobulin A at enteric sites. Despite receiving up to three doses of IPV ( $\pm$  bOPV), only 32% of the total 181 vaccinees had detectable type 2-specific stool neutralization after the primary vaccine series. In contrast, the mOPV2 challenge induced brisk type 2-specific intestinal responses in all vaccine groups, and significantly higher stool neutralization ( $p < 0.0001$ ) and immunoglobulin A concentrations ( $p < 0.0001$ ) were measured after two weeks. Overall, type 2-specific mucosal neutralization and immunoglobulin A levels were positively associated with sustained breastfeeding and inversely correlated with viral shedding. Although IPV-bOPV and IPV-only immunization schedules consistently elicit high rates of seroconversion against all three poliovirus types, these results suggest vaccine regimens lacking OPV2 may induce only modest intestinal mucosal immune responses against type 2 poliovirus.

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### **IMMUNOGENICITY AND PROTECTIVE EFFICACY OF A LIVE ATTENUATED ETEC VACCINE CANDIDATE AGAINST VIRULENT ENTEROTOXIGENIC ESCHERICHIA COLI (ETEC) IN A HUMAN ETEC CHALLENGE MODEL**

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ETEC is a common cause of bacterial diarrhea in children as well as in travelers and military personnel deploying to ETEC endemic countries. A licensed vaccine for ETEC is not currently available. We have previously demonstrated that an oral, live attenuated, three-strain recombinant ETEC vaccine, ACE527, was safe and well tolerated. However the protective efficacy (27%) against severe diarrhea was not statistically significant. We have extended these observations in a new phase 2b efficacy study where we added double mutant heat labile toxin (dmLT) as an adjuvant and also added a 3rd dose to the primary immunization series. Immunized subjects were challenged with the ETEC H10407 at 6-7 months after immunization. Serum IgA and IgG; antibody in lymphocyte supernatant (ALS); fecal IgA and memory B cell responses to LT-B, CFA/II, CS3, and CS6 following vaccination and challenge were evaluated to monitor vaccine immunogenicity and to identify immune correlates of protection. Functional assays such as, toxin neutralization and adherence blocking assays are currently getting evaluated. Results of this recent Ph2b study indicated that the adjuvanted group was significantly protected against severe diarrhea (PE=65.9%  $p=0.01$ ) and against diarrhea of any severity (PE=58.5%  $p=0.02$ ). Overall, the immune responses in the adjuvanted and vaccine alone groups were not significantly different. We found strong indications which suggests that CFA/II response may have contributed in protection. There were also measurable anti-LT, anti-CS3 responses in these subjects. Most of the subjects exhibited antigen-specific Memory B cell responses; however, the responses were short lived and there was no

significant association with protection. The apparent diminished responses to key antigens in the adjuvanted immune subjects following challenge with H10407 gave a further indication that colonization may be modified in this group. Further investigations looking at T cell responses, as well as expanded immune profiling using proteome arrays are underway to find novel ETEC antigens that might show clearer association with protection.

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### WHEN IS A CONTROL NOT A CONTROL? —ANALYSIS OF DIARRHEA AND ENTERIC INFECTION AMONG CONTROLS IN THE GLOBAL ENTERIC MULTICENTER STUDY, KENYA, 2008-2012

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Most case-control studies of risk factors for diarrheal disease focus on cases and use eligibility criteria to ensure controls are diarrhea-free. However, growing attention to the impact of subclinical enteric infections in children suggests these controls may offer valuable insights into underlying rates of infection and disease. This study examined enteric infection, diarrhea, and health outcomes in control children (n=2,534) of the Global Enteric Multicenter Study (GEMS) Kenya site. Data were obtained from laboratory results of stool samples collected from controls at enrollment, a subsequent 14-day memory aid for diarrhea, and a 60-day follow-up visit. Controls reported being diarrhea-free for  $\geq 7$  days before GEMS enrollment, but 69% of stool samples from controls tested positive for  $\geq 1$  enteric pathogen, compared with 81% of samples from cases (p<0.001). Almost 39% of controls reported diarrhea within 14 days of enrollment; 459 (50%) of whom reported diarrhea within the first 5 days. Controls with and without diarrhea in the 14-day period did not differ significantly in detection of enteric pathogens in stool at enrollment, with the exception of typical enteropathogenic *E. coli* (6% in controls with diarrhea v. 4% in those without). At 60-day follow-up, controls who reported diarrhea in the 14-day period were more likely to have visited a health facility for diarrhea (28% v. 7%) or fever (23% v. 16%), and to have significantly lower height-for-age Z (HAZ) scores than controls without diarrhea during that period. Almost 20% of controls were negative for both diarrhea and enteric pathogens. These controls had significantly improved HAZ scores over both a) controls with diarrhea and enteric pathogens in stool (27%) and b) controls without diarrhea but with enteric pathogens in stool (42%). Overall, the high incidence of diarrhea after enrollment and of subclinical enteric infection in controls, and their links with poorer clinical outcomes at follow-up, highlight complexities in assessing diarrheal disease contributors with a single entry point for participants, relevant for design of future epidemiologic studies of diarrheal disease.

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### IMPACT OF MENINGOCOCCAL SEROGROUP A CONJUGATE VACCINE ON MENINGITIS EPIDEMIOLOGY—BURKINA FASO, 2011-2015

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Historically, *Neisseria meningitidis* serogroup A (NmA) caused large meningitis epidemics in sub-Saharan Africa. In 2010, Burkina Faso became the first country to implement a national meningococcal serogroup A conjugate vaccine (MACV) campaign. We examined nationwide laboratory-confirmed case-based meningitis surveillance data in Burkina Faso in the five years (2011-2015) following MACV introduction to evaluate vaccine impact on meningitis epidemiology. We calculated incidence (cases per 100,000 persons) and described reported NmA cases. In 2011-2015, Burkina Faso reported 18,538 cases of suspected meningitis with average annual incidence of 22.2, lower than the reported average annual incidence (81.2) in the 4 years prior to MACV introduction. Among reported cases, 98% received lumbar punctures (LPs); 62% of specimens were tested by at least one confirmatory test. Among 11,164 tested meningitis cases, 4,503 (40%) were laboratory-confirmed: *H. influenzae* (2%), *N. meningitidis* (40%), and *S. pneumoniae* (57%). Average annual incidence of meningococcal meningitis was 3.8 (range: 2.0-10.2 annually) and was highest among infants aged <1 year (8.4). *N. meningitidis* serogroup W caused the majority (64%) of meningococcal meningitis among all age groups. Only six confirmed NmA cases were reported in 2011-2015. Five cases were in children who were age ineligible (n=2) or otherwise not vaccinated (n=3) during the 2010 MACV mass vaccination campaign; one case had documented MACV receipt, representing the first documented MACV vaccine failure. Meningitis incidence in Burkina Faso remains relatively low following MACV introduction; however, data suggest a substantial burden remains and NmA transmission persists. MACV integration into Burkina Faso's routine immunization program is essential to provide continued protection. Sustained investment to strengthen meningitis surveillance will allow monitoring of evolving epidemiology and vaccine effectiveness.

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### BIOMARKERS AND IMMUNE MODULATION IN BURULI ULCER DISEASE

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Buruli ulcer disease (BUD), caused by *Mycobacterium ulcerans*, is a chronic debilitating disease of the skin causing necrotic lesions and ulcerations. The disease mainly affects children and adolescent and cases are reported in 33 tropical and subtropical countries with West Africa, including Ghana, being most affected. As for other mycobacterial infections, T cell mediated immune responses are important for protection and recovery during

treatment, but studies investigating antigen-specific immune responses and immune modulation in BUD patients are scarce. The aim of the study was to characterize *M. ulcerans* specific CD4+ T cell responses and analyze immune modulation in BUD patients. We performed a case-control study on children with BUD and compared them to healthy controls and a follow-up study on BUD patients before and after treatment with antibiotics. Whole blood was stimulated with antigens prepared from *M. ulcerans* and cytokine production by T cells was analyzed by intracellular flow cytometry. In addition, we evaluated markers of activation and inhibition on T cells to determine systemic immune modulation in BUD patients. Results were associated with disease severity and progression to *i.*) investigate potential biomarkers for the disease and *ii.*) to dissect mechanisms of *M. ulcerans*-specific protective immune responses. We show that TNF $\alpha$ -single cytokine producing CD4+ T cells are increased in BUD patients, are associated with severity and healing progress indicating a potential use as biomarker. We will further present results on the TH2 and regulatory cytokine profile of CD4+ T cells in BUD and on regulatory T cell responses. The presented results will provide a complex figure of the antigen-specific immune responses induced in BUD patients, it will provide insights in immune regulation and thereby help to dissect mechanisms of protection and immune pathology in BUD.

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### IMMUNE RESPONSE TO RECOMBINANT PROTEINS OF MYCOBACTERIUM LEPRAE POTENTIAL APPLICATION FOR LEPROSY DIAGNOSIS

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Leprosy is a infectious disease, that remains a serious public health problem. Until now, the diagnosis of leprosy has been based on dermatoneurological examination complemented with smear microscopy and with histopathology. The development of a specific and sensitive serological test is of fundamental importance for early diagnosis. The purpose of this study was to evaluate serum samples from leprosy patients, household contacts and healthy subjects for reactivity against specific recombinant proteins of *M. leprae* (ML0405, ML2055, ML2331, Ag85B, NDO-LID, NDO-HAS and LID) using ELISA. 44 leprosy patients (PB and MB), 61 household contacts (MBC and PBC) and 16 healthy individuals (HC), participated of the study. The comparison between the groups was carried out through the non-parametric Mann-Whitney. The ROC curve was used for analysis of the accuracy values: area under the curve, sensitivity, specificity and likelihood ratios (LR+ and LR-). The group of MB patients had higher IgG production compared to HC and PB groups for all antigens. MBC group showed higher production of specific IgG antibodies to ML2055 and ML0405 in comparison with the HC group. IgM levels were higher in MB patients compared with the HC group only for the ML2331. IgM levels to ML2055 and Ag85B were higher in MBC compared to the HC group. The evaluation of sensitivity and specificity for the ROC curve of the MB group showed that the ML0405 antigen-specific IgG1 levels presented sensitivity of 73% and specificity of 93.8% with LR+ of 11.73 and LR- of 0.28. IgG4 levels to ML0405 presented sensitivity of 80% and specificity of 93.8%, with LR+ of 9.6 and LR- of 0.41. Serum levels of total IgG and IgG1 against LID-1, NDO-LID and NDO-HSA antigens were significantly higher in MB patients in comparison with all other groups. Our results suggest that a serological test using ML0405, ML2055 and LID-1 antigens could be developed as an additional test for the early diagnosis of leprosy. Serological reactivity against ML0405, ML2055 and Ag85B in MBC may represent an eligible strategy for active surveillance and preventive chemoprophylaxis seeking better control of leprosy.

## 754

### HERITABLE GENE EDITING BY TARGETED DELIVERY OF CAS9 NUCLEASE TO THE MOSQUITO GERMLINE

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CRISPR/Cas9 gene editing is a powerful tool for addressing research questions in arthropods. Current approaches rely upon delivering Cas9 ribonucleoprotein complex (RNP) to arthropod eggs by embryonic microinjection. However, embryonic microinjection is challenging, is limited to a small number of species, and is inefficient even in optimized taxa. We have developed a technology called Receptor-Mediated Ovary Transduction of Cargo (ReMOT Control) to specifically deliver the Cas9 RNP to the insect germline by injection into adult females. After injection into the adult female hemolymph, ReMOT Control transduces Cas9 RNP to the developing germline, resulting in "in utero" editing of the offspring chromosomal sequence. In *Aedes aegypti*, ReMOT Control gene editing efficiency is as high as 17%. Once optimized for multiple species, the ReMOT Control technology will dramatically change the landscape of molecular entomology research, allowing easy, flexible genetic manipulation of a wide variety of vector arthropods and non-model species.

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### CRISPR-CAS9 MEDITATED GENE KNOCKOUT OF PLASMODIUM AGONISTS IN ANOPHELES GAMBIAE ENHANCES MOSQUITOES' RESISTANCE TO THE HUMAN MALARIA PARASITE

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In the malaria parasite's journey through the mosquito, *Plasmodium* engages in intimate interactions with the vector's midgut, hemolymph, and salivary glands, during which it relies on numerous mosquito-derived host factors. The mosquito's anti-*Plasmodium* immune responses are on the other hand targeting the parasite through a variety of immune proteins, or restriction factors. These crucial *Anopheles-Plasmodium* interactions represent powerful targets for transmission-blocking by either inhibiting parasite agonists (or host factors) that are required for infection, or over-expressing parasite antagonists (or restriction factors) that suppress infection. *Plasmodium* infection can be suppressed in the *Anopheles* vector by agonist deletion through gene editing. The recently developed CRISPR-Cas9 based genome editing tools for *Anopheles* mosquitoes provide new and promising opportunities for the study of agonist function through gene deletion. We have focused on several validated *Plasmodium falciparum* host factors as targets for CRISPR-Cas9 based germ-line targeted gene knockout (KO), to study their host factor biology, and evaluate them as transmission-blocking targets for the development of a novel malaria control strategy. We have selected three guide RNA (gRNA) target sequences for each gene of interest, and generated several U6::gRNA constructs for embryo microinjection of *A. gambiae*. Several gRNA-overexpressing transgenic lines were obtained and through the crossing with the Vasa::Cas9 strain and outcrossing with the wild type mosquitoes, a couple of KO mutants were generated. Our results have shown that, while some host factors play essential biological roles which are impossible to mutate because of lethality at pre-adult embryonic and larval stages in the KO mutants, other host factors mutants did develop into adults showing profound suppression of human malaria parasite

infection with a significant 82% reduction in oocysts load. Ongoing studies are investigating the biology of *Plasmodium* blocking through gene knockout of *Plasmodium* agonists, as well as the over-expression of anti-*Plasmodium* effectors.

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### DEVELOPING EVOLUTIONARILY STABLE GENE DRIVES IN *ANOPHELES GAMBIAE*

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Gene drives are transgenes capable of biasing their own inheritance. By encoding CRISPR components targeting the homologous wild type locus in the germline, and subsequent repair by Homologous Recombination (HR), they enable super-Mendelian inheritance in the progeny of diploid species. As a result they are capable of spreading themselves - as well as any associated genetic cargo - to allelic fixation in wild populations. Among other possibilities, they promise to enable scientists to unilaterally engineer wild mosquito populations to be refractory to malaria. However research is emerging that 'classic' gene drives create mutations as they propagate, directly preventing further drive spread. These drive-resistant alleles render gene drives unable to copy by HR, and results in the gene drive being selected out of the population. To overcome this hurdle, we are developing evolutionarily stable gene drives that prevent drive-resistant alleles from being created. Here we will outline our progress towards developing evolutionarily stable CRISPR-based gene drives in the malaria mosquito *Anopheles gambiae*. Towards this end I will discuss a new method for gene knock-in into genetically-intractable *loci* as well as preliminary data on the stability and efficiency of these novel gene drive architectures.

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### SI-RNA PESTICIDES TARGETING MULTIPLE MALARIA VECTOR MOSQUITO SPECIES

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Mosquito control is the primary means of preventing malaria and other mosquito-borne diseases. Due to the increase of reported insecticide resistance and rising concern for the negative effects of pesticides on non-target organisms, the current pesticide repertoire is faced with great challenges to sustainability. New biorational pesticides are vitally needed to address established and emerging arthropod-borne infectious diseases. RNAi, which is attracting attention in the agricultural pest control community, has facilitated functional genetic characterization of mosquito development in our laboratory. We are now examining the hypothesis that ingested siRNAs can be utilized as mosquito pesticides. A high-throughput screen conducted in *Anopheles gambiae* enriched for the selection of siRNA pesticides that generate high levels of mortality throughout the larval period, target multiple *Anopheles* species, are non-toxic to humans and other non-target organisms, and which will create an siRNA arsenal to combat resistance that arises from point mutations in any one target sequence. This successful screen identified 50 *Anopheles* siRNA larvicides. Of the 50 larvicidal siRNAs, 10 highly lethal siRNAs with target sequences that are conserved in multiple *Anopheles* mosquito species were prioritized. In silico assays demonstrated that the siRNAs pose little risk for humans or other non-target organisms. Potential field delivery systems for these interfering RNA molecules were then assessed. These studies identified larval soaking, chitosan nanoparticles, non-pathogenic bacteria, and *Saccharomyces cerevisiae* (baker's yeast) as potential delivery systems for *Anopheles* interfering RNA larvicides. Of the delivery mechanisms assessed, yeast interfering RNA larvicides, which can generate up to 100% larval death in laboratory trials even when the yeast are heat-inactivated,

are of particular interest. Future studies will pursue field-testing of these pesticides, as well as extension of our siRNA pesticide screens to adult mosquitoes.

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### CYTOGENETIC MECHANISMS OF HYBRID MALE STERILITY IN THE *ANOPHELES GAMBIAE* COMPLEX

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The success of malaria transmission highly depends on the rate of mosquito reproduction. The development of novel approaches to control the reproductive output of mosquitoes must include the understanding of how reproduction is regulated. Studying the mechanisms of sterility of male hybrids between closely related species of mosquitoes can improve our knowledge of speciation and empower the sterile insect technique. Inter-species crosses in the *Anopheles gambiae* complex most often result in F1 hybrid male sterility confirming the Haldane's rule about sterility or inviability of the heterogametic sex. The genetic basis and mechanisms of hybrid male sterility in mosquitoes are unknown. To investigate them, we performed crosses between laboratory colonies of *An. merus* (MAF) and either *An. gambiae* s.s. (Zanu) or *An. coluzzii* (Mopti and Mali). Our results confirmed sterility of F1 hybrid males. Testes had a near normal morphology and size but produced very few sperms in hybrids between female *An. merus* and male *An. gambiae* or *An. coluzzii*. However, testes were severely underdeveloped in hybrid males from the reciprocal crosses. Using X-specific and Y-specific FISH probes, we followed the process of meiosis in individual species and their F1 hybrids. We discovered a novel sperm phenotype - X+Y bearing sperms - caused by abnormal chromosomal activities in meiosis of male hybrids. Compared with chromosomes in parental species, chromosomes in hybrid males showed various degrees of insufficient condensation indicating a malfunction of proteins associated in chromosome condensation. Moreover, sex chromosomes in hybrid males failed to pair and to enter anaphase I. We hypothesize that fixed differences in the molecular structure of sex chromosome heterochromatin may contribute to the abnormal progress of meiosis and hybrid male sterility in malaria mosquitoes.

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### CHEMOSENSORY GENE EXPRESSION IN THE PROBOSCIS OF *ANOPHELES GAMBIAE* S.L. MOSQUITOES WITH VARYING HOST PREFERENCE

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The *Anopheles gambiae* complex contains several of the primary vectors of malaria in sub-Saharan Africa. Within the complex, *Anopheles coluzzii* is highly anthropophilic, while *Anopheles quadriannulatus* prefers feeding on bovines. Mosquito host searching is modulated by the olfactory organs, and differential expression of chemosensory genes could play a significant role in determining host preference. Previous work has compared chemosensory expression in the antennae and palps of these species. The proboscis remains unstudied, even though olfactory genes are known to be expressed in *Aedes aegypti* proboscises. Five chemosensory gene families that interact directly with odorants may be relevant for host choice: olfactory receptors (*Ors*), ionotropic receptors (*Irs*), odorant binding proteins (*Obps*), chemosensory proteins (*Csps*) and gustatory receptors (*Grs*). We therefore used RNA-Seq to compare their expression in the proboscises of *An. coluzzii* and *An. quadriannulatus*. Overall chemosensory gene expression between the two species is highly correlated, but the expression of a number of genes is considerably enhanced in *An. coluzzii*. *Or6* stands out as the 2nd most highly expressed *Or* in both sexes of this species, while it is almost absent from *An. quadriannulatus*. In addition, several highly expressed *Irs* are between 2.0 and 7.1-fold enhanced in *An.*

*coluzzii*. *Ir7s* is the most highly expressed *Ir* in the proboscis of this species, and is 7.1-fold upregulated. Similarly, two of the most highly expressed *Obps* (*Obp54* and *Obp26*) are 5.0 and 8.4-fold upregulated in *An. coluzzii* proboscises, with *Obp26* being mostly specific to females. No *Csps* showed significantly different expression between the females of the two species. Finally, we detected a large number of *Grs*, although many were expressed at low levels. Several of the most highly expressed *Grs* (*Gr15*, *Gr48-52*) were significantly 1.7 to 12.6-fold higher expressed in the proboscis of *An. coluzzii* females. It is possible that some of the gene expression differences observed here are correlated to host preference in these mosquitoes.

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### GENETIC ARCHITECTURE OF WOLBACHIA-MEDIATED DENGUE VIRUS BLOCKING IN Aedes Aegypti

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The mosquito vector *Aedes aegypti* is responsible for transmitting a range of arboviruses including dengue (DENV) and Zika (ZIKV). The global reach of these viruses is increasing due to an expansion of the mosquito's geographic range and increasing urbanization and human travel. Vector control remains the primary means for limiting these diseases. *Wolbachia pipiensis* is an endosymbiotic bacterium of insects that has the ability to block the replication of pathogens, including flaviviruses such as DENV or ZIKV, inside the body of the vector. A strain of *Wolbachia* called *wMel* is currently being released into wild mosquito populations to test its potential to limit virus transmission to humans. The mechanism that underpins the virus blocking effect, however, remains elusive. Using a full-sib breeding design in conjunction with vector competence assays, we show the first evidence of genetic variation in *Wolbachia*-mediated blocking. We also demonstrate that the trait is highly heritable. In addition, we revealed a significant positive correlation between *Wolbachia* load and DENV copy number in whole mosquitoes. Finally, we used the phenotyped mosquito families to test for correlations between strength of blocking and expression level for several insect immunity genes with possible roles in blocking. Our findings indicate that *Wolbachia*-mediated blocking has the capacity to evolve and is likely to vary over geographic landscapes. The results also indicate that the application of genome wide association approaches should lead to the successful identification of mosquito genes that underpin blocking.

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### ABBV-4083: A CLINICAL CANDIDATE FOR THE TREATMENT OF ONCHOCERCIASIS - EFFICACY IN THE LITOMOSOIDES SIGMODONTIS RODENT MODEL

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A collaboration between AbbVie, academia and DNDi identified ABBV-4083, a tylosin analog, as a clinical candidate for onchocerciasis. By targeting the *Wolbachia* endosymbionts of filariae and depleting them from filarial nematodes, the worms are sterilized and will eventually die. The prototype anti-wolbachial drug, doxycycline, is macrofilaricidal, but the long treatment regimen (4 weeks for onchocerciasis) and its contraindications have limited its broad use and spurred the search for drugs with the same mode of action, but with better safety and higher potency to achieve shortened treatment regimens. Preclinical efficacy studies with ABBV-4083 were performed using the filarial nematode *Litomosoides sigmodontis*. Effects on *Wolbachia* were analyzed one day post treatment in mice that received orally 75 mg/kg/d ABBV-4083

for 3, 7, 10, and 14 days. Depletion of *Wolbachia* from female adult worms was seen beginning with the 7 day treatment regime, reaching a reduction of 93.6% with the 14 day treatment regime in comparison to vehicle controls. Efficacy of ABBV-4083 was further evaluated in jirds with patent *L. sigmodontis* infection to assess long term efficacy on *Wolbachia* reduction and the impact on microfilaremia. 14 days of 100 mg/kg/d ABBV-4083 treatment reduced the *Wolbachia* by 99.7% in female adult worms and 98.1% in thoracic cavity microfilariae 16 weeks post treatment start. In contrast, the same treatment duration with the human bioequivalent dose of doxycycline was not sufficient to maintain *Wolbachia* depletion over time. Thus, blood microfilariae were not detected in ABBV-4083-treated jirds 10 weeks post treatment, while microfilariae levels had increased from their initial decrease in doxycycline treated controls. These results indicate that two weeks of ABBV-4083 treatment are superior to the bioequivalent dose of doxycycline, leading to a long-term *Wolbachia* reduction (>99%) and loss of peripheral microfilaremia. ABBV-4083 is a clinical candidate for onchocerciasis that leads to permanent sterilization of female adult worms, but lacks a strong microfilaricidal efficacy, thus reducing the risk for adverse events.

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### DETERMINING THE OPTIMAL DOSE OF MOXIDECTIN FOR ONCHOCERCIASIS VIA PHARMACOKINETIC-PHARMACODYNAMIC (PK-PD) MODELLING OF DATA FROM HEALTHY VOLUNTEERS AND PATIENTS WITH ONCHOCERCIASIS

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Moxidectin, a second generation macrocyclic lactone, is an effective veterinary endectocide that is currently in development for onchocerciasis in humans. Moxidectin was well tolerated in 244 healthy adults (doses 3 - 36 mg) and 1,105 onchocerciasis patients in 8 clinical trials. Moxidectin's potential as a global health medicine is substantial: it is broadly active, has low intrinsic toxicity, is orally administered (PO), has extensive tissue distribution (Vd/F ~2000L), is minimally metabolised and has low drug interaction risk. Its terminal phase elimination half-life (T<sub>1/2</sub>) of ~23 days supports infrequent dose regimens. Population (pop) PK-PD analyses were performed to determine optimal dose. The pop PK-PD model was developed with data from 210 moxidectin recipients: 112 healthy volunteers (8 mg or 10 mg), and 98 onchocerciasis patients (2, 4 or 8 mg, n=33, 34 or 31, respectively) using NONMEM (7.3). Covariates evaluated included weight, BMI, gender, *O. volvulus* infection, study phase, food and formulation. Individual *post-hoc* estimates were used to compute the AUC<sub>0-∞</sub> in central and peripheral compartments in onchocerciasis patients. Using logistic or linear regression, AUC<sub>0-∞</sub> in central and peripheral compartments were assessed against achieving complete sustained microfilarial response (SMR, defined as achieving zero skin microfilariae at Month 1 sustained to Months 6 and/or 12) and against the ocular mf burden over 12 months. A 3-compartment pop-PK model with 2 transit compartment input was established with allometric weight, BMI, study phase, food and formulation (liquid vs. tablet) effect, and gender as covariates. Increasing moxidectin plasma AUC<sub>0-∞</sub> was associated with SMR for 6 and/or 12 months and increased probability of lower ocular mf burden over 12 months. Predicted peripheral compartment AUC<sub>0-∞</sub> yielded similar results. SMR at 6 months occurred in 95% of moxidectin 8 mg recipients, with a corresponding mean plasma exposure of 2709 ng/

mL.h. Together these results show that anti-mf efficacy is associated with systemic exposure to moxidectin and that an 8 mg PO dose provides a near-maximal complete response at 6 months.

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### A MULTICENTER STUDY OF THE SAFETY OF TRIPLE DRUG MASS DRUG ADMINISTRATION FOR LYMPHATIC FILARIASIS

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Recent clinical trials have shown that a single dose of ivermectin, diethylcarbamazine (DEC), and albendazole (IDA) is dramatically superior to DEC plus albendazole (DA) or ivermectin plus albendazole for clearing *Wuchereria bancrofti* microfilaremia (Mf). Adverse events were more common but not more severe after IDA. We now report results of large community trials that compared the safety of IDA and DA. The trials were conducted in varied endemic settings including two areas with persistent lymphatic filariasis (LF) despite multiple rounds of mass drug administration (MDA, in Haiti and India), and two areas with no prior MDA (in Papua New Guinea and in Indonesia, whose study site is coendemic for *W. bancrofti* and *Brugia timori*). Treatment assignment was randomized by village/locality of residence, and treatment was offered to all residents who were >5 years of age and not pregnant. Participants were followed actively for 2 days and passively for an additional 5 days. More than 21,000 persons have been enrolled to date. 97% of participants were assessed on days 1 or 2 after treatment for adverse events (AEs). Rates of AEs were the same after IDA (10.5%) and DA (10.6%). Severity of AEs were also similar between both groups; 99.4% and 98.9% of AEs were mild or moderate (grade 1 or 2) in IDA and DA arms. There were 3 all-cause serious AEs, all after DA treatment. The most common AEs reported were headache, dizziness, abdominal pain, fever, nausea, and fatigue. AE rates varied by study site. They were significantly more common in persons with Mf, and also significantly more common after IDA than after DA in persons with Mf (40% vs. 24%). However, there was no excess of severe or serious AEs after IDA. IDA has the potential to significantly accelerate LF elimination in countries without co-endemic onchocerciasis or loiasis. Results from the current study suggest that IDA should be safe to use in MDA programs.

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### COMMUNITY RANDOMIZED SAFETY TRIAL OF TRIPLE-DRUG MASS DRUG ADMINISTRATION FOR LYMPHATIC FILARIASIS IN PAPUA NEW GUINEA

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Small studies in Papua New Guinea (PNG) have shown that a single co-administered dose of Ivermectin (IVM), Diethylcarbamazine (DEC), and Albendazole (ALB; IDA) is far more effective at eliminating *W. bancrofti* microfilaremia (Mf) than treatment with standard therapy of DEC plus ALB (DA) used for mass drug administration (MDA) for lymphatic filariasis (LF). Larger community-based studies were required to confirm that IDA has an acceptable safety profile in varying transmission regions that have not previously received MDA, such as many areas of PNG. In this open-label parallel group cluster randomized trial of 24 villages, all community members who were healthy, >5 years, and not pregnant were offered IDA or DA based on their village of residence. Following treatment, participants were actively followed for 2 days and then passively followed for 5 days to screen for adverse events (AEs). More than 2697 participants have been enrolled to date and 91% of these followed up on either day 1 or 2 after treatment. Overall infection rate was 23% (range 8-46% among villages) based on rapid filarial test strip (FTS) positive. Interim analysis on 2697 participants (IDA=1295, 29% FTS+, 6% Mf+; DA=1402, 20% FTS+, 4% Mf+) reveals 21% of participants experienced mild to moderate AEs, with no severe or serious AEs in either treatment group. AE rates were equivalent between sexes and higher among adults compared to children (15% 5-17yr versus 24% >18yr). Headache, nausea, fatigue, fever and dizziness were the most commonly reported AEs. These were more common in participants receiving IDA (23% [21-26, 95%CI]) compared to those receiving DA (19% [17-21, 95%CI, p=0.049]). AEs were also more common in Mf+ individuals particularly the IDA arm (41%) versus the DA arm (29%). Grade 2 AEs were similar in the two arms; 3% versus 2% in IDA and DA groups respectively. There were no differences in AE rates among FTS+/Mf- versus FTS-/Mf- participants for either group. IDA has the potential to significantly accelerate LF elimination in a country such as PNG and results from this study, as well as from other trial sites, suggests that the safety profile of IDA is suitable for use in MDA programs.

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### IDENTIFICATION OF POTENTIAL CLINICAL CANDIDATES WITH MACROFILARICIDAL EFFICACY FOR THE TREATMENT OF ONCHOCERCIASIS

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Mass drug administrations that are currently used to control onchocerciasis are directed against the microfilarial stage blocking temporarily female worm embryogenesis and thus have to be given once to twice per year for the life span of the adult worms to prevent transmission. Identification of a macrofilaricidal compound would therefore reduce program time frames to control and eliminate onchocerciasis. In a joint collaboration with DNDi, academia and Celgene Global Health, we identified potential clinical candidates with such a macrofilaricidal effect. More than 400 compounds were tested *in vitro* against *Onchocerca gutturosa* adults as well as *Litomosoides sigmodontis*, *Brugia malayi*, and *Brugia pangi* microfilariae and adults, identifying 196 compounds with enhanced activity towards the adult stage parasites. 55 lead compounds with *O. gutturosa* EC50 <0.250µM and suitable pharmacokinetic profiles were further tested by oral gavage in mice harboring adult worms of the rodent filarial nematode

*L. sigmodontis*. Two direct-acting lead compounds were identified and assessed for their efficacy against microfilarial and adult stages in patently *L. sigmodontis* infected jirds (5 day treatment once per day and 10 days bi-daily) revealing complete elimination of adult worms 16 weeks post treatment (wpt) with continuous slow decline of microfilaremia until 16 wpt. Subsequently, three potential clinical candidates have been identified that significantly reduced the *L. sigmodontis* adult worm burden in mice by 96% (7d), 83% (7d) and 82% (5d) after bi-daily treatment within four weeks post treatment. These three clinical candidates are currently being evaluated for their impact on adult worm clearance, microfilaremia and embryogenesis in the *L. sigmodontis* jird model. The current study describes the discovery of three potential clinical candidates with macrofilaricidal efficacy that adhere to the current target product profile. Such macrofilaricidal compounds which lack a strong microfilaricidal effect are ideal candidates for the treatment of onchocerciasis, as they possess a reduced risk for microfilariae-driven adverse events.

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### DEVELOPMENT OF ONCHOCERCA VOLVULUS IN HUMANIZED NSG MICE

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The study of *Onchocerca volvulus* has been limited by its host range, with only non-human primates shown to be susceptible to the infection. Small animal models that support the maturation of the parasite have not been identified. Initially, we screened 6 genetically diverse Collaborative Cross mouse strains for susceptibility to infection with *O. volvulus* infective third-stage larvae (L3). None of the Collaborative Cross mice were susceptible to the infection, suggesting that either the murine immune response controls the infection or that mice lack survival and growth factors essential for the parasite. We hypothesized that highly immunodeficient NSG mice would support the maturation of *O. volvulus* and alteration of the host microenvironment through the addition of various human cells and tissues would further enhance the level of maturation. Specifically, NSG mice were humanized with: (1) umbilical cord derived CD34+ stem cells, (2) fetal derived liver, thymus and CD34+ stem cells or (3) primary human skeletal muscle cells. NSG and humanized NSG mice were infected with 100 *O. volvulus* L3 for 4, 8, or 12 weeks. To confirm infection and determine distribution of *O. volvulus*, mice were dissected into 100 regions and qPCR specific to *O. volvulus* O-150 repeat was performed; parasites were found distributed throughout the animal suggesting that the worms survived and migrated in the mice. Necropsies of infected animals were performed and it was observed parasites survived and developed throughout the infection time course and were recovered from a wide range of tissue locations. In each of the different humanized mouse models it was observed that worms matured from L3 to advanced fourth stage larvae, with both male and female organ development. In addition, worms increased in length by up to 5 fold. These novel mouse models for onchocerciasis will enable the development of *O. volvulus* specific biomarkers, new therapeutic approaches and potentially the study of human immune responses to infection with *O. volvulus*.

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### PRE-CLINICAL USE OF FDA-APPROVED SMALL MOLECULE INHIBITORS AS MACROFILARICIDES IN ONCHOCERCA VOLVULUS: A POST-GENOMIC APPROACH

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Post genomic analyses of the *Onchocerca volvulus*, *Brugia malayi*, and *Loa loa* genomes demonstrated that a number of parasite-encoded tyrosine kinases were found to have human homologues with FDA-approved inhibitors. Having previously shown that the tyrosine kinase inhibitor imatinib is microfilaricidal for *B. malayi* at pharmacologically achievable concentrations and affects embryonic development in adult female worms, we sought to find other protein kinase inhibitors that had macrofilaricidal activity. An *in vitro* screen of 8 PKIs against *Brugia malayi* adult parasites identified 3 that were filaricidal to adult females at <10  $\mu$ M. These drugs, sorafenib (b-Raf inhibitor), lapatinib (EGFR inhibitor), and temsirolimus (mTOR inhibitor) were then tested (at 30  $\mu$ M and 10  $\mu$ M) *in vitro* against adult *O. gutturosa* (Og) and *O. ochengi* (Oo) from cattle and against early Ov adults (L5) developed using a novel *in vitro* culturing system, where mobility and viability can be assessed periodically for 29 days. Against Oo adults, sorafenib and temsirolimus had the most macrofilaricidal activity whereas lapatinib had more active against Og. In the Ov L5 viability assay, whereas lapatinib achieved only 50% inhibition of motility and 50% killing at day 29 at 30 $\mu$ M, temsirolimus was highly active, and at 30 $\mu$ M caused 100% inhibition of motility by day 17, and at 10 $\mu$ M 100% inhibition of motility and viability by day 29. Electron microscopy confirmed extensive damage in the killed L5s. Proteomic data and 3-dimensional protein modeling revealed that stage-specific protein expression and computed degree of homology between the Ov and the human target proteins and were predictive of *in vitro* drug efficacies against Ov. Given these results, PKIs should continue to be investigated to assess *in vivo* efficacy as a macrofilaricide using doses approved and tolerable for humans.

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### ALTERATIONS IN THE IL27 PATHWAY ARE CORRELATED WITH THE LOSS OF TRYPANOSOMA CRUZI-SPECIFIC T CELLS IN PATIENTS WITH CHRONIC CHAGAS DISEASE

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We have previously shown that chronically *T. cruzi*-infected subjects bear several features of a process of immune exhaustion including an inverse correlation between disease severity and the magnitude of T cell responses, and alterations in the signaling pathway of the IL-7R. Like IL-7, IL-27 also exerts its function through STAT5. In this study, we performed an analysis of the expression of the IL-27R components (CD130 and WSX1 chains) on CD4+ and CD8+ T cells, and evaluated IL-27-dependent signaling events in relation to T cell responses in patients at different clinical stage of chronic Chagas disease using polychromatic flow cytometry. Subjects with no signs of cardiac disease showed a decrease in CD130+WSX1+CD4+ T cells and an increase in CD130+WSX1+CD8+ T cells compared with either patients exhibiting heart disease or uninfected controls. *T. cruzi* infection *in vitro*, was able to stimulate the downregulation of the IL-27R on CD4+ T cells and the upregulation on CD8+ T cells. IL-27-induced phosphorylation of STAT1, STAT3 and STAT5 was lower in patients with heart disease compared with asymptomatic patients and inversely associated with the frequency of *T. cruzi*-specific IFN- $\gamma$ -producing T cells measured by ELISPOT assay. STAT5-downstream gene expression of *tbx21*, *eomes*, *gzmb* and *cxcl9* assessed by q-RT-PCR was also reduced in patients with cardiomyopathy. These findings support a possible role of the IL-27R signaling pathway in promoting *T. cruzi*-specific T cells responses.

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### INHIBITORY LIGAND PD-L1 ON MHC CLASS II-EXPRESSING NEUTROPHILS AND MURINE LEISHMANIASIS IN HUMAN

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Neutrophils (PMNs) are typically thought to be short-lived effector cells recruited to sites of acute infection by *Leishmania* spp. where they take up, but do not control, parasites. Additionally while neutrophils are highly recruited during early infection, they are present in areas of chronic parasite infection as well. Another long-standing observation from visceral leishmaniasis patients is that direct activation of low-density peripheral blood mononuclear cells (PBMCs) with *Leishmania* antigen does not result in IFN $\gamma$  production by CD4 T cells unless cultures contain anti-IL-10 antibodies. However, stimulation of whole blood (which has not been depleted of normal-density PMNs via density gradient) from these same patients does result in robust IFN $\gamma$  production. This may suggest that low-density PMNs, enriched in the PBMC layer, but not normal-density PMNs, might have an inhibitory effect on CD4 T cell responses. We have recently identified a unique phenotype of neutrophils in circulation and tissue of human patients with chronic cutaneous and visceral leishmaniasis (VL), specifically PMNs that express the molecule MHC class II, often associated with antigen-presentation. We found that MHC class II-expressing PMNs also show increased costimulatory molecules CD80 and CD86. Additionally, patients showed increased low-density neutrophils, which were significantly more likely to express MHC-II and CD80/CD86. However, *in vitro* experiments showed no evidence of direct antigen presentation to, or activation of, T cells by patient neutrophils. Interestingly, we found that MHC class II-expressing neutrophils, already increased in circulation of human VL patients, also show significant expression of the inhibitory molecule PD-L1. PD-L1 is known to bind to PD1 on T cells. MHC II and CD80/CD86 have also been shown to act as inhibitory, rather than activating ligands (binding T cell LAG3 and CTLA4 respectively). Employing a murine model of leishmaniasis, we found that tissue recruited PMNs show increased MHC class II+ PMNs that express PD-L1. These findings may point to a role for tissue-recruited PMNs in inhibiting CD4 T cell responses.

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### TARGETING INHIBITORY RECEPTORS LAG3 AND TIM3 TO ENHANCE ANTI-PARASITIC CD4 T CELL RESPONSES IN VISCERAL LEISHMANIASIS

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Visceral leishmaniasis (VL) caused by protozoan parasite *Leishmania donovani* remains a major public health problem in tropical countries. CD4+ T cells exhaustion is a common phenomenon during chronic leishmania infection which causes reduced IFN- $\gamma$  secretions, critical for controlling the parasite replication. This can be mediated by abnormal expression of immunosuppressive receptors LAG-3 and TIM-3 on the surface of CD4+ T cells. The present study aims to investigate the role of Lag-3 and Tim-3 in patients with VL. Peripheral blood mononuclear cells (PBMCs) were collected from VL patients before and after drug treatment as well as from endemic healthy control. CD4+ T cells were enriched using magnetic beads. Ex-vivo mRNA expression of TIM-3 and LAG-3 was measured in whole PBMCs as well as enriched CD4+ T cells by Real-Time PCR. Surface expression of TIM-3 and LAG-3 were examined by flow cytometry. To know the functional relevance of LAG-3 and TIM-3, whole blood cells of VL patients were stimulated for 24 hours with soluble leishmania antigen in presence of anti LAG-3 or anti TIM-3 antagonistic antibodies or isotype controls and IFN- $\gamma$  secretion was measured in culture

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supernatant. We observed enhanced mRNA expression of TIM-3 and LAG-3 in whole PBMCs as well as CD4+ T cells of VL patients in pre treatment stage compared to post treatment and endemic controls. Similarly, CD4+ T cells showed enhanced surface expression of TIM-3 and LAG-3 as revealed by flow cytometry analysis. We observed an enhanced IFN- $\gamma$  secretion in whole blood culture after LAG-3 blockade compared to isotype controls but there was no any effect of TIM-3 blockade on IFN- $\gamma$  secretion. These results identify LAG-3 as an important immunotherapeutic target to enhance anti-parasitic CD4+ T cell response and treat VL patients.

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### BACTERIAL CO-INFECTION IN MURINE CUTANEOUS LEISHMANIASIS

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Cutaneous leishmaniasis (CL) is caused by *Leishmania* spp. protozoa leading to localized skin lesions and ulceration. In some human infections, response to treatment is best initiated after ulceration. Ulceration introduces bacteria into skin, and secondary bacterial infections are common in CL. The effects of ulceration-induced immune responses on CL are relatively unexplored. We hypothesized that bacteria present during development of CL activate inflammatory responses that contribute to outcome of parasitic infection. Because *Staphylococcus aureus* (Sa) is the most common bacterium in CL lesions, we used a model of Sa-*Leishmania major* (Lm) co-infection. We injected Lm, Sa, or both intradermally into ears of C57BL/6 mice. We monitored lesion volume, and analyzed inflammation by histology, microbial burden by qPCR, expression of immune mediators by qPCR, and ex vivo antigen stimulation of draining lymph nodes (LNs) by cytokine multiplex at 4 weeks post-infection. Through the course of infection, lesion sizes in co-infected ears were two-fold larger than ears injected with Sa or Lm alone ( $p < 0.0001$ ,  $p < 0.001$  at 3 days, 3 weeks of infection). At 4 weeks there were no significant differences in microbial burdens between co-infected vs. singly infected groups. Histology revealed greater neutrophils in co-infected ears (1.5-fold,  $p < 0.01$ ). TSLP transcripts were upregulated in Lm-infected but not co-infected ears ( $p < 0.01$ ). *Leishmania* antigen-stimulated lymph node cells from co-infected mice released over two-fold more IL-17A than cells from mice infected with Lm or Sa alone ( $p < 0.001$ ). These differences occurred although there was no detectable *S. aureus* DNA. These data suggest that co-infection with Sa leads to an increase in neutrophilic inflammation in Lm lesions, which does not alter parasite burden. These pathologic changes could be related to lower TSLP-induced Treg development and/or a more vigorous TH17 type response.

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### TRANSCRIPTIONAL SIGNATURES ASSOCIATED WITH CD8+ T-CELLS RESPONSES DURING VISCERAL LEISHMANIASIS

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Visceral leishmaniasis is one of the major neglected tropical diseases where the role of CD8 has been poorly understood. Following infection, CD8+ T cell activation generates several effector mechanisms for killing the target cell. In addition to the naïve CD8+ T cells, effector/memory CD8+ T cells and CD8+ Treg cells employ different mechanisms for regulating immune responses. Given these various fates for CD8+ T cells in response to infection, there has been a lot of interest in the factors that regulate these processes. We hypothesize that CD8+ T cell function is compromised in VL patients and recovers following successful drug treatment. This has been tested by nanostrating technology for studying the transcriptional

profile of CD8+ T cells in VL patients, and compared after 30 days of drug treatment, as well as with endemic controls to measure mRNA species involved in T-cell development, activation, function and regulation. The results were analyzed for the differences in the expression levels in terms of adjusted p-values and subsequently IPA (Ingenuity Pathway Analysis) was done to uncover the alterations in the signaling pathways. We have found compromised effector and regulatory functions during the disease as compared to the healthy subjects which was further validated at protein level by flow cytometric analysis and functional studies. Moreover, correlational studies provided new avenue for the immunomodulatory roles of CD8+ T cells during chronic infectious diseases. Therefore, these results provide insight for identifying new strategies to manipulate this T cell subset for therapeutic advantage.

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### IMUNIZATION WITH *LEISHMANIA DONOVANI* DOUBLE KNOCK-OUT PARASITES (*LdCen<sup>-/-</sup>MIF<sup>-/-</sup>*) INDUCES LONG TERM MEMORY AND PROTECTION AGAINST VISCERAL LEISHMANIASIS

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Immunogenic potential of the vaccination against visceral leishmaniasis was extensively shown. However, data showing long term memory induced by different vaccine formulations is still scarce. In the current study, the immunogenicity and protection of a vaccine against leishmaniasis (based on *Leishmania donovani* gene deletion mutant strains deficient for centrin and MIF genes- *LdCen<sup>-/-</sup>MIF<sup>-/-</sup>*) were assessed. BALB/c mice were immunized with *LdCen<sup>-/-</sup>*, *LdMIF<sup>-/-</sup>* or *LdCen<sup>-/-</sup>MIF<sup>-/-</sup>* parasites, and the immune responses were compared to a control group (PBS). Our results showed that *LdCen<sup>-/-</sup>MIF<sup>-/-</sup>* immunized group presented higher percentage of CD4+ and CD8+ central memory T cells, increased CD8+ T cell proliferation after specific stimulation at 12wpc. Also, this group presented higher production of antibodies (IgG), and IFN-γ and TNF-α producing CD4+ T cells after challenge. In addition, *LdCen<sup>-/-</sup>MIF<sup>-/-</sup>* immunized group showed lower number of parasites in spleen and liver at 4 and 12 weeks post challenge with wild type strain of *L. infantum*. These data indicates that parasites manipulated to not express certain genes could improve long term and protection when used as vaccine for visceral leishmaniasis. More importantly, focusing on the mechanisms involved on protection and long term immunity could improve the development of vaccines against VL.

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### RESPIRATORY VIRUS ASSOCIATED WITH WHEEZING IN EARLY LIFE: A BIRTH COHORT STUDY IN A LOW-INCOME URBAN COMMUNITY IN DHAKA, BANGLADESH

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Wheezing in early life is associated with an increased risk of asthma and viral acute respiratory illnesses are primarily responsible for wheezing in young children. We estimated the incidence and impact of respiratory viruses in early childhood wheezing. From May 2015-February 2017, children in a low-income community in Dhaka were enrolled during the first week of life. Field staff visited households twice weekly and referred children with respiratory symptoms to the study clinic. Physicians collected nasal wash samples from children with acute respiratory infections (ARI), defined as cough and/or runny nose; or pneumonia, defined as cough with tachypnea, and identified wheezing on lung auscultation. Samples were tested for respiratory syncytial virus (RSV), humanmetapneumovirus (HMPV), influenza viruses, human parainfluenza viruses (HPIV) and adenoviruses by rRT-PCR. We used Cox's regression, adjusted for clustering at child level, to calculate hazard ratios (HR) to estimate the association between experiencing a viral respiratory illness before six months of age and subsequent episodes of wheezing. We followed 481 children for 527 child-years. Of 2114 respiratory illness episodes, 1824 (86%) were ARI, 290 (14%) were pneumonia. Wheezing was associated with 12% (248/2114) of episodes and of these 149 (60%) were ARI and 99 (40%) were pneumonia. Thirty-three percent (151/481) of children had at least one episode of wheezing; 10% (49/481) had at least two episodes. Respiratory viruses were detected in 50% (123/248) of wheezing episodes. RSV was detected in 19% (48/248) of episodes, HPIV in 15% (38/248), influenza viruses in 6% (15/248), adenovirus in 5% (13/248) and HMPV in 4% (9/248). Viral respiratory illness episodes were more likely than non-viral episodes to present with wheezing (16% vs. 9%, p<0.001). Children with at least one viral respiratory illness episode before six months of age were at increased risk of wheezing after six months of age (HR=2.3, 95% CI 1.2-4.4, p=.01). Young children in Dhaka frequently suffered from respiratory illnesses with wheezing and viral infections may increase the risk of wheezing.

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### SPATIOTEMPORAL DYNAMICS OF COMMON RESPIRATORY VIRUSES CAUSING HOSPITALIZATIONS FOR ACUTE RESPIRATORY INFECTIONS AND PNEUMONIA IN CHILDREN IN NHA TRANG, VIETNAM

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Pneumonia and acute respiratory infections (ARIs) caused by bacteria and viruses and pneumonia is the most common cause of death in children under 5 years of age. While most etiologies of pneumonia and ARIs are unknown, multiple pneumonia and ARI-causing viruses circulate widely in South East Asia. However, the patterns and drivers of the seasonal and spatial transmission dynamics are unknown. Using hospital-based surveillance in children, we identify the seasonal patterns of multiple circulating viruses causing hospitalizations for ARIs and pneumonia in Nha Trang, Vietnam and examine clustering of hospitalizations over the study period. We find strong seasonal transmission of respiratory syncytial virus (RSV), peaking in the late summer months, and of WHO-confirmed pneumonia, peaking in the winter months. We examine the relationship of hospitalizations with rainfall, temperature, and dew point, and find significant associations between RSV and the previous mean 7 days rainfall and temperature (odd ratios: 1.98 [95% CI: 1.31, 2.99, p = 0.0013] and 1.23 [95% CI: 1.08, 1.4, p = 0.0015], respectively), and a significant out-of-phase relationship between human parainfluenza 3 and temperature and dew point. We find significant clustering of hospitalizations at local spatial scales (between 1 and 2 km). Influenza A, human metapneumo

virus, and human parainfluenza virus 3 exhibit the strongest clustering up to about 2 km. Our results are important for understanding the spatiotemporal risk associated with circulating pathogens in Southern Vietnam.

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### VIRAL DETECTION IN SEVERELY MALNOURISHED UNDER-FIVE CHILDREN WITH PNEUMONIA AND ASSOCIATED OUTCOME IN AN URBAN HOSPITAL, BANGLADESH

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In Bangladesh, pneumonia has a higher mortality among malnourished children aged <5 years. Evaluating the etiology of pneumonia among these children might help providers develop empiric treatment guidelines. During April 2015 to April 2016, we enrolled children aged < 5 years admitted with severe malnutrition and clinical or radiological pneumonia in icddr,b's Dhaka hospital and followed them for 30 days from the day of admission. We collected nasopharyngeal wash samples and tested for respiratory syncytial virus (RSV), human metapneumovirus (HMPV), influenza viruses, human parainfluenza viruses (HPIV 1, 2, 3) and adenoviruses by real-time RT-PCR and tested for bacterial pathogen by blood culture. We enrolled 234 children. Their median age was 8 months (IQR: 5-13) and 149 (64%) were male. Their weight/age Z-score was -4.3 (SD 1.1), weight/length -2.9 (SD 1.4) and 33 (14%) had bipedal edema. Respiratory viruses were detected in 110 (47%) and bacteria in 10 (4%). The most frequently detected viruses were RSV (32; 29%) followed by adenoviruses (23; 21%), HPIV3 (17; 15%), influenza A virus (14; 13%), HMPV (10; 9%), HPIV2 (1; 1%) and co-detections (13; 12%). Six (5%) children had mixed infections (3 HPIV3 with *Staphylococcus aureus*, *Enterococcus*, *Pseudomonas*; 2 adenovirus with *Salmonella Typhi* and *Pseudomonas*; HMPV with *Klebsiella*). Recurrence of pneumonia occurred in 16/234 (7%) children of which 13 (81%) had viral pathogen and 4 (25%) bacterial pathogen (2 *Streptococcus pneumoniae*, *Pseudomonas*, coagulase-negative *Staphylococci*) and 3 mixed infection (19%). Of all the children with detectable viruses, at time of discharge 84/110 (76%) had improved, 6 (5%) died (2 HPIV3, 1 HPIV3+adenovirus, 1 HPIV3+RSV, 1 HMPV, and 1 adenovirus), 18 (16%) were recovering and 2 (2%) were lost to follow-up. Nine of 16 (56%) deceased children had no detectable pathogen while 3 (19%) had bacterial infection and 2 (13%) mixed infection; all died within 10 days of symptom onset. All the children were treated with antibiotics without any antiviral. In severely malnourished children with pneumonia in Dhaka hospital, viral pathogens were common with RSV predominance.

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### PNEUMONIA ETIOLOGY INVESTIGATING LUNG ASPIRATE SAMPLES USING MULTI-PLEX PCR

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The etiology of pneumonia is difficult to determine but its pursuit is essential given pneumonia is the most common cause of child mortality. We conducted population-based surveillance for pneumonia, including the collection of lung aspirate samples, among those aged ≥2 months in a demographic surveillance area in rural Gambia between 20 March 2011 and 31 August 2012. 7-valent pneumococcal conjugate vaccine (PCV7) was introduced in September 2009 and replaced by PCV13 in May 2011. Standard samples were collected and microbiologic investigations

performed to guide treatment. A 33-plex real-time PCR assay was used to detect bacterial and viral pathogens. A standardized diagnosis of clinical pneumonia was made in 3262 patients, of whom 2291 were aged 2-23 months, 729 were aged 2-4 years, 242 were aged 5 years and greater and 55 died. Chest radiographs were taken for 3069 patients. Of 379 (12%) patients with, and 2690 (88%) without WHO-defined pneumonia with consolidation, 11 (2.9%) and 36 (1.3%) died respectively. Lung aspirates were collected and analysed from 165 patients. Pathogens were detected in 126 (76%) samples, one pathogen in 48 (29%) and two or more in 78 (47%). Bacteria only co-infections were detected in 55 (33%) samples and bacterial-viral co-infections in 18 (11%). Common pathogens were *S. pneumoniae* (n=59, 36%), *S. aureus* (n=23, 14%), *H. influenzae* non-type b (n=13, 8%), Bocavirus (n=14, 8%), Influenza virus (n=12, 7%), *H. influenzae* type b (n=10, 6%), Parainfluenza virus (n=9, 5%) and Respiratory syncytial virus (n=5, 3%). The proportion of children aged less than 5 years in whom *S. pneumoniae* was isolated was significantly less among those who had received ≥2 doses of PCV (17/69) compared to zero doses (16/30); risk ratio 0.57 (95% CI 0.35, 0.93). Soon after the introduction of PCV in The Gambia the predominant etiologic pathogens causing pneumonia with substantial consolidation were *S. pneumoniae*, *S. aureus* and *H. influenzae*. PCV effectively prevented pneumococcal pneumonia. Following the widespread use of PCV in high-mortality settings, robust studies of pneumonia etiology are needed, that include detection of co-infections.

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### QUANTIFYING THE BURDEN OF LOWER RESPIRATORY INFECTIONS: RESULTS FROM THE GLOBAL BURDEN OF DISEASE STUDY 2016

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The global burden of lower respiratory infections (LRIs) remains substantial despite tremendous decline over the last several decades. The Global Burden of Diseases, Injuries, and Risk Factors Study 2016 (GBD 2016) is a systematic, scientific effort to measure the burden LRIs. GBD 2016 estimates the morbidity and mortality due to LRIs and four etiologies for all countries, age groups, and both sexes from 1990-2016. Mortality is modeled in a Bayesian ensemble platform that uses verbal autopsy and vital registration data and country-level covariates. Morbidity is modeled using a meta-regression tool that employs surveys, hospital records, and scientific literature to produce estimates. Deaths, years of life lost (YLLs), years lived with disability (YLDs), and disability-adjusted life years (DALYs) are used to measure the burden of LRIs. LRIs were the second leading cause of death among children under 5 years old in 2016, responsible for 720,000 deaths (95% Uncertainty Interval: 651,600-801,700) in this age group, and the fifth leading cause among all ages, responsible for 2,718,000 deaths (95% UI: 2,478,000-2,869,000). About 75% of LRI deaths occurred in children under-5 and adults over-70. Despite growing populations, the number of LRI deaths decreased by 17% between 1990 and 2016. This reduction is due primarily to reductions in under-5 mortality (65% reduction). LRI mortality in adults 70+ years old increased 94% during this time period. The under-5 LRI mortality rate varied by region and was highest in the Sahel of sub-Saharan Africa. Nearly 50% of under-5 LRI deaths occurred in just five countries: India, Nigeria, Pakistan, DR Congo, and Ethiopia. While under-5 LRI mortality was concentrated in low-income countries, LRI mortality in adults 70+ years was highest in India, Japan, China, the United States, and Brazil and these five locations were responsible for nearly 50% of the deaths in this age group. Despite dramatic declines in under-5 LRI mortality, interventions that focus on preventing and treating LRI remain necessary to accelerate reductions in LRI mortality including those targeting both children and elderly adults.

### HANDHELD POINT-OF-CARE LACTATE MEASUREMENT PREDICTS MORTALITY IN UGANDAN CHILDREN HOSPITALIZED WITH PNEUMONIA

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Globally, pneumonia is the leading cause of death of children <5. Rapid bedside tools to triage and risk-stratify pneumonia patients may improve patient care and outcomes. We conducted a prospective cohort study of children <5 admitted for pneumonia in Uganda. Lactate was measured at admission and daily during hospitalization using a handheld point-of-care device, the Lactate Scout Analyzer. Clinical data and outcome (survival or death) were recorded and clinical risk scores were calculated. Statistical analysis employed receiver operator characteristic (ROC) curves, Kaplan-Meier curves, and linear mixed-effects models. From Sept 2013 to July 2015, we enrolled 155 children with pneumonia admitted to the Jinja Regional Referral Hospital and Kambuga District Hospital. In-hospital mortality was 22/155 (14%). Median [range] lactate level was 2.4 [0.9-25] mmol/L among children who survived versus 5.3 [1.6-20] mmol/L among children who died ( $p=0.0001$ ). Lactate was an informative prognostic marker of mortality, with area under the ROC curve (AUROC) greater than or equal to that of any single clinical sign or composite clinical risk score (AUROC 0.76, [95%CI 0.65-0.87],  $p=0.0001$ ). Lactate augmented the ability of the leading clinical risk score, Pediatric Early Death Index for Africa (PEDIA), to predict mortality (AUROC 0.82 [95%CI 0.69-0.94],  $p=0.001$ ). Lactate level at admission accurately risk-stratified children, with 5-day mortality of 2%, 11% and 26%, among children with lactate of <2.0, 2.0-4.0, and >4.0 mmol/L, respectively ( $p=0.001$ ). Lactate levels decreased over time in patients who recovered (estimated rate 1.1 [95%CI 0.62-1.5] mmol/L per day,  $p<0.0001$ ), and were higher overall in patients who died (estimated difference 2.2 [95%CI 0.98-3.4] mmol/L,  $p=0.0005$ ). Handheld point-of-care lactate measurement is a superior predictor of mortality than any clinical sign or risk score among hospitalized pediatric pneumonia patients. This may be a valuable, convenient tool for triage and risk stratification in resource-limited hospitals for pneumonia, the leading killer of children <5 worldwide.

### CHEST ULTRASOUND VERSUS X-RAY FOR PULMONARY TUBERCULOSIS IN SOUTH AFRICAN CHILDREN

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Globally, around 1 million children develop tuberculosis (TB) annually. Chest X-ray (CXR) is a main diagnostic tool for diagnosis of pulmonary TB (PTB) but has poor inter-reader agreement. Chest ultrasound (US) is free of ionizing radiation and increasingly used by clinicians for pediatric lung diseases. We compared clinician-performed chest US with CXR in children with suspected PTB. Children <13 years with suspected PTB, presenting to a children's hospital in Cape Town between 2014-2015 were enrolled. A clinician, who had 4 days of US training, performed chest US using a grey-scale machine. The anterior, lateral and posterior chest was scanned using a linear transducer (5-10 MHz) and the mediastinum was scanned using a micro-convex array transducer (7.5 MHz). Two clinicians evaluated the US clips independently for consolidation, effusion or mediastinal lymphadenopathy; two independent radiologists reviewed the CXRs. Of

169 enrolled children (median age 27 months), consolidation occurred as commonly on US as on CXR (72.5% versus 73.1%,  $p < 0.01$ ), with moderate agreement between US and CXR ( $\kappa = 0.40$ ). Pleural effusion was more common on US (20.6% versus 14.3% on CXR,  $p < 0.01$ ) with substantial agreement between US and CXR,  $\kappa = 0.67$ . There was poor agreement for lymphadenopathy on CXR versus US ( $\kappa = 0.08$ ); lymphadenopathy was more common on CXR (68.0%) than US (51.7%,  $p = 0.58$ ). US had a higher inter-reader agreement for consolidation ( $\kappa = 0.89$ ) or effusion ( $\kappa = 0.85$ ) than CXR (respectively  $\kappa = 0.45$  and  $\kappa = 0.40$ ), while CXR had a higher inter-reader agreement for lymphadenopathy ( $\kappa = 0.38$  versus  $\kappa = 0.07$ ). In a subgroup of 39 microbiologically confirmed PTB cases, consolidation or effusion was more common on US (consolidation 86.5% versus 73.0%,  $p < 0.01$ ,  $\kappa = 0.43$ ; effusion 25.6% versus 15.4%,  $p = 0.04$ ,  $\kappa = 0.38$ ). Lymphadenopathy was more common on CXR (45.5% versus 36.4% on US,  $p = 0.69$ ,  $\kappa = 0.07$ ). This study shows that chest US is potentially a better imaging modality to identify consolidation or effusion in children with PTB with a higher inter-reader agreement than CXR. However, these findings are not exclusive for PTB; comparing PTB and pneumonia is needed.

### DENGUE VIRUS IGG ANTIBODIES AND ITS ASSOCIATION WITH CLIMATE VARIABLES. A COUNTRY-BASED STUDY (MÉXICO)

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The rapid expansion of dengue in the world is a major concern to health authorities. Forecasting the development from endemic to hyperendemic areas is a major goal to evaluate the burden of the disease and to considerate the application of public health interventions such as vaccination. Particularly, endemic regions are characterized by a continuous exposure of the population to Dengue virus, which in theory result in high levels of antibodies against the virus. However seroepidemiological studies are expensive and limited to communities well known as hyperendemic locations. It seems that environmental conditions optimal for vector breeding could be associated with a continuous exposure to the virus and as a consequence with high levels of anti DENV antibodies. Based on the results of a cross-sectional study conducted in Mexico to assess DENV antibodies seroprevalence, we analyzed the relationship between specific dengue seroprevalence at community level and environmental variables, as altitude, average annual temperature and average annual precipitation in each place where blood samples were taken. As the Primary Sampling Units were schools, we used as data source for environmental data the National System of School Information, of the Ministry of Education in Mexico. Through logistic models, the association between seroprevalence and environmental variables was assessed and confidence intervals were obtained. These analyses were done using software Stata V.14 and the complex design was taken into account in all the estimations. We found that average annual temperature is positively and significantly associated with seroprevalence (OR 2.66; CI95%: 1.68-4.24) while altitude is also significantly associated but in a negative direction (OR 0.91; CI95%: 0.86-0.96). No statistical association was identified in relation to annual precipitation (OR 1.0; CI95%: 0.99-1.01). As the study was cross-sectional, more information is needed to calibrate a model that helps to forecast dengue prevalence in areas where transmission is increasing and, also, to weigh up the effect of the global warming in dengue transmission.

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**TRENDS IN DENGUE AMONG UNITED STATES TRAVELERS, 2010-2016**

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Dengue is a tropical emerging arboviral disease that can result in life-threatening disease. Importation of the four dengue viruses (DENV-1-4) into the U.S. via travelers returning from the tropics has resulted in recent dengue outbreaks in Texas, Florida, and Hawaii. In 2010, dengue became a nationally reportable condition in the U.S. A descriptive analysis was performed using dengue cases reported from state and local health departments to ArboNET, a surveillance system used to monitor trends in arboviral diseases in the U.S. Laboratory-positive dengue cases were defined as clinically suspected cases with either (i) DENV nucleic acid detected by RT-PCR in a clinical specimen; or (ii) detection of anti-DENV IgM in serum. A total of 4,478 travel-associated, laboratory-positive dengue cases were reported to ArboNET during 2010-2016. DENV infection was defined by detection of nucleic acid identified in serum by RT-PCR in 28% of cases, and by detection of IgM antibody in 72% cases. Among RT-PCR-positive cases, the most common DENVs identified were DENV-1 (n= 106, 47%), DENV-2 (n=59, 26%), DENV-3 (n=37, 16%) and DENV-4 (n=25, 11%). The greatest number of dengue cases were reported in 2016 (n=835, 19%), followed by 2013 (n=793, 18%) and 2015 (n=746, 17%). Jurisdictions with the most reported cases were California (n = 681, 15%), Florida (n = 654, 15%), and New York City (n = 623, 14%). Median age of dengue case-patients was 41 years (range: 1-89 years), 90% were adults, and 50% were male. Among cases with a known destination of travel, the most common were Dominican Republic (n=438; 10%), India (n=437, 10%), and Mexico (n=411, 9.5%). The reported rate of hospitalization was 28% and 17 (0.4%) of all cases were fatal. ArboNET reporting of dengue cases among travelers has trended upward in recent years, reflecting the increase in incidence worldwide and an ongoing risk of DENV importation potentially leading to local transmission and outbreaks where competent *Aedes* vectors are present. Continued efforts to improve reporting will allow rapid detection of cases and response as well as messaging to at-risk travelers.

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**ASSOCIATED FACTORS TO DENGUE INFECTION IN SUBJECTS FOLLOWED UP DURING 2.5 YEARS FROM AN ENDEMIC MEXICAN AREA**Ruth A. Martínez-Vega<sup>1</sup>, Irma Yvonne Amaya-Larios<sup>2</sup>, Fredi A. Díaz-Quijano<sup>3</sup>, José Ramos-Castañeda<sup>2</sup>*<sup>1</sup>Universidad de Santander, Bucaramanga, Colombia, <sup>2</sup>Instituto Nacional de Salud Pública, Cuernavaca, Mexico, <sup>3</sup>OLFIS, Colombia, Colombia*

The immunity and the mobility of subjects could be determinant factors for Dengue virus (DENV) infection. To determine associated factors to DENV infection in subjects followed up during 2.5 years from two Mexican endemic localities. Between August and November of 2014 we visited subjects from a cohort of older than 5 years old enrolled between June and November 2011, and they were followed until November of 2016. We interviewed and took blood sample every 6 months from subjects who accepted to participate. ELISA capture IgM and IgG and ELISA indirect IgG (Panbio) were performed to evaluate the incidence of DENV infection during this period. Binomial regression was performed to evaluate associated factors to DENV infection. An adjustment of standard errors was made considering the 75 sample clusters (houses in an area of 50 mt radio). 862 (72%) of 1197 subjects from original cohort and 104 subjects were new inclusions. 400 were selected for serology, of which 12 (9.6%) were primary infections, 17 (18.9%) were secondary infections and 17 (9.2%) were post-secondary infections. The associated factors were to live in Axochiapan compared with Tepalcingo (Risk Ratio [RR] 0.52; IC95% 0.29-0.93; p=0.028), previous immune status as secondary

infection compared with primary infection (RR 2.09; IC95% 1.01-4.32; p=0.046) and the different place to home where they spend the most time as staying in the house of relative compared with school or workplace (RR 3.89 IC95% 1.05-14.32, p=0.014), other places as church, markets were not associated. Other variables like age, gender and time spend at home were not associated. Dengue transmission in endemic settings seems to depend on specific life styles as suggested by the increase in risk to be infected in seropositive subjects and the mobility of subjects.

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**DENGUE IN PREGNANT WOMEN: CHARACTERIZATION OF CASES IN BRAZIL, 2007-2015**Laura B. do Nascimento<sup>1</sup>, Cláudio M. Siqueira<sup>1</sup>, Giovanini E. Coelho<sup>2</sup>, João B. Siqueira, Jr.<sup>1</sup>*<sup>1</sup>Federal University of Goiás, Goiânia, Brazil, <sup>2</sup>Ministry of Health- Brazil, Brasília, Brazil*

This study was undertaken to characterize the probable cases of dengue in pregnant women reported in Brazil, from 2007 to 2015. A descriptive study was carried out of sociodemographic, epidemiological, clinical and laboratory characteristics, with data from the Sinan Information System. The annual incidence of dengue in pregnant women ranged from 3.3 (2009) to 816.6 (2010) cases per 100,000 live births; of the 43,772 probable cases of dengue in pregnant women residents of 3,218 municipalities in the five major regions of the country, 81.6% were investigated, 34.1% were laboratory confirmed, and 1.7% were severe; rates of hospitalization and lethality were 5.4% and 1.6%, respectively; the risk of death due to dengue was higher in the pregnancy than the population of non-pregnant women of childbearing age (ratio=3.95 [95%CI=3.07;5.08]), higher in the third trimester of pregnancy (ratio=8.55 [95%CI=6.08;12.02]). The results show the burden of dengue in pregnant women and their vulnerability to worsening of the disease and death.

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**EVALUATION OF DENGUE ANTIBODIES IN SERONEGATIVE SUBJECTS FROM A PHASE III EFFICACY TRIAL DEMONSTRATES A POSITIVE CORRELATION IN GENERATION OF SEROTYPE-SPECIFIC AB WITH INCREASING AGE FOLLOWING VACCINATION**Anthony Byers<sup>1</sup>, Alina Munteanu<sup>1</sup>, Robert Small<sup>1</sup>, Lilibeth Lanza<sup>1</sup>, Del Leistritz-Edwards<sup>1</sup>, Michael Peredelchuk<sup>1</sup>, Matthew Bonaparte<sup>2</sup>, Aravinda de Silva<sup>3</sup>, Bruno Guy<sup>4</sup>, Janice Moser<sup>1</sup>*<sup>1</sup>Sanofi Pasteur, Orlando, FL, United States, <sup>2</sup>Sanofi Pasteur, Swiftwater, PA, United States, <sup>3</sup>University of North Carolina, Chapel Hill, NC, United States, <sup>4</sup>Sanofi Pasteur, Marcy l'Etoile, France*

Sanofi Pasteur's tetravalent dengue vaccine (CYD-TDV) has shown efficacy against symptomatic dengue in endemic areas of Asia-Pacific and Latin America with subjects between the ages of 2-14 and 9-16 years (y), respectively. The vaccine showed efficacy of 60.3% (95% confidence interval [CI], 55.7;64.5) for all participants, but demonstrated differing efficacy based on age: 65.6% (95% CI, 60.7;69.9) for those aged 9y or older and 44.6% (95% CI, 31.6;55.0) for those younger than age 9 years. Based on this and other analyses, countries throughout Asia-Pacific and Latin America have approved the CYD-TDV vaccine for use in individuals 9-45y. To assess the impact of subject age independent of their dengue serostatus, we evaluated the specificity and affinity of the serum antibody (Ab) response in a subset of CYD-TDV vaccinated subjects from the Asia-Pacific phase III trial (NCT01373281) over 4 age ranges (2-5, 6-8, 9-11, 12-14y), who were confirmed seronegative at the onset of the trial by Vero PRNT50. Ab specificity was assessed via a combined dengue virus-depletion flow cytometry-based U937 DC-SIGN neutralization assay that qualitatively determined whether serum neutralizing Abs (nAbs) to each of the 4 dengue serotypes from these subjects were homotypic and/or heterotypic (cross-reactive). In agreement with previous studies, CYD-

TDV vaccination primed dominant homotypic nAb responses to serotype 4 while the nAb responses to the other 3 serotypes were mostly cross-reactive. To get sufficient data to compare between age groups, we then focused on anti-serotype 4 responses, and interestingly, we observed a significant positive correlation in the level of dengue serotype 4-specific homotypic nAb with the increase in the age of the subjects. Alternatively, evaluation of serum Ab affinity via a biolayer interferometry-based assay did not find any statistical differences in Ab affinity for any of the 4 serotypes across the age ranges tested. These data display a positive effect of increased age on the generation of CYD-TDV-induced serotype-specific homotypic nAb responses, not biased by pre-existing dengue antibodies at the time of vaccination.

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### EVALUATION OF THE EFFECT OF PRE-EXISTING IMMUNITY AGAINST DENGUE ON NEUTRALIZING ANTIBODY RESPONSE INDUCED BY A LIVE ATTENUATED TETRAVALENT DENGUE VACCINE CANDIDATE, KD-382, IN CYNOMOLGUS MONKEYS

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One of the challenges in dengue vaccine development is inducing neutralizing antibody responses against all four serotypes simultaneously and ensuring that the induced neutralizing antibodies last for a long time. Our tetravalent dengue vaccine candidate currently under development, KD-382, is a live attenuated vaccine using a classical host range mutation strategy and thus is expected to induce a strong neutralizing antibody response similar to the antibody response induced by natural infection. To date, we have shown that dengue-naïve cynomolgus monkeys administered a single dose of KD-382 seroconverted for all four serotypes (Tetra-NAb response). However, when considering vaccine use in dengue endemic regions, it is thought that some vaccinees will be immune to dengue as a result of previous natural dengue infections. Therefore, it is important to evaluate the effect of pre-existing dengue immunity on neutralizing antibody responses induced by KD-382. We administered KD-382 (5, 5, 5, 5Log<sub>10</sub> PFU/dose) to 15 cynomolgus monkeys pre-administered with one of the monovalent parental wild-type strains (DENV3: 6Log<sub>10</sub> PFU/dose, DENV1, 2, 4: 5Log<sub>10</sub> PFU/dose) or vehicle 60 days before KD-382 vaccination (3 monkeys per group). Neutralizing antibody titers against each of the four parental wild-type strains were measured by a focus reduction assay 30 days after KD-382 administration. All 3 monkeys given the vehicle followed by KD-382 (not pre-immune/naïve) seroconverted (FRNT50=1:>10) against all four serotypes (Tetra-NAb response). Also, all 12 monkeys given one of the monovalent parental wild-type strains (pre-immune) followed by KD-382, regardless of the pre-immunized serotypes, showed a Tetra-NAb response. KD-382 successfully induced Tetra-NAb responses under both naïve and pre-immune conditions, suggesting that KD-382 could be a useful vaccine for both naïve vaccinees and vaccinees that are seropositive due to previous natural dengue infection at base-line.

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### SINGLE ADMINISTRATION OF LIVE-ATTENUATED TETRAVALENT DENGUE VACCINE CANDIDATE, KD-382, INDUCED LONG-LASTING (>2 YEARS) NEUTRALIZING ANTIBODY AGAINST ALL FOUR SEROTYPES IN CYNOMOLGUS MONKEYS

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One of the challenges in dengue vaccine development is inducing neutralizing antibody responses against all four serotypes simultaneously and ensuring that the induced neutralizing antibodies last for a long time. Our tetravalent dengue vaccine candidate currently under development, KD-382, is a live attenuated vaccine using a classical host range mutation strategy and thus is expected to induce a strong neutralizing antibody response similar to the antibody response induced by natural infection. To date, we have shown that dengue-naïve cynomolgus monkeys administered a single dose of KD-382 seroconverted for all four serotypes (Tetra-NAb response). However, it is important to show how long neutralizing antibodies induced by KD-382 can be expected to last. We administered a single dose of KD-382 (5, 5, 5, 5Log<sub>10</sub> PFU/dose) to 6 cynomolgus monkeys (male and female), and are measuring the neutralizing antibody titer against parent strains for each serotype over time. This study has currently been running for over two years, and we have confirmed that neutralizing antibody titers against all four serotypes (FRNT50=1:>10) persist for at least 25 months. The geometric mean neutralizing antibody titers at 25 months for serotype-1, serotype-2, serotype-3 and serotype-4 were 3.2, 2.6, 2.3 and 2.9 (Log<sub>10</sub> FRNT50), respectively. The persistence of neutralizing antibodies for over two years suggests that, in cynomolgus monkeys, KD-382 can induce a strong neutralizing antibody response against all four dengue serotypes simultaneously with only a single administration, and thus has high potential as a dengue vaccine. We will continue our observations, but as no decrease in neutralizing antibody titer has been observed so far, we expect that neutralizing antibodies will last for even longer.

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### EFFECT OF EXPOSURE HISTORY ON DENGUE INFECTION AND DISEASE: A STATISTICAL APPROACH AND ITS APPLICATION TO THE DENGUE COHORT IN NICARAGUA

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A cohort of children has been established in Nicaragua since 2004 to study the epidemiology, virology and immunology of the dengue virus, an mosquito-borne arbovirus with four co-circulating serotypes. Each subject was bled annually and followed up for dengue-fever-like symptoms. Serum samples were tested by ELISA, and a subset of ELISA-positive samples were serotyped with other lab-methods. We proposed a novel statistical competing-risk framework to assess the infection risks of all four serotypes during the study years and the effects of exposure history on both infection and probability of disease given infection. This framework couples the cohort data with surveillance data to inform the model about the complete exposure history at the individual level both before and during the study years. The model is fitted with a data-augmented MCMC sampling algorithm. An efficient sampling approach for individual exposure history was also proposed. We demonstrated by extensive simulation studies that the proposed algorithm can provide unbiased estimation of key model parameters. By applying our method

to the Nicaragua pediatric cohort, we found that there were three outbreaks triggered by three different serotypes during the study years. The probability of disease given infection was much lower for DENV-4 than for other three serotypes. We found that the time since most recent infection (as a proxy of decay of immunity) showed different effects on the pathogenicity for younger and older children, suggesting age may play an important role on the immune response after dengue infection, which has important implications on dengue vaccine policy.

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### GEOSPATIAL ANALYSIS OF DENGUE EMERGENCE IN RURAL AREAS IN THE SOUTHERN PROVINCE OF SRI LANKA: 2012-2013

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Dengue is a major cause of acute febrile illness in Sri Lanka, historically considered an urban disease. In 2012-2013, we documented acute dengue surprisingly associated with self-reported rural residence in the Southern Province. In this analysis, we used geographic information systems (GIS) and spatial statistical analysis to assess this association among patients hospitalized with febrile illness in the Southern Province. Febrile patients were enrolled from June 2012-May 2013 in a cross-sectional surveillance study at the largest tertiary care hospital in the Southern Province. Acute dengue was diagnosed in a subset of febrile patients by paired serology and virologic testing. Patients' addresses were used to determine their grama niladhari (GN) divisions, the lowest administrative division in Sri Lanka. Each patient was geocoded to the geographic center of the GN division and placed randomly within a 10 km radius of that center. Geographic distribution of acute dengue infection was modeled using a spatial generalized additive model (GAM) predicted onto a grid of coordinate pairs covering the Southern Province to map spatial variation in odds of dengue infection. Permutation tests were applied to identify areas where local odds of dengue infection significantly differed from the average odds of dengue. Of 760 patients, 312 (41%) had laboratory-confirmed acute dengue. Dengue was highly spatially heterogeneous (local odds ratio (OR) 0.29-4.97 of acute dengue infection compared to the cohort average). There was a higher than average odds ( $p < 0.05$ ) in the rural northeast region of the Southern Province and a lower than average odds in the more urbanized southwest region of the Southern Province, including cities such as Galle and Matara. Our study further affirms the emergence of dengue in rural southern Sri Lanka. Increased travel, changes in land use or demographics, and favorable living conditions for mosquito vectors may account for these changes. Our study highlights the need for real-time, geospatial analyses to identify unrecognized clusters of dengue infection and to optimize public health surveillance and control activities.

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### SPREAD OF DENGUE 1 AND 2 IN MACHALA, ECUADOR: EVIDENCE OF A DYNAMIC EPIDEMIC GENETICALLY RELATED TO THOSE OF SURROUNDING COUNTRIES OF COLOMBIA, VENEZUELA AND PERU

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Dengue is one of the major public health concerns in Ecuador, where it is endemic and provides the greatest burden of all mosquito-borne febrile illnesses. A surveillance platform has been established in the city of Machala to identify and characterize disease caused by arboviruses. Here, samples are collected from febrile subjects at four sentinel clinics and the central hospital of the Ministry of Health. A total of 14 DENV1 and 35 DENV2 sequences were successfully obtained from these samples collected in 2014 (previously published) and 2015. The sequences were aligned together with other reference dengue sequences from the surrounding countries and phylogenetic trees of full genome and E gene were inferred. For DENV1, the trees indicated existence of two different lineages in 2014, suggesting at least two different introductions of this serotype into the city of Machala, one lineage most closely related with sequences from Colombia and the other one to sequences from Venezuela. In 2015, no samples belonging to the Venezuela lineage were collected, suggesting possible extinction of this lineage in Machala. For DENV2 all sequences belonged to the same lineage, indicating that the majority of this serotype in the city originates from the same introduction and that this lineage continues to persist in the city. DENV2 lineage, like DENV1, was closely related to sequences from Colombia and Venezuela, but also to sequences from Peru. Our results suggest frequent flow of DENV between Ecuador and surrounding countries and show an epidemic in the city of Machala that is dynamic, with introduction and persistence of multiple lineages, as well as lineage extinction. In addition, our results show the importance of continuous surveillance, where genetic information can be used to track epidemic dynamics and patterns of dengue movement in this region.

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### THE GLOBAL CD4 T CELL RESPONSE AGAINST DENGUE VIRUS

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Dengue disease is a large public health problem that mainly afflicts tropical and subtropical regions. Understanding of the correlates of protection against dengue virus (DENV) is poor and hinders the development of a successful human vaccine. Here we map HLA DRB1 restricted DENV-specific epitopes in individuals previously exposed to dengue virus in the general population of Nicaragua and compare it to the breadth and specificity of CD4+ T cell responses previously investigated in Sri Lanka. The 22 DRB1 alleles analyzed to date cover 81.7% of alleles with phenotype frequency  $\geq 5\%$  worldwide and account these alleles combined afford 92% phenotypic coverage worldwide (92% of individuals express at least one of the alleles). The capsid protein followed by nonstructural NS3, NS2A, and NS5 proteins were the most targeted proteins. We further noticed a wide variation in magnitude of T cell responses as a function of the restricting DRB1 allele. Comparing these patterns to those in the general population of Sri Lanka, we found a general correlation between restricting HLA allele and magnitude of CD4+ T cell responses. Based on these results we devise a pool of epitopes that affords improved coverage worldwide at the level of CD4 T cells response, broadly covering different HLA DRB1\* alleles and different DENV serotypes. These results are of relevance for both vaccine design and the identification of robust correlates of protection in natural immunity.

## NEW BIOMARKERS OF LIVER INVOLVEMENT BY DENGUE INFECTION IN ADULT VIETNAMESE PATIENTS

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Liver injury with marked elevation of aspartate amino transferase enzyme (AST) is common in dengue infection. New plasma biomarkers are reported in drug-induced liver injury mainly in the centrilobular area such as Glutamate Dehydrogenase (GLDH), Hydroxyphenylpyruvate dioxygenase (HPPD), and Glutathione S Transferase- $\alpha$  (GST). Arginase-1 (ARG-1) indicates liver injury mainly in the portal area. Paraoxonase-1 (PON-1), 4- and Gamma-glutamyl transferase (GGT) are other measurements to detect liver injuries. The cross sectional study was conducted in adult dengue patients from October 2015 to December 2016 at the Hospital for Tropical Diseases of Ho Chi Minh City, Vietnam. A total of 87 dengue patients were recruited into three equal groups according to plasma AST levels (lower 80, 80-400, higher 400 IU/ml). The new liver enzymes in the blood samples were measured from 4th to 6th day of their illness by commercial ELISA kits. Thirty nine patients (44.8%) had dengue without warning signs (D), 37 (42.5%) had dengue with warning signs (DWS), and 11 (12.6%) had severe dengue (SD) on the discharged day according to the 2009 WHO classification. Plasma AST level was significantly higher in SD group [mean:1192.4 (standard deviation: 416.3)] compared to the DWS group [322.6 (332)] and D group [(143.3 (165.0)]. Our univariate analysis revealed that ARG-1, HPPD, and GGT levels were significantly associated with dengue severity, whereas GLDH, GST, and PON-1 were not associated with disease severity. Additionally, multivariate logistic regression indicated that plasma ARG-1, HPPD, and GST levels were independently correlated with SD against DWS/D patients, while HPPD and GLDH were associated with SD/DWS against D patients. Dengue-induced liver injury is initiated in the centrilobular region towards the portal area during disease progress. Moreover, these new biomarkers can serve as good prognostic predictors.

## BRIDGING EFFICACY OF THE CYD-TDV TETRAVALENT DENGUE VACCINE FROM CHILDREN/ADOLESCENTS TO ADULTS IN HIGH ENDEMIC COUNTRIES BASED ON NEUTRALIZING ANTIBODY RESPONSE

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The CYD-TDV dengue vaccine has recently been licensed in multiple endemic countries based on vaccine efficacy (VE) to prevent symptomatic, virologically-confirmed dengue (VCD) demonstrated in two placebo-controlled Phase 3 trials in 2–14 year olds (y.o) in Asia (CYD14 - NCT01373281) and 9–16 y.o in Latin America (CYD15 - NCT01374516). Neutralizing antibody titers at baseline and post-vaccination (Month 13) measured by the PRNT50 strongly correlated with VE. These titer data may be useful for predicting VE in adults, thus constituting a basis for bridging/extrapolation of VE. Two Phase 2 trials of CYD-TDV measured PRNT50 titers pre-vaccination (baseline) and one month post-vaccination (Month 13): CYD22 in Vietnam (n=32 9–16 y.o and n=18 18–45 y.o - NCT00875524) and CYD47 in India (n=115 18–45 y.o - NCT01550289). PRNT50 distributions were compared between age cohorts by geometric mean ratios. In addition, epidemiological bridging methods were applied to estimate VE against any serotype (DENV-Any) and against each DENV serotype over 25 months of follow-up post first vaccination in a hypothetical 18–45 y.o cohort in CYD14+CYD15 (Bridging Population 1) and in the 18–45 y.o CYD47 cohort (Bridging Population 2). Baseline and Month 13 geometric mean PRNT50 titers to each serotype were significantly greater in 18–45 than 9–16 y.o for all comparisons (CYD22 18–45 vs. CYD22 9–16 and vs. CYD14+CYD15 9–16; CYD47 18–45 vs. CYD14+CYD15 9–16) in these endemic regions. Based on the multiple bridging methods VE against DENV-Any was estimated to be 75.3–86.0% with 95% confidence intervals spanning 52.5–100% for Bridging Population 1 and 68.4–77.5% with 95% confidence intervals spanning 42.3–88.5% for Bridging Population 2. VE was estimated to be 56.9–76.9%, 68.3–85.8%, 91.4–95.0%, and 93.2–100% against serotype 1, 2, 3, 4 for Bridging Population 1 and 44.5–66.9%, 53.2–69.2%, 79.8–92.0%, and 90.6–95.0% against serotype 1, 2, 3, 4 for Bridging Population 2. In conclusion, VE to prevent dengue disease is predicted to be at least equal to or higher in 18–45 than 9–16 y.o in the above described endemic populations.

## ZIKA AND DENGUE VIRUS-SPECIFIC AND CROSS-REACTIVE MEMORY B CELL RESPONSES

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Since 2015, Zika virus (ZIKV) has spread rapidly to many countries in the Americas, where it co-circulates with other flaviviruses that also cause human disease, such as dengue virus (DENV). DENV is comprised of four serotypes, where the amino acid sequence of the envelope is 65-70% conserved among the serotypes and 54-59% conserved with ZIKV. The antigenic similarity of ZIKV and DENV has raised important biological concerns regarding the effect of immunological cross-reactivity on protection and potential enhancement of ZIKV and DENV infections and vaccines. To date, the cross-reactivity of ZIKV and DENV B cell responses is not fully understood in the context of natural human infections. Here, we use a novel ELISPOT-based assay, designated the Quad-Color Fluorospot assay, that enables analysis of DENV serotype specificity vs. cross-reactivity of the memory B cell (MBC) population at a single-cell level, adding a fifth color to include ZIKV. We analyzed a unique set of peripheral blood mononuclear cells from the Nicaraguan Pediatric Dengue Cohort Study, ongoing since 2004, to which Zika was added in January 2016. Samples were collected 2 weeks and several months after RT-PCR-confirmed ZIKV infection (ZIKV+) from children who were documented to be previously DENV-immune or DENV-naïve. We also included a set of dengue patients who were ZIKV-naïve. Preliminary analysis of early convalescent samples (day 14-15) from 10 patients showed that despite the antigenic similarity between DENV and ZIKV and regardless of prior DENV exposure, MBCs

from ZIKV-infected subjects were highly reactive and specific to ZIKV, with lesser cross-reactivity to DENV. When analyzing total activated MBCs, DENV-naïve ZIKV+ patients displayed a higher frequency of ZIKV-specific MBC spots compared to DENV-immune ZIKV+ patients and as expected, DENV-naïve ZIKV+ patients displayed a lower frequency of DENV-specific MBC spots compared to DENV-immune ZIKV+ patients. Analysis of a larger set of patients at convalescence and 6 months post-illness is ongoing. These studies provide much-needed information on how the human memory B cell response reacts to ZIKV and DENV over time.

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### PHASE-III DENGUE VACCINE TRIAL SIMULATIONS QUANTIFY SENSITIVITIES OF VACCINE EFFICACY ESTIMATES TO UNMEASURED HETEROGENEITIES

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There are an increasing number of investigational dengue vaccines in clinical development that have the potential to be important tools to reduce the burden of dengue. Vaccine efficacy (VE) estimates obtained in late-phase field trials are crucial for assessing vaccine suitability for public health implementation. Various known heterogeneities in dengue virus (DENV) transmission, which typically are not measured or fully balanced by randomization during vaccine trials, have the potential to confound estimates of VE against clinical endpoints. We quantified the extent to which VE estimates may be sensitive to heterogeneities associated with DENV transmission and vaccine action by analyzing simulated results from “virtual” phase-III trials in Iquitos, Peru. To ensure that we captured all known heterogeneities, we simulated city-wide DENV transmission and vaccine trial protocols using an agent-based model calibrated with entomological and epidemiological data from long-term field studies. We simulated several scenarios, including a baseline scenario in which the trial was designed to have 90% power of detecting VE with a lower bound of the 95% confidence interval > 30% against virus-confirmed clinical disease. Assuming leaky protection, we found that estimates of overall VE were 5-10% lower than the per-exposure protection used in the simulation due solely to the fact that the number of exposures was not measured in the simulated trial. In scenarios with equal per-exposure protection by serotype, estimates of serotype-specific VE were capable of varying by as much as 10-15% due to heterogeneity in exposure by serotype. The extent of these and other sensitivities of VE estimates to unmeasured heterogeneities tended to be exacerbated when the simulated vaccine had a relatively short duration of protection, particularly in the presence of temporal heterogeneity in serotype-specific exposure. Our results highlight both the sensitivity of VE estimates to unmeasured heterogeneities and the potential for agent-based models to quantify the extent of that sensitivity for locations under consideration as possible vaccine trial sites.

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### DISSECTING THE QUALITY OF NEUTRALIZING ANTIBODY RESPONSES INDUCED BY THE NIH LIVE ATTENUATED TETRAVALENT DENGUE VACCINE TV003

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Dengue virus (DENV) is responsible for about 390 million infections annually. A safe and effective vaccine against DENV should stimulate prolonged and balanced immune responses to all the four serotypes. Proper understanding of the correlates of protection using dengue human infection models (DHIM) prior to engaging in efficacy trials in endemic areas is crucial for accelerating effective vaccine development. The Laboratory of Infectious Diseases at the National Institutes of Health developed a live attenuated tetravalent DENV vaccine TV003 currently in Phase 2 and 3 evaluations. A randomized placebo-controlled trial in which TV003 or placebo recipients were challenged six months later with a DENV-2 Tonga 74 strain, (rDENV2Δ30) was conducted to better understand the protective efficacy of TV003. All the recipients were protected from dengue infection defined as rDENV2Δ30 viremia, rash, and neutropenia. In order to understand possible correlates of protection, we compared the properties of neutralizing antibody responses elicited by TV003 against all four DENV serotypes. We hypothesized that serotype-specific antibodies provide long-term protection based on natural infection studies. To test this hypothesis, we estimated the contribution of serotype cross-reactive and type-specific antibodies to neutralization, by depleting specific populations of DENV-reactive antibodies from 6 months post vaccinated immune sera. Most of the subjects developed neutralizing antibodies to all 4 serotypes of DENV. Depletion results demonstrated that greater than 50% of neutralization responses were mediated by type-specific antibodies against all 4 serotypes. Further, epitope specific mapping studies were performed using the recombinant chimeric viruses as tools to further predict the regions targeted by type-specific neutralizing antibodies. We discuss the implications of these results in terms of testing the vaccine in large efficacy clinical trials.

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### A SINGLE GLYCOSYLATED AMINO ACID IN DENGUE VIRUS NS1 PROTEIN IS REQUIRED FOR TRIGGERING HUMAN ENDOTHELIAL CELL PERMEABILITY

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Dengue virus (DENV) is a mosquito-borne flavivirus that causes up to 96 million dengue cases annually. Infection with any of the four serotypes (DENV-1-4) can result in inapparent infection, classic dengue, or severe forms of the disease associated with vascular leak leading to shock and death. We recently described a novel role for DENV nonstructural protein 1 (NS1) in triggering permeability of human endothelial cells *in vitro* and systemic vascular leak *in vivo*. We also showed that DENV NS1 disrupts the endothelial glycocalyx layer (EGL), resulting in hyperpermeability. However, the molecular determinants of NS1-induced endothelial permeability have not yet been identified. Unlike NS1 from most flaviviruses, DENV NS1 displays only two, rather than three, conserved N-linked glycans (Asn-130 and Asn-207), required for hexamer stability and secretion. We examined the importance of NS1 glycosylation status on endothelial barrier dysfunction *in vitro* and demonstrated that mutation of the N-glycosylation site 207 (N207Q) completely prevents (i) NS1-induced hyperpermeability (measured by transendothelial electrical resistance, TEER) and (ii) EGL disruption (measured by cleavage of sialic acid, a major component of the EGL) in human pulmonary microvascular endothelial cells, without affecting NS1 binding to the surface of endothelial cells or expression or secretion of the protein. In contrast, the N130Q mutation greatly reduced secretion of NS1. We are currently assessing the effect of the N207Q mutation on other pathogenic functions of NS1 *in vitro* as well as in our *in vivo* model of dermal endothelial permeability. We are also expanding the study to NS1 from other flaviviruses such as Zika virus and West Nile virus. In summary, we have identified a single amino acid,

N207, at one of the two glycosylation sites of DENV-2 NS1 as the key determinant required for NS1 to trigger endothelial permeability *in vitro*. This information is crucial for understanding DENV pathogenesis, as well as for developing antiviral therapies and NS1-based vaccines.

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### BOOSTING EXPLAINS PATTERNS IN RATIOS OF INAPPARENT AND SYMPTOMATIC DENGUE VIRUS INFECTIONS

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Dengue is the most prevalent arthropod-borne viral disease worldwide, and the four dengue virus (DENV) serotypes circulate endemically in many tropical and subtropical regions worldwide. Numerous studies have shown that the majority of dengue virus (DENV) infections are inapparent and that the ratio of inapparent to symptomatic infections (*I/S*) fluctuates substantially year-to-year. In the Pediatric Dengue Cohort Study (PDCS) in Nicaragua, established in 2004 and as such the longest continuously running longitudinal dengue cohort study in the field, the *I/S* ratio has varied greatly, from 16.5:1 in 2006-7 to 1.2:1 in 2009-10. We hypothesize that in dengue-endemic areas, frequent boosting of the immune response can be protective against symptomatic disease and can explain fluctuating *I/S* ratios. We define boosts as exposures to homotypic or heterotypic DENV serotypes that do not lead to extensive viremia and result in a <4-fold rise in antibody titers (below the threshold of classification as an inapparent infection), thus leaving a modest trace on the immune response. Evidence for frequent boosting in endemic areas comes from studies that show that neutralizing antibody titers marginally increase between primary and secondary infections, even though traditionally, cross-reactive neutralizing antibodies are thought to wane following a primary infection, as observed in non-endemic settings. We formulate mechanistic, epidemiological models to examine the epidemiological effects of protective homologous and heterologous boosting of the antibody response in preventing subsequent symptomatic DENV infection. We fit these models to the PDCS dataset using Bayesian approaches and discriminate between models using Bayesian selection criteria. We show that models that assume that frequent boosts can protect against symptomatic disease can recover fluctuations in the *I/S* ratio, whereas a model without boosting cannot. These results have important implications for dengue vaccination policy, as effective vaccination has the potential to decrease the protective effects of boosting.

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### CHANGES IN THE FORCE OF INFECTION OF DENGUE FROM 1994 TO 2015 IN A PEDIATRIC DENGUE COHORT STUDY IN NICARAGUA

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Dengue virus (DENV) is the most prevalent vector-borne disease of humans, infecting up to 400 million individuals annually. The force of infection, the rate at which susceptible individuals are infected in a population, is an important measure of the burden of disease and a critical metric for vaccination policy. We used age-stratified seroprevalence and case data from 12 years of the Pediatric Dengue Cohort Study in

Nicaragua to estimate the annual force of infection from 1994-2015. Seroprevalence data revealed a consistent decline in the rate at which children acquire DENV-specific immunity over time: in 2004, ~50% of children >5 years old were seropositive, but by 2014, children ≥11 were ≥50% seropositive. We estimated high force of infection in 1994-1995 and 1998-1999 and a gradual decline thereafter, with the exception of an uptick in 2009-2011. Two hypotheses proposed to explain changes in the force of infection are 1) transition from epidemic to endemic transmission and 2) population demographic transition. In 1994-1998, DENV3 was introduced for the first time into Nicaragua; in 2009-2011, DENV3 once again caused large epidemics. The age distribution of the Nicaraguan population has also shifted over this time period, with a decline in birthrate and increase in life expectancy. We used mathematical models to simulate these hypotheses and test the most likely determinants of changes in the force of infection in Nicaragua. We show that the initial large decline in the force of infection can be explained by the virgin-soil introduction of DENV3 in 1994-1998, in which high numbers of dengue cases were reported and a large fraction of the population was infected and became subsequently immune to DENV3. We show that the subsequent gradual decline in the force of infection can be attributed to changing demographics in Nicaragua, specifically lower birth rates. The decline in DENV transmission intensity in Nicaragua is consistent with trends seen globally. These results suggest that changes in immunity and demographics over time substantially impact dengue transmission intensity in Nicaragua. \*L. Katzelnick and R. Ben-Shachar contributed equally.

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### THE LIVE ATTENUATED DENGUE VACCINE TV005 IS WELL TOLERATED AND HIGHLY IMMUNOGENIC IN FLAVIVIRUS NAIVE SUBJECTS 50 - 70 YEARS OF AGE

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Over the past several decades dengue has become hyper-endemic in all tropical and sub-tropical regions of the world. Current estimates report nearly 100 million symptomatic and 300 million asymptomatic dengue infections annually. More recently, dengue and severe dengue are being reported in elderly populations in endemic regions, indicating a possible need for vaccination in these populations. For this reason, we sought to evaluate the safety and immunogenicity of the live attenuated tetravalent dengue vaccine TV005 in flavivirus-naïve adults 50 – 70 years of age in a placebo-controlled, double-blind study. Twenty-eight subjects were enrolled in the Baltimore/Washington area and randomly assigned to receive a single dose of TV005 (20) or placebo (8). The ages of enrolled subjects ranged from 51 – 70 years. Fifteen subjects were male (53%). Subjects were evaluated in the clinic approximately every other day for the first 16 days post-vaccination and again on days 21, 28, 58, 90, and 180. Adverse events were collected through day 28. Blood for safety assessment and viremia was collected through day 21. The vaccine was well-tolerated in this age group. The only adverse event that occurred significantly more frequently in TV005 recipients was an asymptomatic rash (65%). Thirteen subjects (65%) had vaccine virus recovered from the blood; 8 subjects had > 1 virus recovered. TV005 induced a tetravalent antibody response in 89% of vaccinees and a trivalent or better response in 95%. These data demonstrate that TV005 is well tolerated and highly immunogenic in an elderly population. TV005 will be further evaluated in a Phase 2 trial in an elderly population in Taiwan.

## 802

### FLAVIDOT: AN AUTOMATED VIRUS PLAQUE COUNTER FOR MEASUREMENT OF THE SEROLOGICAL NEUTRALIZATION RESPONSE AGAINST ZIKA AND DENGUE VIRUSES

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Immunoassays, such as plaque reduction neutralization test (PRNT), allow us to quantify the neutralization capability of antisera. This assay is considered the gold standard for estimating the amount of infective viral particles in a sample but requires researchers to count individual plaques in each of the wells either by eye or utilizing expensive equipment and proprietary software. This process is time-consuming (even more so on 96-well plates), difficult to standardize among analysts, and not easy to customize when working with a wide variety of viruses. We report the development of a free, open-source, automated alternative, the Flavidot plaque counter. The Flavidot is capable of counting viral plaques from a variety of phenotypes, is easy to customize for different staining methods, and has a user-friendly interface. The counter was created using R statistical software, and Shiny to provide a user interface where different parameters may be adjusted. The counter was optimized by analyzing plaque images of 150+ DENV strains of all four serotypes. PRNT assays were performed on 96-well plates coated with C6/36 cells. The program's performance was tested on C6/36 and Vero cells infected with DENV and ZIKV. We compared the plaque counts obtained by unaided eye and the counts produced by the Flavidot. Counts obtained by Flavidot automatic settings were similar to those obtained by eye (Analyst 1, average plaque difference between methods: -1.89 [95%CI -4.00 - 0.22]; Analyst 2: -2.64 [95% CI -5.02 - 0.25]), which is consistent with the average plaque difference in counts by eye between analysts (-0.75 [95%CI -2.29 - 0.79]). The Flavidot plaque counter was developed as a tool to assist researchers using PRNTs on DENV and ZIKV to quantify protective antibodies. It is easy to install and requires no prior coding experience. With further improvements, this counter may be used in clinical settings, providing analysts with the speed and consistency needed to respond to emerging global health threats.

## 803

### DENGUE SEROTYPE AND DISEASE SEVERITY TRENDS AMONG INFANTS AND YOUNG CHILDREN IN INDIA, 2012-2015: IMPLICATIONS FOR DENGUE VACCINE STUDIES

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India is a major epicenter for dengue. We aimed to provide baseline information for dengue vaccines by studying clinical and serotype patterns among children with dengue infection hospitalized at a tertiary-care center in southern India. Dengue was confirmed by positive NS1 and/or anti-dengue-IgM direct ELISA. Dengue severity was graded using the 2009 WHO classification. Dengue serotypes were determined by RT-PCR and sequencing. Between 2012-2015, among 1,064 children with suspected dengue, laboratory-confirmed dengue was seen in 726; 157 in the Nov 2012-Dec 2013 period, and 569 in Sept 2014-Dec 2015. The male:female ratio remained constant at 1.2:1. Mean age decreased over time: 9.1yrs

(2012-13) and 7.1yrs (2014-15). The proportion of infants (age<1yr) increased over time; 4.2% to 8.6% (p<0.01). Severe dengue occurred among 25.1% and 28.8% of children during the two time periods. Dengue severity increased with age (p<0.001); infants were more likely to have severe dengue than older children (62.2% vs 24.9%, p<0.001). Moderate-to-severe thrombocytopenia, present in 62.8%, was significantly associated with severe dengue (p<0.001) and DENV-1 serotype (p<0.05). In 2012-13 (PCR and sequencing-confirmed, n=113), serotype 3 (38.9%) and 2 (38.1%) predominated; DENV-1 was less common (19.5%). In 2014-15 (n=401), DENV-1 (64.6%) predominated, followed by DENV-2 (21.2%), DENV-3 (6.7%) and DENV-4 (2.0%). Co-infections with multiple serotypes were seen in 5.5% (n=22). There was no correlation between disease severity and multiple-serotype co-infection. Our results indicate a high burden of dengue among Indian children, particularly infants, and increasing disease severity with dynamic serotype replacement, highlighting the need for a safe vaccine for young children. Ongoing population-based epidemiological surveillance is critical for dengue vaccine studies.

## 804

### BURDEN OF DENGUE IN OUAGADOUGOU, BURKINA FASO

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To address a gap in knowledge about dengue burden in Africa, the Dengue Vaccine Initiative conducted passive health care facility-based fever surveillance and seroprevalence study in Ouagadougou, Burkina Faso from Dec 2014 to Apr 2017. In the passive fever surveillance component, 3184 patients between 1-55 years of age, with current fever or history of fever ( $\leq 7$  days) with negative malaria rapid test, were enrolled at 5 health care centers; 505 were found to be positive either by dengue IgM ELISA or dengue rapid test (NS1 or IgM). Most of these cases occurred in the 15-29 year old age group. During the 26-month study period, dengue cases peaked in Sep-Dec 2015 and 2016. Selected acute serum samples, collected between Dec 2014 and June 2016, underwent further testing using Bioneer's DENV/ZIKV/CHIKV triplex PCR commercial kit. There were 157 acute samples selected for testing; 20 of these samples also had seroconversion by IgM ELISA between acute and convalescent visits; 17 were NS1 Ag positive by rapid test. Twelve (60%) of the 20 IgM seroconverters were dengue PCR positive; 14 (82%) of the 17 NS1 Ag positive patients were dengue PCR positive. In the seroprevalence component, blood samples were collected at 6 month intervals in a cohort aged 1-55 years, with 3 sets of blood samples collected in 3,066 subjects who were initially enrolled in June 2015. At enrollment, 66.4% of subjects were IgG ELISA positive. In the 2nd (n=2,473) and 3rd bleeds (n=2,248), 70.4% and 67.4% of subjects were IgG positive, respectively. The seroconversion rate was highest at 7.2% in 10-14 year olds, followed by 5-9 year olds (7.1%) and 30-34 year olds (6.9%). During the study period, a dengue outbreak was declared by WHO in Ouagadougou from Sep 2016 to Jan 2017. An additional seroprevalence blood collection was being performed in Mar/Apr 2017 on about 2,000 subjects who had participated in all three previous seroprevalence bleeds. Our study indicates that substantial endemic transmission of dengue is occurring in Burkina Faso.

## PERSISTENCE OF A NOVEL DENGUE VIRUS 2 COSMOPOLITAN GENOTYPE LINEAGE THAT EMERGED IN INDONESIA IN 2011, IDENTIFIED IN THE WESTERN AUSTRALIAN TRAVELLER COHORT

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Dengue virus (DENV) is found throughout the tropics. Over 70% of the global disease burden is borne by those living in Southeast Asia and the Western Pacific region. We have sequenced the Envelope gene of DENV-2 isolated from returning travellers entering Western Australia between 2011 and 2015, most of whom had visited Indonesia. A phylogenetic tree was estimated using maximum likelihood method. In previous studies we identified a novel lineage of DENV-2 Cosmopolitan genotype circulating in Indonesia, predominantly in Bali. The current study has found that this lineage emerged in Indonesia in early 2011 and has persisted into 2015, identified in travellers sampled between 2011-2013 and also 2015. A second lineage within the Cosmopolitan genotype that is most closely related to strains from Nepal, Malaysia and Singapore was found to have emerged in 2014 in Bali and continues to persist into 2015. Furthermore, other co-circulating lineages of DENV-2 were also identified, most of which are shown to be closely related to lineages known to have been circulating in Indonesia since at least 2008. The distribution and prevalence of these DENV-2 lineages should continue to be monitored. Febrile returning travellers can act as sentinels to provide timely data to identify circulating DENV genotypes and lineages, reveal epidemiological and evolutionary trends, and inform our understanding of epidemic virulence.

## DEVELOPMENT OF NOVEL SEROLOGICAL ASSAYS TO DISCRIMINATE BETWEEN DENGUE VIRUS AND ZIKA VIRUS ANTIBODY RESPONSES

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The recent emergence of Zika virus (ZIKV) in dengue-endemic regions/countries raises new challenges for dengue vaccine development and implementation. Cross-reactivity exists at the immunological level between these two related flaviviruses, which may bias the results of serological assays in areas where they co-circulate. In particular, analysis and follow up of dengue vaccine efficacy and effectiveness studies involves serological survey of anti-dengue responses, and such cross-reactivity may result in misleading interpretation. Therefore, easy, cheap, sensitive and specific assays are needed to discriminate between anti-dengue and anti-Zika responses. It is also important to differentiate these responses from those directed against yellow fever and Japanese encephalitis viruses, in Latin America and Asia respectively. Such specific assays would also be of benefit to the ongoing development of a Zika vaccine. Several assays are being evaluated; in particular, an ELISA aimed at measuring anti-NS1 antibody responses against these different flaviviruses is being developed, addressing both sensitivity and specificity. Sera from animals or humans obtained after infection by a single flavivirus or after multiple infections have been used in this regard, and preliminary data will be presented and discussed.

## DENGUE PREVALENCE IN A MILITARY VS CIVILIAN POPULATION

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Dengue is the most common arbovirus infection in the world. In the Philippines, dengue is hyperendemic and high rates of the disease are reported (220,518 suspected cases of dengue in 2016). Newly available interventions can impact the force of transmission and may increase the average age of primary dengue infection and subsequently, occurrence of secondary dengue infection in adult populations. Though dengue has been described in the Philippine civilian population, the burden of disease among military personnel has not yet been characterized. Acute blood samples of patients presenting with dengue-like illness at the Armed Forces of the Philippines Medical Center, Manila were tested using DENV RT-PCR. Statistical tests of association were used to determine differences in demographic factors and logistic regression was used to determine signs or symptoms which can be predictors of dengue infection. From Nov 2012 to Nov 2016, 209/645 (32%) patients tested positive for DENV by RT-PCR, age-range for the confirmed dengue cases was 6 mos. – 55 yrs., male to female ratio 1.32; 129/209 had DENV serotype data: 54 (42%) DENV-3, 38 (29%) DENV-2, 25 (19%) DENV-1, and 12 (9%) DENV-4. We further analyzed the data according to age and military or civilian status. Our findings showed dengue as primarily affecting patients <18 y.o. and though the number of confirmed dengue cases was lower in the active duty military (OR 0.66) versus the civilian adult population, the disease burden in the military population was still substantial. Since only acute blood samples were tested and no serological testing of paired acute/ convalescent samples was done, this could lead to a lower rate of laboratory-confirmed dengue and underestimate the burden of infection.

## IMMUNOGENICITY AND SAFETY OF TAKEDA'S DENGUE VACCINE CANDIDATE IN CHILDREN AND ADOLESCENTS AGED 2-17 YEARS FROM PANAMA, THE DOMINICAN REPUBLIC AND THE PHILIPPINES: 18-MONTH RESULTS FROM A PHASE 2 RANDOMIZED PLACEBO-CONTROLLED TRIAL

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Dengue is the most common mosquito-borne viral disease in humans. A dengue vaccine for those aged 9 years and older was licensed recently, but there remains urgent medical need for a vaccine that is safe and effective against all four dengue serotypes (DENV-1-4) in recipients of all ages. In December 2014, a phase 2, randomised, double-blind, placebo-controlled trial of Takeda's tetravalent dengue vaccine candidate (TDV) was initiated at three sites in dengue endemic countries (Panama, the Philippines, and the Dominican Republic) to determine its safety and immunogenicity in ~1800 healthy participants aged 2-17 years old. Participants were randomised to receive placebo or TDV in different dose schedules (either: one dose at 0 months and one dose at 3 months; one dose at 0 months; or one dose at 0 months and a booster dose at 12 months) (ClinicalTrials.gov: NCT02302066). The study is ongoing and all participants will be followed up for a 48 month period. The primary objective of this study is to assess geometric mean titres of neutralising antibodies to DENV-1-4 in

the per-protocol set at different time points. Secondary objectives include the analysis of the serious adverse events and the virologically confirmed dengue cases overall, and the analysis of the non-serious adverse events and seropositivity rates for one or more serotypes in the immunogenicity subset. Detailed safety and immunogenicity analyses following the administration of TDV in different schedules will be presented, with discussion on how these data support the continued development of this vaccine candidate.

## 809

### CIRCULATING DENGUE VIRUS AND CLINICAL CHARACTERISTICS IN PATIENTS WITH ACUTE FEBRILE ILLNESS FROM HUANUCO, PERU

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Dengue infections are a major health problem and there is an increasing necessity to strength its epidemiological surveillance in Peru due to the low laboratory-confirmation rate (<50%). This study was undertaken to assess the frequency of Dengue virus (DENV) serotypes and describe its clinical presentation in patients with acute febrile illness from Huánuco, an endemic area in the center of Peru. A total of 268 serum samples from thirty five health care centers were studied for the presence of DENV via RT-PCR, NS1 antigen, IgM and IgG antibodies from November 2015 to July 2016. PCR positive samples were sent for commercial sequencing and serotype 1 to 4 identification. Physicians used a standardized questionnaire to collected the demographic and clinical symptoms information. Dengue virus RNA was detected in 25.0% (67/268) of samples via qPCR. For the anti-Dengue antibody tests, the NS1 antigen was detected in 19.03% (51/268), IgM antibodies were found in only 10.45% (28/268), and IgG antibodies were detected in 15.67% (42/268) of patients. The most common symptoms accompanying fever were: Headache (100.0%), Arthralgias (85.07%), Myalgias (94.03%), Headache (97.01%), retro-orbital pain (73.13%), Low back pain (53.73%), Skin rash (29.85%), hyporexia (52.24%), Sore throat (34.33%) and sickness (50.74%). In conclusion, Dengue is currently a predominant serotype in Huanuco, Peru. The clinic isn't sufficient to diagnose cases of DENV, for this reason it's necessary to have a laboratory diagnosis. Further investigations should be conducted to evaluate the use of RT-PCR as a reliable method for DENV detection as well as for serotype surveillance in Peru.

## 810

### HETEROGENEITY IN EVOLUTIONARY RATES MAY REFLECT ECOLOGICAL AND BIOLOGICAL DIFFERENCES BETWEEN DENGUE GENOTYPES

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Dengue virus (DENV) evolutionary rates have not been comprehensively compared across all genotypes despite changes in global DENV incidence and the availability of DENV sequence data. Redressing this knowledge gap may have implications for the current understanding of DENV evolution. We obtained all unique complete envelope sequences for DENV serotypes 1 - 4 from GenBank comprising over 70 years of human DENV infections ( $n = 6784$ ). Sequences were genotyped by maximum likelihood phylogenies inferred by RAxML or PhyML packages using appropriate nucleotide substitution models informed by JModelTest2. 12 individual genotype datasets (DENV-1 I, IV, V; DENV-2 Asian-I, American, American-Asian and Cosmopolitan; DENV-3 I,II,III; DENV-4 I,II) then underwent evolutionary rate estimation by the BEAST package using comparative

clock and demographic models. To adjust for potential rate inflation by over-sampled focal epidemics, we repeated analyses after down-sampling to a maximum of 10 sequences by year. Under a strict clock and constant demographic model, the median genotype evolutionary rate was  $9.83 \times 10^{-4}$  substitutions/site/year (IQR 8.22 -  $10.24 \times 10^{-4}$ , range 4.38 -  $11.76 \times 10^{-4}$ ). There was striking heterogeneity between genotype evolutionary rates among the DENV-1, -2 & -4 serotypes, with as high as two-fold differences between genotype rates. While down-sampling reduced rate point estimates across all genotypes, DENV-2 and DENV-4 intra-serotype rate differences were robust after adjusting for sampling skew and comparative clock and demographic model analyses. In conclusion, There are substantial differences in the evolutionary rates of some DENV genotypes. We show that such differences are at least partially driven by skewed sampling of increasingly frequent epidemics, and may perhaps reflect intrinsic biological or ecological differences between DENV genotypes. Rate estimates for some genotypes were substantially higher than historical estimates in prior study and may reflect long term changes in the global epidemiology of DENV.

## 811

### CONGENITAL OCULAR PATHOLOGY IN RHESUS MACAQUES INFECTED WITH ZIKA VIRUS IN UTERO

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Congenital Zika virus syndrome (CZS) continues to threaten women infected with Zika virus (ZIKV) during pregnancy with no known correlate to predict the severity of the syndrome, no treatment and no available vaccine. We developed the first rhesus macaque model of ZIKV infection during pregnancy to study the mechanisms of CZS pathogenesis and assess potential interventions. Five rhesus macaques were inoculated subcutaneously with 104 PFU of Asian-lineage ZIKV, to model mosquito transmission, during either the first ( $n=3$ ) or third ( $n=2$ ) trimester of pregnancy. All 5 animals were infected by 1 day post-inoculation (dpi). Notably, 3 animals infected in the first trimester exhibited a longer duration (24-70 dpi) of plasma viremia than seen in any non-pregnant animal (<17 dpi) infected with the same dose and virus, as also documented in human cases. One pregnancy resulted in a fetal demise at 49 dpi, when both the mother and fetus were euthanized and underwent necropsy. Fetuses from the other 4 pregnancies were surgically removed and necropsied at ~155 days of gestation. All 5 fetuses were vertically infected, with Zika viral RNA (vRNA) found in multiple tissues and evidence of inflammation in the placenta, decidua, fetal membranes, fetal lung, lymph nodes and spleen. Animals infected during the first trimester also showed moderate ocular pathology and inflammation of the fetal retina, choroid and optic nerve. The fetus that died *in utero* showed severe ocular pathology including a scleral and chorio-retinal coloboma as well as vRNA detected in 27/36 tissues tested by qRT-PCR and in 4/10 tissues tested by *in situ* hybridization (ISH). Maternal viral tissue distribution was also assessed in the fetal demise case. vRNA was present in 4 lymph nodes, spleen and uterus (6/63 tissues tested). The uterus and decidua was also positive for ZIKV by ISH. Altogether, these data suggest that ZIKV readily crosses the placental barrier leading to infection and pathology in the rhesus macaque that is similar to that seen in human infection.

## 812

### DECREASED TRANSMISSION OF ZIKA VIRUS IN Aedes Aegypti MOSQUITOES CO-INOCULATED WITH AN INSECT-SPECIFIC FLAVIVIRUS

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Previous studies have demonstrated that an insect-specific flavivirus, Nhumirim virus (NHUV), can suppress the *in vitro* growth of West Nile

virus (WNV) and similarly decrease WNV transmission rates in NHUV/ WNV co-inoculated *Culex quinquefasciatus* mosquitoes. To assess whether NHUV might similarly interfere with flaviviruses that infect *Aedes aegypti*, the ability of NHUV to suppress *in vitro* viral growth of Zika virus (ZIKV), Dengue-2 virus (DENV2) and Chikungunya (CHIKV) was assessed. *Aedes albopictus* (C6/36) cells were concurrently inoculated with ZIKV, DENV2 or CHIKV and NHUV or infected with NHUV 1-5 days prior to ZIKV, DENV or CHIKV infection. Results demonstrated that ZIKV titers were reduced 100,000-fold in cultures either pre- or concurrently inoculated with NHUV/ ZIKV as compared to only ZIKV. A 1,000-fold reduction in DENV2 titer was observed for concurrent infections with NHUV/ DENV compared to DENV alone. Interestingly, DENV2 virus was not detected in C6/36 cells infected with NHUV 3 days prior to DENV2 (<1.8 log<sub>10</sub> PFU/mL). In contrast, the alphavirus, CHIKV, exhibited only a 10-fold reduction in titer observed only when cells were pre-inoculated with NHUV 3 days prior to CHIKV infection that was short-lived with titers rebounding within 72 hours, indicating a potential low-level non-specific interference. To assess whether NHUV could impact ZIKV transmission, *Ae. aegypti* mosquitoes were intrathoracically inoculated with ZIKV alone or NHUV/ZIKV and the resulting ZIKV infection and transmission rates assessed. Infection rates of 100% were observed in both groups; however, the transmission rate for mosquitoes from the NHUV/ZIKV dually-inoculated group was 41% compared to 78% for ZIKV-only inoculated mosquitoes ( $p < 0.0001$ ). Differences in ZIKV titers from bodies or saliva were not observed. These results indicate NHUV mosquito infection could be used as a model to assess superinfection exclusion mechanisms and to study the potential for the mosquito virome to impact vector competence of medically important flaviviruses.

## 813

### ZIKA VIRUS INFECTION AMONG A POPULATION-BASED AMONG A POPULATION BASED COHORT OF PREGNANT WOMEN IN PANAMA CITY, PANAMA, 2016-2017

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Zika virus (ZIKV) infection during pregnancy has been recognized as a cause of microcephaly and other adverse outcomes. Because a high proportion of infected persons experience mild or no symptoms, and access to testing is often limited, it has been difficult to estimate the complete burden of ZIKV infection. We are conducting a cohort study of pregnant women in Panama to estimate the incidence of ZIKV symptomatic and asymptomatic infection, and assess birth outcomes and cognitive and psychological development up to two years of age. Participants have been recruited from two peri-urban health centers and are being followed throughout pregnancy. Blood samples are collected at recruitment, 28 weeks' gestation, and at one week post-partum. We are conducting active surveillance for ZIKV disease symptoms via weekly telephone calls, and collecting blood and urine samples from symptomatic patients who report fever or history of fever >38°C, rash, or conjunctivitis. Laboratory testing is conducted using serological and molecular methods, including IgM and neutralizing antibody testing and rRT-PCR. To date, 488 women have been recruited at a median gestational age of 16 weeks (IQR: 11-23). Overall, 735 blood samples have been collected. Among the 488 women, 411 (84.2%) have no evidence of ZIKV infection and 13 (2.7%) had a probable ZIKV infection (Zika IgM positive, dengue IgM negative). Among the remainder, both Zika and dengue IgM antibodies were detected or results were inconclusive. Additional confirmatory testing is pending. Eight of the 488 women reported symptoms but testing was negative. Our study demonstrates that probable asymptomatic ZIKV

infections have been identified among pregnant women in Panama. Study participants are being followed to determine the overall risk for ZIKV infection during pregnancy and to assess outcomes and child development following infection.

## 814

### PERFORMANCE OF THE CDC TRIOPLEX REAL TIME RT-PCR DURING THE 2016 ZIKA EPIDEMIC IN PUERTO RICO

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The emergence of Zika virus in 2015 presented a challenge to diagnostics in areas where transmission of dengue and chikungunya viruses has been detected. In response to this public health emergency, the Centers for Disease Control and Prevention developed the Trioplex Real Time RT-PCR Assay designed for the simultaneous detection of dengue, chikungunya and Zika virus RNA in a variety of human clinical specimen types. We determined the analytical and clinical performance characteristics of the Trioplex in detecting each target virus serum, urine and whole blood-EDTA specimens. The assay was adapted to RNA extraction, PCR equipment and procedures commonly found in US and international public health laboratories. To determine the limit of detection (LoD) of the Trioplex for each target virus on each these modalities, inactivated dengue, chikungunya and Zika viruses were suspended in human serum, urine or whole blood and diluted to undetectable levels. The overall LoD for the assay was determined to approximate 103 genome copy equivalents per milliliter of specimen for every target virus. The assay modality that achieved the highest sensitivity included RNA extraction from 1 mL of specimen. Independent studies confirmed that the sensitivity of Trioplex is similar to other CDC and non-CDC tests; and that the Trioplex modality with the highest sensitivity compares to the most sensitive commercial tests. In order to determine the clinical sensitivity of Trioplex during the first 6 days of illness, 373 concurrently collected serum, urine and whole blood samples from patients with positive Zika IgM after 7 days were tested. In 373 confirmed cases in Puerto Rico, the Trioplex detected 85% (317/373) in serum, 55% (206/373) in urine and 70% (243/345) in whole blood. Testing simultaneously collected serum and urine provides an additional 3% sensitivity over serum alone; whereas the value of testing serum-whole blood provides an additional 5% over serum alone. The high sensitivity of the Trioplex demonstrates the utility of the assay resolving Zika cases in endemic areas. More than 30 thousand Zika cases in Puerto Rico have been confirmed with the Trioplex.

## 815

### EXPERIMENTAL INFECTION OF JAMAICAN FRUIT BATS (*ARTIBEUS JAMAICENSIS*) WITH ZIKA VIRUS

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Zika virus (ZIKV) emerged in the New World with the 2014 outbreak in Brazil. It has spread to much of South America, Central America, Mexico and the Caribbean, with thousands of cases. While disease presentation may be subclinical to mild and include symptoms of maculopapular rash, conjunctivitis, and arthralgia, infection of pregnant women can lead to fetal microcephaly. Additionally ZIKV infection can induce Guillain-Barre syndrome. In the 1950s and 1960s bat species were investigated as possible reservoirs for ZIKV. In total, five different species of bats were found to be susceptible to the virus. Bats seroconverted, had viremia, and, in one experimental infection study bats developed fatal neurological disease. This warranted further investigation of ZIKV in bats to determine their use as an animal-model, and to better understand the potential role of bats in viral ecology. Nine Jamaican fruit bats (*Artibeus jamaicensis*) were subcutaneously inoculated with 7.5x10<sup>5</sup> pfu of ZIKV strain PRVABC59 and monitored over the course of 28 days, during which

there were no conspicuous signs of disease. Bats were euthanized at 2, 5, 10 and 28 days post-inoculation to assess the course of infection and antibody responses. Bats seroconverted by day 28 by ELISA with ZIKV-infected, fixed Vero E6 cells. Low levels of viral RNA were detected in one brain and one urine sample. IHC detected ZIKV antigen in lung and testes of one bat, and brains and salivary gland of two others. Pathology was consistently observed in the lungs, heart, testes and brain. Pneumonia was observed in four bats, cardiomyocyte necrosis in three bats, degeneration and lymphocyte infiltration in the testes of two bats, and neuronal degeneration in the hypothalamus and cerebellum in three bats. These results provide evidence that Jamaican fruit bats are susceptible to ZIKV and may serve as an animal model to study neurological components and sexual transmission of the virus. Low viral load in urine and tissues suggests the role of bats in viral ecology may be minimal.

## 816

### ANALYSIS OF THE EFFECT OF ZIKA VIRUS INFECTION DURING PREGNANCY ON PLACENTAL DEVELOPMENT AND BIRTH OUTCOMES

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Since its recent emergence and pandemic spread in the Americas, Zika virus (ZIKV) infection during pregnancy has been associated with microcephaly and other congenital birth defects. Despite these devastating effects, the full extent of the impact of Congenital Zika Syndrome is yet unknown. ZIKV was first detected in Nicaragua in January 2016, and ZIKV transmission peaked in Managua from July 25 to August 14, 2016. ZIKV RT-PCR testing was performed for national surveillance on all pregnant women who presented to government health centers with Zika-like symptoms. Nicaragua began enrollment in the Zika in Infants and Pregnancy (ZIP) study, a multi-site study that enrolls pregnant women in their first trimester, in November 2016. The Nicaraguan Zika Positives (NZP) study began in January 2017 as a complementary study to include women infected during the peak of the epidemic. The NZP enrolls pregnant women confirmed as rRT-PCR ZIKV+ by the Ministry of Health or with a history of Zika-like symptoms during their pregnancy. For those who displayed Zika-like symptoms but did not have a ZIKV+ rRT-PCR result, recent ZIKV infection was confirmed via the NS1 Blockade-of-Binding ELISA (BOB) assay. Women complete questionnaires on demographics and risk factors and provide blood, urine, and vaginal secretion samples upon enrollment. At birth, additional samples, including placental tissue and cord blood, are collected. Infants are evaluated at birth, 3, 6, and 12 months by pediatric specialists to assess their overall health, hearing, vision, and neurological development. Biological samples (blood, urine) are collected at each infant visit and are assessed for evidence of ZIKV infection (IgM ELISA, BOB ELISA) and for possible confounding infections such as syphilis, CMV, toxoplasmosis, and HSV. Placental and amniotic membrane tissues have been analyzed to evaluate placental development and pathology and identify sites of ZIKV replication. To date, 26 women have been enrolled and 19 have given birth. This study is providing important information regarding the impact of ZIKV infection during pregnancy on placental, fetal, and infant development.

## 817

### INFLAMMATORY MONOCYTES ARE THE MAIN TARGET OF ZIKA VIRUS INFECTION IN PERIPHERAL BLOOD MONONUCLEAR CELLS FROM PEDIATRIC PATIENTS IN NICARAGUA

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The ongoing Zika pandemic in the Americas is linked to congenital birth defects and Guillain Barré Syndrome. Leukocytes play an important role in host immune responses early in flaviviral infection, including dengue virus (DENV), where monocytes and dendritic cells are the main target of infection in humans. However, the targets of Zika virus (ZIKV) infection in peripheral blood mononuclear cells (PBMCs) are not known. Therefore, we infected PBMCs with ZIKV *in vitro* and characterized cell targets and phenotypic changes 24, 48 and 72 hours post-infection. To phenotype cells, we used markers for monocytes (CD16, CD14, CCR2, HLA-DR), dendritic cells (CD11c, CD123, CD1a, CD1c), T cells (CD4, CD8, CD45RA, CD45RO), and NK cells (CD56). ZIKV infection was detected using monoclonal antibodies against nonstructural protein 3 (NS3) and envelope protein (E). Analysis by flow cytometry and immunofluorescence microscopy revealed that monocytes are the main target of ZIKV infection, with many monocytes acquiring an intermediate phenotype (CD14+CD16+). Next, we analyzed by flow cytometry PBMCs collected on days 3, 4, and 5 post-onset of symptoms from ZIKV-infected pediatric patients in a hospital-based study in Nicaragua. Viral load was measured by qRT-PCR in serum and PBMCs. Patients with confirmed acute dengue virus (DENV) infection were used for comparison. Similar to *in vitro* experiments, we found that inflammatory and intermediate monocytes (CD14+CD16-CCR2+ and CD14+CD16+) are the main target for ZIKV infection in humans, but we also detected a few infected dendritic cells. In comparison to DENV infection, the frequency of ZIKV-infected cells was lower but the percentage of CD14+CD16+ cells was significantly higher. To characterize the immune response genes in ZIKV-infected monocytes, we isolated monocytes from healthy blood donors, infected them with ZIKV *in vitro*, and analyzed gene expression 48 hours post-infection. Many inflammatory chemokines and cytokines were upregulated after infection, suggesting that monocytes play an important role in orchestrating the innate immune response to ZIKV upon infection.

## 818

### LONGITUDINAL ANALYSIS OF CROSS-NEUTRALIZATION BETWEEN DENGUE AND ZIKA VIRUSES IN TWO PEDIATRIC STUDIES IN NICARAGUA

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The four dengue virus serotypes (DENV1-4) and Zika virus (ZIKV) are antigenically related mosquito-borne flaviviruses that cause human diseases of major medical and public health importance worldwide. The cross-reactivity between DENV and ZIKV has raised questions about cross-protection and concerns of cross-enhancement of disease, yet few data exist characterizing the long-term antibody response. Here, we studied the extent of cross-neutralization between DENV and ZIKV using samples from two long-term studies in Nicaragua: a hospital-based study with a longitudinal arm extending for 18 months and a 13-year ongoing community-based cohort study with annual healthy blood samples. We

selected confirmed dengue cases, stratified by serotype (DENV1, 2, 3) and immune status (primary, secondary) (n=5/group), and analyzed plasma samples collected at acute, convalescent, 3-, 6-, 12- and 18-month time-points from the hospital study, as well as 1-3 years post-infection from the cohort study, using a Vero cell-based focus reduction neutralization assay. We also analyzed samples at convalescence and 3 months post-ZIKV infection. We found that ZIKV infections in both DENV-naïve and previously DENV-exposed individuals induce high levels of neutralizing antibodies to ZIKV, with much lower cross-neutralization of DENV1-4. We also found that in both primary and secondary DENV infections, neutralizing antibody titers to ZIKV were ~10-fold lower than to the homologous infecting DENV, and also lower than to heterologous DENV serotypes. We noted differences by serotype, with less cross-neutralization of ZIKV by DENV1-immune plasma. However, for all DENV infection antisera, cross-neutralizing antibody titers against ZIKV were consistently maintained over time, both 0-18 months and 1-3 years post-DENV infection. DENV/ZIKV-naïve samples used as controls did not neutralize (<10) DENV1-4 or ZIKV in our assay, demonstrating that we are not observing background signal. These findings improve our understanding of cross-neutralization over time between DENV and ZIKV, with implications for natural immunity and vaccines.

## 819

### A NOVEL ANTIBODY-BASED ASSAY DISCRIMINATES ZIKA VIRUS INFECTION FROM OTHER FLAVIVIRUSES

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Zika virus (ZIKV) is a mosquito-borne flavivirus that emerged recently as a global health threat, causing a pandemic in the Americas. ZIKV infection mostly causes mild disease, but is linked to devastating congenital birth defects and Guillain-Barré syndrome in adults. The high level of cross-reactivity among flaviviruses and their co-circulation has complicated the use of serological assays to differentially detect clinical and subclinical ZIKV and dengue virus (DENV) infections, accentuating the urgent need for a specific and sensitive serological test. We previously generated a ZIKV NS1-specific human monoclonal antibody, which we used to develop an NS1-based competition ELISA. Well-characterized samples from RT-PCR-confirmed Zika patients and individuals exposed to other flavivirus infections or vaccination were used in a comprehensive analysis to determine the sensitivity and specificity of the NS1 blockade-of-binding (BOB) assay, which was established in laboratories in 5 countries (Nicaragua, Brazil, Italy, United Kingdom and Switzerland). Of 158 sera/plasma from RT-PCR-confirmed ZIKV infections, 145 (91.8%) yielded greater than 50% inhibition. Of 171 patients with primary or secondary DENV infections, 152 (88.9%) scored negative. When the control group was extended to patients infected by other flaviviruses, other viruses, or healthy donors (n=540), the specificity was 95.9%. We also analyzed longitudinal samples from DENV-immune and DENV-naïve ZIKV infections and found inhibition was achieved within 10 days post-onset of illness and maintained over time for many months post-infection. We have used the

NS1 BOB ELISA to evaluate potential ZIKV infections in pregnant women months after exposure, to determine the rate of infection in our pediatric cohort study in Nicaragua, to perform age-stratified serosurveys in children and adults, and to investigate circulation of ZIKV in other parts of the world. Thus, the Zika NS1 BOB assay is a simple, robust, sensitive, specific, and low-cost solution for Zika surveillance programs, sero-prevalence studies, and intervention trials in flavivirus-endemic areas.

## 820

### PASSIVE TRANSFER OF ZIKA VIRUS-LIKE-PARTICLE-INDUCED IMMUNE SERA TO AG129 MICE PROTECTS AGAINST LETHAL ZIKA VIRUS CHALLENGE

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Zika virus (ZIKV) infection poses a serious public health threat due to its association with neurological complications, including birth defects in developing fetuses and Guillain-Barre Syndrome in adults. We sought to develop a ZIKV vaccine based on virus-like-particles (VLPs) generated in transiently transfected HEK293 mammalian cells. The genetic construct consists of the structural protein genes of ZIKV placed downstream from a heterologous signal sequence. To better understand the humoral responses and potential correlates of protection (CoP) afforded by the VLP vaccine, a dose response study was first carried out in immune-competent mice (C57Bl6 x Balb/c) using a dose titration of VLPs formulated with and without aluminum hydroxide as an adjuvant. Serum samples from VLP-immunized mice were tested in neutralization assays using reporter virus particles (RVPs), where alum was observed to provide a significant dose-sparing effect of approximately 25-fold. Further, to assess the protective capacity of the immune sera, we performed passive transfer experiments in AG129 mice which are susceptible to ZIKV infection and pathogenesis. Of note, mice that received the immune sera prior to ZIKV infection demonstrated significantly reduced viral replication (greater than 99%) as measured by RNA genome equivalents in the blood, and remained healthy, as measured by weight maintenance and survival. Control mice succumbed to infection. These results underscore the significant protective effect of the antibody responses elicited by this VLP vaccine candidate. Additionally, these studies will help define optimal vaccine formulations, contribute to translational efforts in developing a ZIKV VLP vaccine for clinical development, and address immunologic CoP.

## 821

### KNOWLEDGE, PERCEPTIONS AND SOCIOECONOMIC STATUS DRIVING DENGUE PREVENTION PRACTICES IN URBAN COASTAL ECUADOR

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Dengue is a major mosquito-borne febrile illness in the tropical and subtropical regions with dengue virus (DENV) infection ranging from asymptomatic to life-threatening hemorrhagic fever and shock. Since household-level vector control remains the primary means of controlling the disease, it is important to identify local social and behavioral factors that affect dengue prevention actions at the household-level. Our study measured the impact of socioeconomic factors and perceptions about dengue on the implementation of dengue prevention actions at

the household level in the city of Machala, Ecuador, which features a high incidence of DENV. From January 2014 to September 2016, we investigated 416 households. Households were identified through an active dengue surveillance study and included homes with people who had confirmed dengue disease (identified through Ministry of Health sentinel clinics), and neighboring homes within 200 meters. Each head of household completed a questionnaire that included demographics and questions about dengue knowledge, attitudes, and prevention. The data was evaluated using descriptive statistics (significance when  $p < 0.05$ ). We found that the total number of implemented dengue preventions increased during the three years of the study and positively correlated with knowledge of dengue transmission. Individual prevention measures positively correlated with perceiving dengue as a serious problem in the community, having attained higher education level, being of older age, owning the house, and having received dengue training. Fewer preventions were implemented by those who perceived dengue prevention as difficult or impossible. We conclude that socioeconomic status, the level of awareness about and attitude towards dengue, as well as dengue prevention training strongly influence the implementation of dengue prevention on the household level. These results will inform public health interventions in the region.

## 822

### AUSTRALIAN ARBOVIRUSES ASSOCIATED WITH UNDIAGNOSED UNDIFFERENTIATED FEBRILE ILLNESS

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There are at least 13 Australian arboviruses that have been associated with human infection but routine tests are not available to diagnose infection with most of these and their role in human pathology, undifferentiated febrile illness (UFI) or other serious diseases is unknown. Following the development of a commercial ELISA to diagnose Ross River virus infections, the number of cases in Australia rose from 20-50 per year to more than 9000 in 2016. The aim of this study was to determine how prevalent infection of humans with these "orphan" viruses was and whether any of them are the cause of UFI. Screening of 1000 age and gender stratified plasma samples from healthy blood donors for IgG antibodies against, Ross River, Barmah Forest, Alfuy, Kokobera, Stratford, Kunjin and Edge Hill viruses by indirect ELISA produced a large number of non-specific reactions so, an age and gender stratified sub-sample from the plasma panel was tested in plaque reduction neutralisation tests. Additionally, 492 UFIs samples from patients in northern Australia were screened by indirect immunofluorescence for the presence of IgM antibodies against these viruses. There was a linear increase in the prevalence of neutralising antibodies against these viruses with annual seroconversion rates of Ross River virus, 1.4% ( $r^2=0.7$ ); Barmah Forest virus, 0.5% ( $r^2=0.7$ ); Kunjin virus, 0.4% ( $r^2=0.7$ ); Edge Hill virus, 0.4% ( $r^2=0.5$ ); Kokobera virus, 0.3% ( $r^2=0.9$ ); Alfuy virus 0.2% ( $r^2=0.5$ ); Murray valley encephalitis virus, 0.1% ( $r^2=0.3$ ) and Stratford virus 0.6% ( $r^2=0.9$ ). Of the 492 samples from UFI patients 5.2% contained IgM antibodies against one or more of these Australian arboviruses: RRV (0.8%); BFV (0.4%); ALF (1.4%); EHV (1.8%); KOK (1.4%); KUNV (2.4%); MVE (1%); STRV (1.2%). These results suggest that a number of the "orphan" Australian arboviruses have been infecting humans at a regular rate for decades and some of them may be associated with UFIs. There should be ongoing, and systematic, testing of UFI patients for evidence of recent infection with these orphan arboviruses to determine how significant the burden of the disease they cause is.

## 823

### POLICI: AN ONLINE TOOL FOR VISUALIZATION OF POPULATION-LEVEL YELLOW FEVER IMMUNIZATION COVERAGE IN AFRICA

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Yellow fever (YF) is a vector-borne viral haemorrhagic fever found in Africa and Latin America, primarily spread by *Aedes* species in Africa. Despite the existence of an effective, life-long and safe vaccine since the 1930's, YF is still a major cause of mortality in Africa. Decades of inconsistent and incomplete vaccination have left the endemic region of Africa a patchwork mosaic of population level vaccination coverages both within and between countries, making outbreak risk assessments and planning of efficient vaccination campaigns difficult. Population level vaccination coverages at the first administrative level were previously calculated as part of a model calculating the burden of YF in R, but despite the usefulness of this information the necessity of specialist programming knowledge limited its accessibility. Through integrating this information with the R web-based graphical user interface (GUI), Shiny, we are able to present this pertinent information online and in an interactive way without the need of specialist information or software, making it accessible to relevant stakeholders in global health.

## 824

### A COHORT STUDY TO DETERMINE THE INCIDENCE OF ZIKA VIRUS INFECTION AMONG NEWBORNS, SANTOS, BRAZIL, 2016-2017

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Zika virus has been recognized as a teratogenic infection since its emergence in Brazil in 2015, being implicated in severe neurological anomalies of the fetus and newborn. In order to assess the risk of transmission from an infected mother to the newborn, the risk of congenital anomalies among the infected newborns, and their relation to the timing of infection during pregnancy, a cohort study of mothers and their newborn babies was set up in Santos, state of Sao Paulo, Brazil. Data from a concurrent study has shown that Zika emerged in Santos in September, 2015. Enrollment in the cohort started in June, 2016. All women that come to the study's hospital for delivery are being invited to participate. The ones who agreed to participate signed an Informed Consent Form. Since November, 2016, recruitment has been extended to the antenatal clinic. Participants respond to a structured questionnaire, and have a blood sample collected for Zika IgG serology (Euroimmun), or PCR (when presenting Zika suggestive symptoms). A cord blood and an urine sample are being collected for PCR and serology of the newborns. From June, 2016 until February, 2017, 730 mothers and their 743 babies were enrolled. 15.1% of the mothers presented a positive IgG serology for ZIKV, while 5.6% had an inconclusive result. PCR and IgM serology of their babies were all negative. No ZIKV related congenital anomalies were reported in the study's hospital. The pattern of severe congenital anomalies occurring after a ZIKV outbreak, as previously seen in other sites in Brazil and elsewhere, has not been observed in this study's sample so far. The limitations on the specificity of serologic assays for ZIKV diagnosis have to

be considered in the interpretation of these results. The full spectrum of ZIKV infections of pregnant women and the congenital infection of the fetus and newborn are yet to be fully understood.

## 825

### PERSISTENCE OF ZIKA VIRUS IN SEMEN OF MEN LIVING IN AN ENDEMIC AREA

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Zika virus (ZIKV) jeopardizes sexual health and reproductive health services. Sexual transmission of ZIKV is associated with viral persistence in semen after acute infection. Therefore, gamete preservation programs have been suspended in areas with active vector-borne transmission, like Trinidad and Tobago. However, data on ZIKV persistence in semen are scarce. We conducted a prospective observational study to assess the proportion of men living in an endemic area, who had persistent shedding of ZIKV in semen after confirmed infection. Symptomatic men with confirmed ZIKV infection and age  $\geq 18$  years, were recruited at Trinidad IVF Fertility Centre from June to October 2016. Clinical data and serial semen samples were collected. ZIKV infection was confirmed at the Institute of Tropical Medicine in Antwerp, Belgium, by ZIKV-specific real-time reverse transcriptase polymerase chain reaction (RT-PCR) on urine or semen samples, or by a virus neutralization test on serum. Follow-up of ZIKV shedding in semen was continued until ZIKV RNA could no longer be detected by RT-PCR in 2 consecutive samples. Cycle threshold (Ct-) values were used as a semi-quantitative measure of viral loads. Isolation of ZIKV from semen in culture (as a proxy for infectivity) was attempted in samples with low threshold Ct-values. Twenty-three men with confirmed ZIKV infection were recruited, mean age 36 years (range 25-53). The initial semen was collected at median 13 days post-infection (dpi) (range 2-25). ZIKV RNA was detected in 9/23 men (39.1%, 95%-CI [22.2-59.2]). ZIKV was isolated from three semen samples collected at 9, 12 and 17 dpi, with respective Ct-values 19, 21 and 18. Loss of ZIKV RNA detection had occurred in 4/9 men by 61 dpi, and in all men by 103 dpi. After acute infection, ZIKV-RNA was frequently detected in semen from men in Trinidad and Tobago. Our data suggest that clearance of ZIKV from semen occurred in 9/9 men. Testing of semen from ZIKV-infected men might assist in reducing the individual risk for sexual transmission of ZIKV.

## 826

### PRIOR INFECTION WITH DENGUE VIRUS SEROTYPE 3 DOES NOT ENHANCE SUBSEQUENT ZIKA VIRUS INFECTION IN RHESUS MACAQUES

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Antibody dependent enhancement (ADE) is an antibody-mediated enhancement of disease that occurs with secondary infections of heterologous serotypes of dengue virus (DENV). The emergence of Zika virus (ZIKV) in the Americas, a close DENV relative, has led to the question of whether ZIKV might function as a "fifth serotype" of DENV, causing enhanced pathology in persons with previous DENV exposure. An important question related to ADE is whether it also contributes to significant fetal injury following congenital ZIKV infection. We recently developed a rhesus macaque model to study ZIKV. To examine whether exposure to DENV enhances ZIKV infection, we infected 3 Indian-origin

rhesus macaques, previously infected with DENV serotype 3 (DENV-3) with an Asian lineage ZIKV isolate from French Polynesia (ZIKV-FP). We compared infection of these 3 animals with 2 animals that were infected with ZIKV-FP alone and 3 animals that were mock-infected using quantitative RT-PCR to assess viral burden, complete blood counts (CBC) to assess blood count responses to ZIKV infection, and blood chemistry panels to evaluate changes in blood biochemical parameters throughout infection. We observed no significant difference in ZIKV vRNA burden between the animals previously infected with DENV-3 compared to ZIKV alone or controls. We also did not detect rash, fever, significant weight loss, nor any significant changes in CBC values for any animals. Chemistry panels showed increases in LDH and SGOT in animals that were infected with ZIKV alone and decreases in blood glucose in DENV/ZIKV and ZIKV alone animals. Overall, our results were not indicative of enhanced ZIKV infection. Longitudinal analysis of cytokine and chemokine profiles from plasma samples of these animals are in progress with results expected in early summer.

## 827

### GAPS ON RISK COMMUNICATIONS APPROACH OF NATIONAL ZIKA VIRUS OUTBREAK RESPONSE IN COLOMBIA, BRAZIL, EL SALVADOR, GUATEMALA, HONDURAS AND DOMINICAN REPUBLIC

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To explore the needs, attitudes and practices of women, men, and youth related to Zika virus prevention. A cross-sectional survey using a mobile-technology-based structured questionnaire was administered to 3361 inhabitants of 6 countries (Brazil, Colombia, Dominican Republic, Guatemala, Honduras and El Salvador) during July-September 2016. The study used a 2-stage, 30-cluster sampling method. The study found that two-thirds of respondents attributed ZIKAV transmission to mosquito bites and contaminated water, only 4 and 1 percent respectively were aware that Zika can be spread by sexual intercourse and from a pregnant woman to her fetus. Four out of 10 respondents knew that there is a causal relationship between Zika and microcephaly in newborns. Most respondents agreed that Zika virus represents high or moderate risk in their communities and that it is a disease than can be prevented. However, participants' opinions were divided almost equally when asked if Zika virus infection could be treated. Participant responses on the use of preventive measures showed variation among the six countries. Participants in Guatemala, the Dominican Republic and El Salvador displayed a preference to adopt individual protective measures (mosquito net and mosquito repellent), while participants in Honduras and Colombia exhibited a preference towards water management. Participants in Brazil were evenly divided between protective measures (29%) and water management (29%). Aligned with the general lack of awareness on sexual transmission, there was no practice of sexual-related protection among participants in all six countries. Zika-related risk communication process in the six Latin American countries included in the study have been mostly focused on vector control. These findings clearly identify information priority gaps that need urgently to be addressed by regional and national stakeholder involved in public health activities to protect the most vulnerable population against Zika disease and its complications.

## 828

### DECIPHERING DURABLE NEUTRALIZING ANTIBODY RESPONSES TO ZIKA VIRUS

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In 2015, Zika virus (ZIKV) became a global health emergency as it spread throughout Latin America causing thousands of cases of birth defects.

ZIKV is a flavivirus closely related dengue, and is presumably amenable to vaccine-mediated prevention. However, a greater understanding of humoral immunity to this virus is needed to design interventions that are optimally protective and do not enhance disease with ZIKV or dengue infection. To address these issues, we aim to study long term humoral immunity that is attributable to memory B cells (Bmem) and create tools for assessing ZIKV vaccine-induced immune responses. We identified travelers with prior ZIKV exposure (2 dengue-naïve, 2 dengue-immune), immortalized and cloned Bmem for recombinant monoclonal antibody (mAb) expression. Screening of polyclonal culture supernatants revealed a ZIKV-reactive Bmem frequencies ranging 0.2-1.0%. Bmem from primary ZIKV cases produce mAb that are almost entirely type-specific. Interestingly, ZIKV type-specific clones are also readily detected on a dengue-immune background, which means that ZIKV immunity in secondary flavivirus infection is not dominated by cross-reactive Bmem clones in contrast to what is observed in heterologous secondary dengue infection. We are further characterizing neutralizing mAb derived from a primary ZIKV case to determine epitope specificity, ability to protect against *in vivo* challenge, and representation in large panels of ZIKV-immune human serum. As with dengue, our data indicates that ZIKV-specific neutralizing antibodies recognize complex structural epitopes present on the intact virion but not recombinant E protein monomers.

829

### ZIKA VIRUS INFECTION OF RHESUS MACAQUES VIA MOSQUITO BITE

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Zika virus (ZIKV) is primarily transmitted by *Aedes aegypti* mosquitoes, but animal models of ZIKV pathogenesis rely on needle inoculations. Saliva delivered to the host by a biting mosquito may have a critical impact on the initial infection of the skin and may modulate the local innate and adaptive immune response, so needle inoculation may fail to fully recapitulate important biological aspects of natural ZIKV infection. We previously established a rhesus macaque model of ZIKV infection using subcutaneous (sc) inoculation to initiate infection. Here, we show that *Ae. aegypti* can be used to initiate systemic ZIKV infections in rhesus macaques. To assess differences in ZIKV replication between virus delivery by needle versus mosquito vector, we infected rhesus macaques with the Puerto Rican ZIKV isolate PRVABC59 by either sc inoculation (n=3) or by exposure to infected mosquitoes (n=4). All three sc-inoculated macaques were productively infected, with viral load dynamics similar to what we have reported previously. Four of four animals also were productively infected by mosquito bite, with noticeable differences in peak viral load and the time to peak viral load. In addition, we used *in vitro* methods to estimate the ZIKV dose inoculated by mosquitoes and found that *Ae. aegypti* saliva titers ranged from 101.5 to 103.2 PFU. Analysis of ZIKV tissue distribution is ongoing, but preliminary analyses demonstrate the presence of viral RNA in multiple lymphoid tissues of those animals infected via mosquito bite at 14 days post feeding. Data on ZIKV tissue distribution in sc-inoculated animals, as well as a characterization of the ZIKV variant repertoire present in mosquito saliva compared to needle inoculated virus, is anticipated later this spring and can be accessed in real-time at: <https://zika.labkey.com/project/OConnor/ZIKV-023/begin.view>. This tractable laboratory model demonstrates the feasibility of using direct mosquito feeding on nonhuman primates to better understand ZIKV transmission, systemic spread, and factors that influence disease outcomes in natural infections.

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### LOW PREVALENCE OF ANTIBODY PERSISTENCE 10 YEARS AFTER HEPATITIS E VIRUS INFECTION AMONG PREGNANT WOMEN IN NORTHERN BANGLADESH

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Hepatitis E Virus (HEV) is a major cause of viral hepatitis in developing countries with up to 30% case fatality among pregnant women. HEV is vaccine preventable yet is responsible for over 10% of all maternal mortality in Bangladesh. In South East Asia, HEV causes yearly outbreaks despite being highly endemic, suggesting that antibody persistence after infection is low. From 2001-2010, 103 pregnant women from Gaibandha, Bangladesh experienced an asymptomatic HEV infection. In 2016 (6 to 15 years after the infection), these women were revisited to examine long-term antibody persistence. We were able to revisit 86 of the 103 (83.5%) women. Antibody loss was high; 88.4% (95% CI: 80.0-94.3%) did not have detectable antibodies at follow-up. 30.3% of those who lost antibodies owned goats compared to 0% with persistent antibodies (X2 p=0.04). A higher percentage of those with persistent antibodies reported taking medicine for gastric illness (50% positive versus 19.7% negative, X2 p=0.03). None of the other demographic or exposure risk factors examined were associated with antibody persistence, including time since infection. Only a few years after infection, 88.4% of pregnant women no longer had detectable antibodies. This was much higher than previous findings of 20.0% (95% CI: 12.0, 28.0) antibody loss 10 years after HEV infection in the general population in Southern Bangladesh. It is unclear why the antibody persistence is so different in these two rural populations with similar health indicators but may highlight the importance of environmental re-exposures. In the less flood-prone Gaibandha, in Northern Bangladesh, environmental re-exposure to HEV may not occur frequently, whereas in Southern Bangladesh, an area surrounded by rivers and highly prone to severe floods, re-exposure to HEV may provide immune-boosts, preventing antibody loss. Since antibody titres waned quickly after infection in this population, adolescent immunization and frequent booster doses may be necessary to protect pregnant women from the severe consequences of HEV infection.

831

### ARBOVIRUS SURVEILLANCE

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Arboviral diseases affect hundreds of millions of people every year. Yellow fever, West Nile, dengue, chikungunya, and Zika are persistent threats globally to people living in or visiting affected areas. Timely and accurate surveillance of arboviruses at multiple geopolitical scales is critical to prevention and control. However, surveillance systems are often shaped by historical and practical considerations rather than specific public health objectives. We developed a method to leverage 15 years of dengue clinical data and laboratory testing data from Puerto Rico to design an efficient arbovirus surveillance system that is expected to provide timely, accurate, and efficient situational awareness of island-wide, regional, and serotype-specific dengue activity. Among 105 healthcare providers reporting in the Puerto Rico dengue surveillance system, we identified a set of 22 providers that are as informative as all 105 with respect to the specified public health awareness goals. Out-of-sample comparisons with historical data suggest that this streamlined system will perform robustly. We also identified an alternative strategy for targeting surveillance efforts for emerging threats when historical information is unavailable. As public health agencies throughout the Americas strive to track and

respond to the evolving threats of dengue, chikungunya and Zika, timely and accurate surveillance is paramount. A carefully targeted surveillance system can streamline data collection, improve situational awareness, and guide allocations of surveillance resources for dengue and other arbovirus threats. This data-driven design process can be easily and broadly applied to establish and improve arbovirus surveillance, and its data-free alternative provides a valuable tool for rapid implementation in response to emerging threats.

## 832

### SENTINEL SURVEILLANCE OF INFLUENZA VIRUS IN MALI

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To better define the burden of influenza in developing countries, the Center for Vaccine Development of MALI (CVD-Mali), Minister of Health Mali instituted national influenza sentinel surveillance in 2014 in both rural and urban sites. A total of five Influenza like Illness (ILI) sites and three Serious Acute Respiratory Infection (SARI) sites have been activated respectively in 5 Reference Health Center and 3 Hospitals. Samples were collected at all sites four days a week by following the WHO case definition, and delivered to the laboratory. Samples were transported in VTM at 2-4°C and delivered within 72 hours. The samples were processed at the laboratory approximately every 48 hours. The RNA extraction was performed per CDC standard methods (QIAamp @ viral RNA kit) and tested immediately by real-time RT-PCR CDC protocol for the detection and characterization of influenza virus. Data analysis was performed using the Biosystems™ software applied to the system ABI7500 real-time PCR. Possible outcomes include influenza A, B, H1N1 (swine flu) and H5N1 (bird flu). A weekly report is sent to all sites and the Ministry of Health, CDC, WHO country office and WHO Influenza Network. From November 24th, 2014 to December 29th, 2016, 2313 samples were received in the laboratory from 1988 ILI and 325 SARI cases. The diagnostic confirmed 162 Influenza B, 237 Influenza A (80 A (H1N1) pmd2009, 157 Influenza A (H3N2) and 1914 were negative to Influenza. Among SARI cases, 22 were positive to influenza (5 influenza B, 6 Influenza A (H1N1) pmd2009 and 11 Influenza A (H3N2)). Seasonal peaks of Influenza are observed in the year: from November to March with predominant of Influenza B and H1N1pmd2009 and from July to September with most H3N2 cases. The year round of surveillance shown that young children from 2-15 years old are most vulnerable. Two years of surveillance data have demonstrated that influenza is a public health problem. Vaccination would be a useful control strategy.

## 833

### ASSOCIATION BETWEEN SECRETOR STATUS AND NOROVIRUS INFECTIONS IN A BIRTH COHORT IN SOUTH INDIA

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Noroviruses bind to histo blood group antigens (HBGA) found on the surface of gut epithelial cells, as well in saliva and other secretions. Expression of the HBGA antigen is in part controlled by *FUT2* gene; individuals who express the HBGA antigens on the gut epithelial cells are

known as “secretors”. In this study, we evaluated the rate of norovirus infections in the first 3 years of life in 10 secretor positive and 5 secretor negative children. These 15 children were part of a birth cohort study conducted in Vellore, India, involving 373 children who were followed up to the age of 3 years. The 373 children had 1856 diarrheal episodes which were tested for norovirus GI and GII, rotavirus and other common enteropathogens. Of the 10 secretor positive children, 5 had at least 1 episode of norovirus diarrhea during the 3 years of follow-up. The 5 secretor negative children had no norovirus diarrhea in 3 years. All surveillance stool samples collected every 2 weeks from birth till 3 years of age from the 15 children (approximately 70-80/child) were tested by quantitative multiplex real-time PCR for detecting norovirus genogroups GI and GII. Overall, 14.7% (63/428) of surveillance samples from the 5 secretor positive children who had norovirus diarrhea were positive for noroviruses (16 GI, 45 GII, 2 mixed), 15.2% (71/468) surveillance samples from 5 secretor positive children without norovirus diarrhea were positive for noroviruses (23 GI, 46 GII, 2 mixed), and 13.5% (47/349) of samples from the 5 secretor negative children who had no norovirus diarrhea, were positive for noroviruses (11 GI, 36 GII). In conclusion, there was no significant difference in the rate of norovirus infections between the secretor positive and secretor negative children, suggesting that noroviruses do infect secretor negative children.

## 834

### DETECTION OF HUMAN ANELLOVIRUSES (TORQUE TENO VIRUS, TORQUE TENO MIDI VIRUS, AND TORQUE TENO MINI VIRUS) FROM THE ACUTE RESPIRATORY INFECTION CONSORTIUM (ARIC) NATURAL HISTORY STUDY

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Human anelloviruses are a recently discovered virus family and include torque teno virus (TTV), torque teno midi virus (TTMDV) and torque teno mini virus (TTMV). Human anelloviruses are genetically diverse, cause chronic infections in humans, and are highly prevalent worldwide with epidemiology studies reporting infection prevalence above 75% in adults in several countries. The link between human anellovirus infection and clinical disease or pathology in humans is unclear, however studies have suggested the possible association of anellovirus infection and a variety of conditions including cancers, hepatitis, autoimmune disorders, and respiratory diseases. Using patient samples collected as part of the Acute Respiratory Infection Consortium (ARIC) Natural History Study at U.S. military treatment facilities in which otherwise healthy subjects were enrolled within three days after influenza-like illness (ILI) onset, we 1) determined the prevalence of human anelloviruses from nasopharyngeal swabs collected from patients with both etiologically diagnosed and undiagnosed acute respiratory infections using conventional PCR and targeted deep sequencing and 2) report progress on a real-time PCR (qPCR) assay to detect and quantify torque teno virus from patient samples during the acute and convalescent phases of acute respiratory infection. Overall, we report high prevalence of all three human anelloviruses (TTV, TTMDV, TTMV) from ILI patient samples and describe a qPCR assay that

can be used in future studies to investigate the prevalence of TTV and improve our understanding of the clinical relevance of TTV as an emerging viral diagnosis in humans.

### 835

#### SECRETOR STATUS AND ITS ASSOCIATION WITH THE ROTAVIRUS DIARRHEA AND ANTIBODY RESPONSE IN A BIRTH COHORT FROM SOUTH INDIA

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Individuals expressing the histo blood group antigen (HBGA) on the gut epithelial cells, which is in part controlled by the *FUT2* (*fucosyl transferase 2*) gene, are called secretors. The relation between HBGA functionality and rotavirus infection is not well documented. This study investigates the association between the *FUT2* secretor status and rotavirus diarrhea among a birth cohort from an Indian slum. The cohort of 373 children was followed up to the age of 3 years to understand the natural history of rotavirus infection and disease. Genomic DNA was isolated from buccal epithelial cells or blood by amplification of a region of the *FUT2* gene and genotyping for two single nucleotide polymorphisms (G428 and G385) was carried out. All the diarrheal and surveillance stool samples (collected every two weeks) were tested for rotavirus G and P types by a hemi-nested multiplex PCR. Of the 373 children, secretor status was assessed for 305 kids, of which 200 (65.6%) kids were secretor positive while 105 (34.4%) were secretor negatives. Of the 305 children with known secretor status, 232 (76.1%) had at least 1 episode of rotavirus diarrhea during the 3 years, and there was no significant difference between secretors and non-secretors ( $p=0.418$ ). However, Children with G428 homozygous mutation for inactivation of the *FUT2* (se428 se428) were at a significantly lower risk (43/232) of rotavirus diarrhea ( $p=0.04$ ). No significant association between secretor status and the number of rotavirus infections during the 3 years of follow-up was observed. The rotavirus genotype distribution was similar among the secretors and non-secretors, with G2P[4], G1P[8], G10P[11], G1P[4] and G9P[8] being the common circulating strains. However, both IgG and IgA antibody response to rotavirus infection was significantly lower among secretor negative children ( $p<0.001$ ) when compared to secretor positive children. To conclude, although secretor status does not significantly affect the rate of rotavirus infection in children followed up to 3 years of age, the secretor positive children mount a substantially higher immune response to rotavirus infection than secretor negatives.

### 836

#### EFFECT OF AGE AT VACCINATION ON ROTAVIRUS VACCINE EFFECTIVENESS IN BOLIVIAN INFANTS

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Efficacy of rotavirus vaccines is lower in developing countries versus developed countries. One hypothesis is that higher levels of maternal antibodies in developing countries could interfere with vaccine response, suggesting that delayed dosing could be beneficial. The present analysis aims to assess whether rotavirus vaccine effectiveness (VE) varies by age at vaccination during routine use in Bolivia. Data were merged from two post-licensure evaluations of monovalent rotavirus vaccine (RV1) in Bolivia, where RV1 is recommended at two and four months of age. For each dose, children were classified as receiving each dose "early," "on-time," or "late." Stratified unconditional logistic models were used to estimate VE, using unvaccinated children as the referent. VE was calculated as  $(1 - \text{odds ratio}) \times 100\%$ . Models were adjusted for hospital, age, and time since RV1 introduction (via including terms for month and year of birth). VE for two doses of RV1 tended to be higher in infants receiving the first dose early (VE 90%; 95% confidence interval [CI] [67%, 98%]), when compared to

infants receiving their first dose on time (62% [48%, 72%]) or late (53% [32%, 66%]). Estimates of RV1 VE for two doses were not substantially different when comparing children by age at second dose. We found high effectiveness of RV1 when given at an early age, supporting the current WHO recommendations for administration of rotavirus vaccines.

### 837

#### ROLE OF MAMMALIAN IMMUNE RESPONSES IN VECTOR-ENHANCED ORBIVIRAL TRANSMISSION

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*Culicoides sonorensis* biting midges are vectors of several emerging and re-emerging orbiviruses including bluetongue, epizootic hemorrhagic disease, and African horse sickness viruses. They feed primarily on domestic sheep and cattle, but opportunistically feed on a variety of wildlife and on humans. The ability to obtain blood meals from this wide host range speaks to the versatility of midge salivary components, 45 of which we recently identified including anti-hemostatic factors and protease inhibitors, as well as proteins to immunomodulate host defenses. The extreme efficiency with which *Culicoides* midges can transmit orbiviruses, and the basis of anecdotal evidence for vector-enhanced orbivirus transmission by midge feeding, are unclear. One reason is that very little is known about the natural effects of *Culicoides* bites on mammalian hosts, particularly in the initial hours post feeding; a critical time for transmitted viral particles to establish infection. To gain insight as to why *C. sonorensis* are such efficient vectors for orbiviruses, we used a mouse model to characterize immune responses in the first three days after midge feeding. Analysis of skin, lymph node cell populations and cytokines by histology, flow cytometry, and real time PCR indicated a Th-mediated cellular response with significant mast cell activation, hemorrhaging, edema and vasodilation, as well as rapid dermal infiltration of CD16/32+ granulocytes, known infection targets of orbiviruses. In contrast to the hostile mammalian immunological environment we hypothesized would confront any bite-transmitted virus particles, effects on the dermal vasculature and lymphoid tissues in response to midge feeding led to a local, then systemic immunological state highly favorable for orbivirus infection and dissemination. The interactions of these beneficial innate immune responses with bite-transmitted virus is likely key to the transmission competency of this vector and play an important role in vector-enhanced transmission. These beneficial elicited responses may well extrapolate to other competent vector - arboviral disease associations.

### 838

#### CLINICAL AND EPIDEMIOLOGICAL CHARACTERISTICS OF ONCOLOGICAL PATIENTS INFECTED WITH HUMAN T-CELL LYMPHOTROPIC VIRUS (HTLV-1) AT THE NATIONAL CANCER CENTER OF PERU, 2010-2015

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Peru is a country where Human T-cell lymphotropic virus type 1 (HTLV-1) is endemic. The objective of the study is to describe clinical-epidemiological characteristics of HTLV-1 infected patients at the national cancer center of Peru, Instituto Nacional de Enfermedades Neoplásicas (INEN) during the period 2010-2015. This is an observational study of HTLV-1 cases diagnosed by positive confirmatory test in cancer patients. Clinical and sociodemographic information was obtained from medical records. 205 oncological cases with HTLV-1 infection were collected. The average age was 57.11 years and the male-female ratio was 0.38. The highland of Peru was the most common place of birth (58.5%). The most common neoplasms were non-Hodgkin's lymphoma (13.7%) for hematologic

malignancies and cancer of the cervix (56.1%) for solid tumors. 41.9% of patients had HLTV-1 associated disease: onychomycosis (24.4%), pulmonary tuberculosis (9.8%) and scabies (4.4%). ( $P < 0.01$ ). Have been breastfeeding was the most common risk factor of infection ( $P < 0.01$ ). The clinical-epidemiological characteristics of HTLV1-infected patients are similar to those reported in the Peruvian population. The high frequency of HTLV-1 infection in patients with cervical cancer could be related to either the immunosuppression by the retrovirus.

### 839

#### SEASONALITY AND GEOGRAPHIC DISTRIBUTION OF ROTAVIRUS DIARRHEA IN CHILDREN <5 YEARS FROM A NATIONAL SURVEILLANCE STUDY

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Rotavirus is the leading cause of diarrhea related hospitalization among infants and young children worldwide. In India, rotavirus diarrhea accounts for over half a million hospitalizations among under five children annually. We did a prospective, multi-center hospital based surveillance to investigate the seasonality and geographic distribution of rotavirus gastroenteritis among under five children in India. The seven sites included Vellore and Trichy (Tamil Nadu), Kolenchery (Kerala), Tirupati (Andhra Pradesh), and Hyderabad (Telangana) which are from the southern part of India while Ludhiana (Punjab), and New Delhi are from the north. During the surveillance period from July 2012 to June 2016, 6935 children with acute gastroenteritis were enrolled into the study and stool samples were collected from 6550 children. All the samples were tested for rotavirus positivity using EIA and the overall positivity was (34.9%). During the study period, the maximum number of rotavirus related hospitalizations were reported from December 2013 through February 2014 (241 [10.5%]). Overall, a seasonal variation in rotavirus diarrhea was observed, with the maximum number of cases (791 [31.5%]) seen during winter season (December-February), which was followed by summer season (March - May) (634 [27.6%]), and rainy season (September-November) (516 [22.5%]). The pattern was similar between the northern and southern sites of India. There is a high burden of rotavirus gastroenteritis among under five children in India which shows a seasonal variation with higher positivity rates during the winter months.

### 840

#### CURRENT MEDICAL TREATMENT FOR MIDDLE EAST RESPIRATORY SYNDROME: A SYSTEMATIC REVIEW

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Middle East Respiratory Syndrome (MERS) is a novel viral respiratory disease caused by MERS-Coronavirus (MERS-CoV), and the first reported case was in Saudi Arabia in 2012. There is no specific treatment for MERS, and it ranges from supportive treatment to antiviral treatment like interferon (IFN) a 1a, IFN b 1a, and ribavirin. We conducted a systematic search on ten databases Studies published after 1/1/2012 and reporting information about treatment of MERS-CoV infection were included in our review. We used Mann-Whitney U, Chi2 and Fisher's exact tests to investigate the relation between the mortality outcome and independent variables. Classification tree model was used to find the best predictors of the mortality. We included 16 papers including ten case reports, two case series, and four observational studies. Despite receiving treatment with ribavirin plus IFN, the mortality rate was as high as 71% of 68 patients in IFN-treatment group and exactly the same (71% of 48 patients) in patients who received supportive treatment only. There was a significant difference between died and survived patients in chronic respiratory disease (CRD), diabetes mellitus (DM), hypertension, renal replacement therapy and ventilation. Indicating that having CRD, hypertension or DM and being ventilated increase the risk of mortality (for all of these factors). While there was no significant difference between died and survived patients in gender, ribavirin, corticosteroid, oseltamivir, IFN beta 1a, IFN alpha 2b, IFN alpha 2a, congestive heart failure (CHF), other comorbidities (p less than 0.05). There was a significant difference between died and survived patient in age, being older in died patients, and time from admission to antiviral treatment start being larger in died patients as well (p less than 0.05). The IFN treatment has shown no efficacy over supportive treatment only. Antiviral treatment delay, older age group, and co-morbidities pre-existence (hypertension, diabetes mellitus, chronic kidney disease, dialysis dependent) were associated with worse outcome.

### 841

#### OROPUCHE VIRUS IDENTIFICATION AS AN EMERGING ETIOLOGICAL AGENT RESPONSIBLE FOR ACUTE FEBRILE DISEASE IN A EASTERN MIDDLE REGION OF THE PERUVIAN JUNGLE

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Oropuche virus (OROV) is a member of the Bunyaviridae family from the Orthobunyaviridae genus, this viral RNA genus has more than 170 agents distributed in 18 different serogroups. OROV has been identified as a causal agent of the "Fiebre Oropuche", a febrile/tropical disease transmitted by arthropod vectors. Currently, the only notified cases of Fiebre Oropuche have been reported in Brazil, Panama, Peru and Trinidad y Tobago with a high incidence during the periods with increased precipitations as it favors the vector reproduction. This study was undertaken to assess the frequency of Oropuche (OROV) and describe its clinical presentation in patients with acute febrile illness from Huánuco, Peruvian Jungle, Peru. A total of 268 serum samples from thirty five health care centers were studied for the presence of OROV via RT-PCR

from November 2015 to July 2016. PCR positive samples were sent for commercial sequencing. Physicians used a standardized questionnaire to collect the demographic and clinical symptoms information. OROV virus RNA was detected in 17.35% (46/268) of samples via qPCR. The most common symptoms accompanying fever were: Arthralgias 65.22% (30/46), Myalgias 76.09.0% (35/46), Headache 86.96% (40/46), retro-orbital pain 60.87% (28/46), Low back pain 34.78% (16/46), Skin rash 32.61% (15/46), hyporexia 50.0% (23/46), Sore throat 34.78% (16/46) and sickness 47.83%(22/46). In conclusion, OROV is currently a predominant virus in Huanuco, Peruvian Jungle, Peru. The clinic isn't sufficient to diagnose cases of ORV, for This reason it's necessary to have a laboratory diagnosis. Further investigations should be conducted to evaluate the use of RT-PCR as a reliable method for OROV and for surveillance the other arbovirus in Peru.

## 842

### ANALYSIS OF THE *BABESIA MICROTI* PROTEOME IN INFECTED RED BLOOD CELLS BY A COMBINATION OF NANOTECHNOLOGY AND MASS SPECTROMETRY

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Babesiosis has been recognized in recent years as an emerging infectious disease in humans. The disease is caused by red blood cell (RBC)-infecting protozoan parasites of the genus *Babesia*. Transmission occurs primarily by ticks or less frequently through blood transfusion. There is an increased need to develop better tools for the design of drugs, vaccines, and biomarkers of infection for babesiosis. Here, we used a combination of nanotechnology and mass spectrometry (MS) to describe the proteome profile of *Babesia microti* in infected RBCs. Protein extracts were obtained from whole blood samples of 14-day infected hamsters showing 30% parasitemia. Proteins were captured from the extracts in bait-loaded nanoparticles, eluted, and analyzed by reverse-phase liquid chromatography-MS. The identification of tandem mass spectra was performed by searching against the NCBI *B. microti* database using the SEQUEST software. Using this approach, we identified ~500 proteins that shared homology with known and putative proteins of the genome-sequenced *B. microti* R1 strain. Ascribed functions of *B. microti* proteins identified in infected RBCs include intracellular signaling, protein translation and modification, protein transport, DNA replication, cytoskeleton support, and lipid and glucose metabolism. We also identified surface antigens shown to play critical functions in the immunopathogenesis of Apicomplexan parasites, such as GPI-anchored proteins and members of the *B. microti* sero-reactive surface antigen family. By applying recent advances in nanotechnology and proteomics, we successfully captured and identified proteins of *B. microti* from whole blood. Moreover, ours is the first evaluation of the proteome of *B. microti* in infected RBCs, information that could be relevant to the study of intracellular parasite survival, discovery of anti-parasitic compounds, and development of diagnostic tests.

## 843

### SEQUENCE CONSERVATION IN THE IMMUNODOMINANT *BABESIA MICROTI* ANTIGENS

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The Apicomplexa tick-borne hemoprotozoan parasite *Babesia microti* is responsible for human babesiosis in the Northeast and upper Midwest regions of the United States and globally. Most healthy adults infected with *B. microti* experience mild to moderate illness; however, the disease

can be fatal in immunocompromised individuals and those who acquire the disease through blood transfusion. Through genome-wide immunoscreening, we have identified over 50 immunodominant *B. microti* antigens that are reactive against plasma/serum from babesiosis patients. Among these, the top 3 ranking antigens - *Babesia*  $\alpha$ -helical cell surface domain (BmBAHCS), Serine Repeat Antigen (BmSERA) and Epidermal Growth Factor (BmEGF) are being pursued as diagnostic and vaccine antigens with promising results. In order to investigate the antigenic polymorphism among these antigens, we have determined the nucleotide sequence and deduced amino acid sequences by PCR-amplification of the full length gene of each molecule from 7 human samples isolated from *B. microti*-infected individuals. This analysis showed that the nucleotide and amino acid sequences were identical for BmBAHCS and BmSERA, but BmEGF showed alterations at two amino acid positions, Lys135 to Asn135 and Gly168 to Ile168. The biological significance of these mutations is not known, although these changes did not influence the reactivity of recombinant BmEGF protein to the serum samples from babesiosis patients. Our data suggest lack of immune pressure among immuno-dominant *B. microti* antigens. Bioinformatics analysis is underway to understand the impact of these mutations on the function of this molecule. The significance of limited or absent antigenic polymorphism in immuno-dominant *B. microti* antigens in parasites in endemic areas will be discussed.

## 844

### IDENTIFICATION OF PROTEIN PROFILES OF *BARTONELLA BACILLIFORMIS* STRAINS FROM ENDEMIC DEPARTMENTS OF PERU

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*Bartonella bacilliformis* is the etiologic agent of Carrion's disease, endemic in andean valleys of Peru, Ecuador and Colombia. In extreme poverty areas of Perú, it constitutes a serious public health problem, affecting mainly the child population. The aim of this research was to determine the variants of the protein profiles of *Bartonella bacilliformis* strains from endemic regions of Perú. For the research, bacterial colonies were collected from strains subcultures from the departments of Ancash, Cusco, Cajamarca, La Libertad, Lima and Piura; which were lysed by sonication to obtain total soluble proteins. The protein components of the bacteria were separated by vertical electrophoresis in 8% sodium dodecyl sulfate gels (SDS-PAGE), for the visualization of the bands the gel was dyed with silver nitrate. The determination of the fractionation Ranges and molecular weights was performed on the ChemiDocXRS+Image Lab software for PC or Mac (Bio-Rad) gels. The strains from Piura, Cajamarca and Lima presented the highest number of bands from 31.30 to 297.93 kDa, with fractionation ranges between 0.98 and 0.04; the highest concentrations were observed between 32 and 41 kDa present in strains from Piura, Cajamarca and Cusco. In strains from La Libertad, Ancash and Lima the 32 kDa protein was not visualized. The knowledge of the antigenic profiles of *Bartonella bacilliformis* circulating strains in endemic Peruvian departments has made possible that the National Institute of Health develop and implement confirmatory, sensitive and specific Western Blot diagnostic method, that recognises specific epitopes for IgM and IgG antibodies, showing four antigenic bands: 136.60 kDa, 122.70 kDa, 60.95 kDa y 56.60 kDa. The test could allow the early and accurate diagnosis of Carrion's disease cases that occur in high and extreme poverty Peruvian endemic areas, for the immediate antimicrobial treatment, having a high impact on the affected population health.

## 845

### MOLECULAR CHARACTERIZATION BY MULTI-LOCUS SEQUENCE TYPING OF *RICKETTSIA ASEMBONENSIS* AND OTHER *RICKETTSIA FELIS*-LIKE ORGANISMS, PERU

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*Rickettsia asembonensis* and Candidatus *Rickettsia senegalensis* are *R. felis*-like organisms (RFLOs). Although it is unclear if these RFLOs are pathogenic to humans, their closest known relative *R. felis* does cause human disease, and thus, it is possible that these potential pathogens are an emergent threat in rural areas of Peru, where rickettsial infections are common and frequently go under-diagnosed and under-reported. *R. asembonensis* was first discovered in Kenya. Since then, multiple *R. asembonensis* strains have been reported worldwide. Candidatus *R. senegalensis* is a novel member of the RFLO group that so far has been reported in African countries, India and the USA. Despite these reports, there is limited genomic information on these strains, which hinders evolution and phylogenetic studies aimed at understanding the relationships among multiple RFLOs around the world. Recently we identified fleas and ticks that were positive for *R. asembonensis* and other RFLOs. Interestingly, no *R. felis* was detected. Here we report multi-locus sequence typing (MLST) characterization of nine diverse RFLO isolates collected in Iquitos and Puerto Maldonado, in Peru. We used genomic information derived from next-generation sequencing data to generate complete open reading frames (ORFs) for two conserved genes (*17-KDa* and *gltA*), and three variable genes (*ompA*, *ompB*, and *sca4*). The sequence information was also used to infer phylogenetic relationships among rickettsial strains. We find that RFLO strains collected in Peru are distinct from other isolates described thus far. This work will help to better understand the epidemiology of rickettsial infections in Peru and in Latin America, where novel rickettsial strains have potential as new emergent human pathogens.

## 846

### THE DETECTION OF SPOTTED FEVER GROUP *RICKETTSIA* DNA IN TICKS AND HUMAN SAMPLES FROM PASTORAL COMMUNITIES IN KENYA

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Undiagnosed febrile diseases are common in Kenya. This study tested the significance of *Rickettsia* spp. infections as a cause of febrile illness in humans living in pastoral communities where the presence of arboviruses in vectors and humans had previously been reported. Human blood and tick samples collected from several geographically dispersed pastoral communities in Kenya were tested for acute infections of *Rickettsia* spp. bacteria by conventional PCR using three primer sets targeting *gltA*, *ompA* and *ompB* followed by sequencing. *Rickettsia* spp. DNA was detected in 14% (39/278) of human blood samples tested using the *gltA* primer set. The human samples were negative on testing with the *ompA* and *ompB* primers. *Rickettsia africae*, *R. raoultii* and *R. aeschlimannii* DNA were detected in the subset of positive blood samples that were sequenced using the *gltA* primer. Of all the tick pools screened, 25% were positive for *Rickettsia* spp. DNA using the *gltA* primer set. The *Rickettsia* spp. identified by sequencing of forty one tick PCR amplicons were *R. aeschlimannii*, *R. africae*, *R. monglotimonae*, *R. raoultii*, *R. montanensis* and *R. parkeri*. The findings in this study suggest that rickettsioses also contribute to cases of

febrile illness in pastoral communities in Kenya. The high percentage of ticks from camels infected with *Rickettsia* suggests that camels may be involved in the maintenance of SFG *Rickettsia* in Kenya. *R. aeschlimannii* DNA was detected in human blood samples - the first time this has been reported in Kenya.

## 847

### INHIBITION OF B-TRYPTASE BY MOSQUITO SERPINS IS MEDIATED BY DISSOCIATION OF THE ACTIVE TETRAMER

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Saliva of mosquito and other blood-sucking arthropods contains a complex mixture of anti-hemostatic, anti-inflammatory, and immunomodulatory compounds that facilitate blood feeding and pathogen transmission. We previously showed that the main anticoagulant of *Aedes albopictus* (Alboserpin) mosquitoes belong to the serpin superfamily of protease inhibitors targeting the coagulation factor Xa. In addition to its anticoagulant activity, we discovered that Alboserpin also inhibits b-tryptase, the most abundant secretory serine protease in human mast cells. The inhibition of b-tryptase by Alboserpin appeared to be reversible, since excess heparin resulted in reactivation of the catalytic activity of this enzyme. These results indicate that Alboserpin inhibition of b-tryptase is mediated by destabilization of active tetramer rather than direct inhibition of the catalytic sites of the enzyme. Alboserpin also showed a potent anti-inflammatory effect on paw edema formation. This anti-inflammatory activity was also confirmed in cultured endothelial cells. Inhibition of the mast cells b-tryptase could attenuate the inflammation and vasoconstriction at the mosquito biting site and represent a strong adaptive evolution in hematophagy.

## 848

### MATRIX-ASSISTED LASER DESORPTION/IONIZATION TIME-OF-FLIGHT MASS SPECTROMETRY FOR RAPID IDENTIFICATION OF MEDICALLY IMPORTANT MOSQUITOES

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Accurate and rapid identification of vectors is vital for the success of control programs. Conventionally, vector identification is done on the basis of morphological keys. Detection of the internal transcribed spacer (ITS2) of the rDNA has also been commonly used. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is now widely used for bacteria and fungi, but less explored for insects. We used MALDI-TOF MS for protein-profiling of the mosquito species from Chandigarh (North India) with the aim of creating their database. Mosquito larvae were collected from different rural and urban areas and reared to adult stages. Using the morphological keys, ITS2 PCR and species confirmation by sequencing, ten species were found, viz. *Anopheles stephensi*, *An. culicifacies*, *An. annularis*, *An. lindesayi*, *Aedes aegypti*, *Ae. albopictus*, *Culex quinquefasciatus*, *Cx. vishnui*, *Cx. tritaenorrhynchus*, *Armigeres subalbatus*. Cephalo-thorax part of the mosquitoes was selected for MALDI-TOF MS analysis on Microflex MALDI-TOF MS (Bruker Daltonics, Germany). The spectra so obtained were then processed using the Biotyper v3.0 and Flex Analysis v3.3. Five specimens of each species were processed in this way and the average spectral profiles so obtained were selected for database creation. Sixty coded mosquito specimens (six from each species) were then subjected to blind-testing. A score of >1.8 was used to denote reliable genus and species identification. The average number of peaks produced by different mosquitoes were 55-60 peaks for *Anopheles*, 80-100 for *Aedes*, 30-60 for *Culex* and 45-50 for *Armigeres* species, and a specific protein signature was obtained for these species. It was found that 58/60 (96.7%) were correctly identified with a score

of >1.8. *E. coli* ATCC 25922 gave a reliable score (>2) every time, when matched against the in-built database of Biotyper. Thus, MALDI-TOF MS appears to be a pragmatic technique for accurate and rapid identification of mosquito species. The database can be further expanded to include larvae and pupae stages and also species from different geographical regions.

## 849

### WANGA IN CELL CULTURE: TOOLS FOR STUDYING ASSOCIATIONS BETWEEN *ANOPHELES* AND *WOLBACHIA*

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*wAnga* is to date the first and only strain of *Wolbachia* that has been identified in natural populations of *Anopheles gambiae* mosquitoes, the principle vectors of malaria (Baldini et al., (2014) Nat. Comm. 5:3985). *wAnga* is negatively associated with natural *Plasmodium falciparum* coinfection (Shaw et al., (2016), Nat. Commun. 7:11772), but there is no evidence that it can cause cytoplasmic incompatibility, limiting its potential use in vector control. Moreover, *wAnga* is also found at very low densities in the sister species *An. coluzzii*. We hypothesize that *wAnga*'s low titers may limit its ability to cause reproductive phenotypes in its host. We have established *wAnga* infections in *An. coluzzii* Mos55 cells and *Drosophila melanogaster* S2 cells, and are using cell culture to facilitate the study of this bacterium. We will make comparisons between *wAnga*'s ability to grow in *An. coluzzii* Mos55 cells and *D. melanogaster* S2 cells, which could provide valuable insight regarding the hospitability of the anopheline environment for *Wolbachia*. We are also working to transfer the *wAnga* strain via cell culture into other anopheline species. We hypothesize that an *Anopheles*-specific strain is more likely to be successfully transferred and stably maintained in other anophelines than *Aedes*- or *Drosophila*- specific *Wolbachia* strains, which have been met with recalcitrance in *Anopheles* thus far. Furthermore, we are striving to manipulate titers of *wAnga* using cell culture systems, with the goal of translating these findings into *in vivo* tools for the study of *wAnga*-induced host phenotypes. We are also taking advantage of the ability of cell culture to facilitate whole genome sequencing efforts by improving the capacity to obtain high purity DNA in higher ratios. This research will provide new knowledge to the field of innovative vector control against *Anopheles* mosquitoes, specifically towards assessing the potential of *Wolbachia* as a vector control agent for malaria transmission.

## 850

### THE ADULT *Aedes aegypti* MOSQUITO MIDGUT PERITROPHIC MATRIX PROTEOME

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The *Aedes aegypti* mosquito is the principle vector of arboviruses such as dengue, chikungunya, yellow fever and Zika virus. These arboviruses are transmitted during adult female mosquito blood feeding. While these viruses must transverse the midgut to be transmitted, the blood meal must also reach the midgut to be digested and subsequently used for egg development. However, this is a high-risk, high-reward process, as aggregation of blood meal metabolites can be toxic to the female mosquito midgut. Understanding the mechanisms that allow for midgut protection may provide novel molecular-based control strategies for mosquitoes and mosquito-borne diseases. The midgut peritrophic matrix (PM), a semipermeable extracellular layer that forms in response to blood feeding and separates midgut epithelial cells from the blood bolus, may serve as one such mechanism. While previous studies suggest the PM is comprised of 20-40 major proteins, only two have been identified to date. We conducted a mass spectrometry based proteomic analysis to identify

proteins that comprise the adult female *Ae. aegypti* midgut peritrophic matrix. Altogether, 474 unique proteins were identified, with 115 predicted secreted proteins. Of these, 57 were associated with catalytic activity, 20 were conserved hypothetical proteins, 8 were hypothetical proteins and 17 were of salivary gland origin. Most interestingly, we identified a conserved hypothetical protein of unknown function with characteristics similar to known peritrophic matrix proteins. This protein may be integral for midgut protection from blood meal derived toxicity, and serve as a novel target for vector control.

## 851

### ELUCIDATING THE ROLE OF LIPOLYTIC PATHWAY IN MOSQUITO REPRODUCTION AND *PLASMODIUM FALCIPARUM* TRANSMISSION

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Female *Anopheles* mosquitoes undergo a number of blood feeding cycles on a vertebrate host in order to produce multiple egg batches during their life span, and these obligatory steps are exploited by *Plasmodium* parasites for their own transmission. Blood feeding is therefore a critical step for both mosquito reproduction and parasite transmission that could be exploited to impact malaria dynamics in endemic areas, especially as these two processes are temporally and physiologically coupled. Previous studies revealed a correlation between blood meal digestion and major changes in transcriptional profiles of metabolic genes involved in lipid biosynthesis, transport, and breakdown, suggesting the occurrence of *de novo* lipid synthesis triggered by blood feeding and followed by lipid mobilization. Here, we aim to elucidate the specific role of blood meal-derived lipids (and/or of lipids synthesized *de novo* after a blood meal) in *Anopheles gambiae* reproduction and *Plasmodium falciparum* parasite development in mosquito stages. To address this, we initially performed targeted lipidomic analyses of various mosquito tissues after a blood meal. Our analyses reveal a coordinated accumulation and depletion of major lipid classes across key mosquito tissues during blood meal digestion, reflective of an engagement of lipogenic and lipolytic pathways. RNA interference (RNAi) against triglyceride (TAG) lipase and associated proteins, involved in lipolytic breakdown of TAGs to yield free fatty acids and diacylglycerol (DAG) identifies lipid mobilization as central in determining reproductive success of the main malaria vector. Specifically, TAG-lipase inhibition, significantly impairs egg development and abolishes fertility, and upon a *P. falciparum* infectious feed had no apparent impact on the number of oocysts per midgut. While further characterization is underway, this study identifies the regulation of TAG/DAG equilibrium as critical for achieving reproductive success of *Anopheles* mosquitoes that could be exploited to control mosquito population and reduce malaria transmission.

## 852

### A FEMALE REPRODUCTIVE PROTEIN AFFECTS THE INTERACTION BETWEEN *ANOPHELES GAMBIAE* MOSQUITOES AND *PLASMODIUM FALCIPARUM* PARASITES

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In *Anopheles gambiae*, male-female molecular interactions following mating are important determinants of female reproductive fitness. *Anopheles* oogenesis and *Plasmodium* parasite development are both temporally and physiologically tied, and an increasing amount of evidence points to reproductive processes playing an important role in

*Plasmodium falciparum* development. Transfer of the steroid hormone 20-hydroxyecdysone (20E) from males to females during mating induces the expression of the gene *Mating-Induced Stimulator of Oogenesis* (*MISO*) in a number of *Anopheles* species and forms a complex with this factor. We find that species that have evolved this mating system have high tolerance to *P. falciparum* infection, and that depletion of *MISO* via RNA interference induces a significant fecundity cost in infected females. We hypothesize that *MISO* has evolved as a key factor to maintain female fitness in the face of *Plasmodium* infection. Our studies suggest male-transferred 20E may modulate aspects of female mosquito biology that are relevant to anopheline vector competence, which could have immense implications for *P. falciparum* transmission. Understanding how male-female molecular interactions following mating affect *P. falciparum* infection in the mosquito vector is key to the development of future vector control tools.

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### HIGHLY CONSERVED PATTERN OF INTERGENOMIC SEQUENCE VARIATION IN INTERNAL TRANSCRIBED SPACER 2 (ITS2) IN ANOPHELES SUBPICIUS SPECIES A ACROSS WIDELY DISTRIBUTED POPULATIONS

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*Anopheles subpictus* s.l. has emerged as a malaria vector in India and Sri Lanka which consists of at least four cryptic species. Ribosomal DNA (rDNA) is extensively used for discrimination of cryptic species that is considered as a gold standard marker for taxonomic resolution of closely related species. Ribosomal DNA is considered highly conserved within an interbreeding population due to concerted evolution acting on this mutigene family and therefore has been extensively used for identification of closely related species especially for the discrimination of cryptic species that are morphologically indistinguishable. It is assumed that all copies of rDNA within an individual are identical, however there are some reports of presence of intergenomic sequence variation in rDNA. This phenomenon may affect the species diagnostic value of rDNA. We sequenced internal transcribed spacer rDNA (ITS2) of *Anopheles subpictus* species A from different geographical populations from Indian subcontinent (Alwar 27.5° N, 76.6° E; Jodhpur 26.2° N, 73.0° E; Delhi 28.7° N, 77.1° E; Kheda 22.9° N, 72.9° E; Ranchi 23.3° N, 85.3° E; Chilka Lake 19.8° N, 85.4° E; Jaffna, Sri Lanka 9.6° N, 80.0° E) and discovered constant pattern of intergenomic variation in rDNA of *An. subpictus* species A with presence of two types of sequences. Cloning and sequencing of ITS2 from individual mosquitoes revealed presence of two types of sequences differing by indel of 12 bases. However, all populations showed identical intergenomic sequence variation with presence of these two types of sequences. We infer that intergenomic sequence variation will not affect species diagnostic value of ribosomal DNA.

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### DISCOVERY OF A NOVEL MOSQUITO JUVENILE HORMONE BINDING PROTEIN ISOLATED FROM THE YELLOW FEVER MOSQUITO, Aedes Aegypti

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Juvenile hormone (JH) is a key regulator of insect metamorphosis and reproductive development. JH binding protein (JHBP) is responsible for transporting JH to target tissues while protecting it from degradation and regulating its bound concentration. Although JHBP has been identified and physically characterized in agricultural pest insects (Lepidoptera), specific function and structure of JHBP remains unknown in medically-important

insects including mosquitoes. Here, we are the first to describe a new type of JHBP isolated from the yellow fever mosquito, *Aedes aegypti* (nJHBP). nJHBP is a member of the odorant-binding protein family (OBP) and is related to the D7 proteins found in mosquito saliva. Unlike Lepidoptera JHBP which is found in all life stages, Western blot analysis revealed that nJHBP circulates only in the hemolymph of pupal and adult mosquitoes. Binding studies using isothermal titration calorimetry showed that nJHBP binds JH II and JH III with high affinity. However, nJHBP did not interact with the eicosanoid fatty acid ligands of the D7 proteins or JH analogues lacking the epoxide group such as methoprene. nJHBP was crystallized in the presence of JH III and found to have a two OBP domain structure. A single molecule of JH III was found in the N-terminal domain binding pocket that is enclosed by a cap structure derived from the C-terminal domain. The lack of clear structural path to the binding site suggests that its binding mechanism is analogous to unrelated Lepidoptera JHBP. Relative quantification of nJHBP mRNA in female mosquitoes indicated nJHBP expression reaches its highest level at 12 hour post-eclosion, corresponding to a reported peak of JHIII synthesis. nJHBP expression level declines after 12 hours and remains low after blood-feeding, which also follows the expression pattern of JHIII. Altogether, these results document a discovery of a novel JHBP in mosquitoes. This finding has a major implication for understanding how JH is regulated in mosquitoes and future studies will aim to further understand the role of nJHBP in regulating JH and its mechanism of action in delivering JH to target sites.

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### VECTORBASE: DATABASE FOR POPULATION BIOLOGY AND OMICS DATA QUERY, BROWSE AND ANALYSES

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VectorBase ([www.vectorbase.org](http://www.vectorbase.org)) is a free, web-based bioinformatics resource center (BRC) for invertebrate vectors of human pathogens, funded by NIAID/NIH. This database is the 'home' of genomes of arthropod vectors and pests (e.g., *Anopheles gambiae*, *Aedes aegypti* and *Culex quinquefasciatus* among other species in Diptera, Hemiptera, Phthiraptera, and Acari), phylogenetically related species, and one intermediate host (the snail, *Biomphalaria glabrata*). In addition to these 40 genomes, it also has transcriptomes, proteomes and population data for an even wider list of species. The population biology data includes lab and field collected information, both from genotypes and phenotypes, covering any biology, ecology, or behavior trait, and even insecticide resistance and population abundance. In addition to the data imported from external databases or directly submitted by users, VectorBase also generates and computes primary data. Over its 13 years of existence, the discovery and interpretation of hosted data has been used for basic and translational research, as expressed in numerous scientific publications, using data in new or re-purpose analyses, descriptions and hypotheses testing. Raw and process data can be exported or download in a variety of different formats, visualized, browsed, queried and analyzed with the site tools or any other external tools. Because VectorBase data, tools and resources are updated every two months, this presentation will highlight this last year major additions. The website has extensive documentation resources for new and experienced users including tutorials, video tutorials (YouTube/youku), practice exercises, answer keys and sample files. In addition to our standard (in-person) workshops, this year we started to offer live webinars. Thesis or publications using this database, are kindly ask to reference the paper or papers where the data was originally published and VectorBase most recent paper, as explained in the website under the "Help" navigation tab. To contact us send a message to [info@vectorbase.org](mailto:info@vectorbase.org).

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### CHOLESTEROL-MODULATED IMMUNE SIGNALLING MEDIATES *WOLBACHIA*-INDUCED INHIBITION OF O'NYONG NYONG VIRUS IN *ANOPHELES* MOSQUITOES

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*Wolbachia* is an intracellular bacterium that infects numerous arthropods. *Wolbachia* has gained considerable attention as a potential disease control agent due to its ability to inhibit pathogen infection when introduced into naturally uninfected mosquito hosts. Field releases of *Wolbachia*-infected *Aedes aegypti* are currently underway to control Dengue and Zika viruses in multiple countries. We used the O'nyong nyong virus (ONNV) model to understand the molecular mechanism of *Wolbachia*-mediated virus interference in *Anopheles* mosquitoes. Enhanced host immunity and competition for metabolic resources are two competing hypotheses for the mechanism of *Wolbachia*-mediated pathogen inhibition in insects. Here we establish that 1) *Wolbachia*-mediated virus inhibition is the result of up-regulation of the Toll innate immune pathway, and 2) cholesterol inhibits *Wolbachia*-induced virus blocking by modulation of Toll signaling rather than competition for cholesterol between *Wolbachia* and virus. *Wolbachia* and cholesterol influence Toll immune signaling in *Anopheles* mosquitoes in a complex manner and provide a functional link between host immunity and metabolism in *Wolbachia*-mediated pathogen interference in mosquitoes.

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### NATIONWIDE INSECTICIDE RESISTANCE STATUS AND BITING BEHAVIOR OF MALARIA VECTOR SPECIES IN THE DEMOCRATIC REPUBLIC OF CONGO (DRC) 2013-2016

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WHO estimates that malaria incidence and deaths in DRC reduced by >40% from 2010-2015, partly due to increased ownership of LLINs. Entomological data was collected nationwide in 7 Provinces between 2013-2016. Human landing catches (HLC) between 18:00 and 06:00 were used to assess mosquito biting times and behavior. HLC was conducted indoors and outdoors in two houses for four consecutive nights per trimester. HLC was conducted monthly in Sankuru and Haut Katanga during 2016. WHO insecticide susceptibility testing of adult *Anopheles gambiae* s.l. was conducted annually using diagnostic concentrations of deltamethrin (0.05%) and permethrin (0.75%). Pre-exposure to the synergist PBO followed by permethrin was done to determine resistance mechanisms. *Anopheles gambiae* s.l. were resistant to permethrin in six of seven Provinces. Conversely, five of seven sites reported susceptibility to deltamethrin. In bioassays with PBO and permethrin the mean increase in mortality was 24%, indicating involvement of metabolic resistance mechanisms. The *kdr*-1014F mutation was present at high frequency (>70%) in Tshopo and Kinshasa and low frequency (<20%) in Sankuru

and Haut Katanga in 2015. In Sankuru, *An. gambiae* s.l. biting rates were consistently high at more than 10 bites/person/night every month. While in Haut Katanga peak *An. gambiae* s.l. biting rates coincided with the rainy season between October and March and *An. funestus* s.l. biting continued through the dry season with 5-15 bites/person/night. *An. gambiae* s.l. biting times were similar in Tshopo, Kinshasa and Sankuru, with the majority of biting occurring late at night between 22:00 and 04:00. In Haut Katanga, considerable *An. gambiae* s.l. biting occurred before 19:00, while *An. paludis* was predominantly an outdoor biter with a large biting peak between 19:00 and 22:00. Malaria vector biting rates remain high in some Provinces of DRC despite increased LLIN coverage. The majority of biting was late at night; therefore LLINs should provide some personal protection. However, the finding of widespread permethrin resistance is concerning and alternative insecticides may be needed.

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### INSECTICIDE RESISTANCE STATUS, INTENSITY AND MECHANISMS OF *ANOPHELES GAMBIAE* S.L. IN SOUTHERN AND CENTRAL MALI BETWEEN 2014 AND 2016

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There is growing concern that insecticide resistance may jeopardize the effectiveness of long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS). Between 2014 and 2016, PMI supported annual IRS in Barouéli and Koulikoro, with Fana District added in 2016. Resistance testing was conducted annually in 14 sites covering southern and central Mali. *An. gambiae* s.l. were collected from larval sites and adult mosquitoes exposed in WHO tube tests to diagnostic doses of permethrin, deltamethrin, bendiocarb and pirimiphos-methyl. Pyrethroid resistance intensity tests were conducted using CDC bottle bioassays at 1x, 2x, 5x, and 10x the diagnostic concentration. Bottle bioassays with pre-exposure to the synergist PBO followed by permethrin were conducted to determine the contribution of mixed function oxidases. PCR was performed to identify species and frequency of *Ace1<sup>R</sup>*, *kdr* 1014F and *kdr* 1014S mutations. WHO tube tests revealed full susceptibility to pirimiphos-methyl in all sites and full susceptibility to bendiocarb except in Niono and Bougouni where possible resistance was recorded. Resistance to permethrin and deltamethrin was widespread with a mean mortality of 38% to permethrin and 64% deltamethrin across all 14 sites in 2016. There were survivors at all sites when permethrin and deltamethrin were tested at 10X the diagnostic dose. No significant difference in pyrethroid resistance intensity was observed between 2014, 2015 and 2016. There was a high frequency of *kdr* 1014F in *An. gambiae* and *An. coluzzii* but a low frequency in the secondary vector *An. arabiensis*. The *Ace1<sup>R</sup>* and *kdr* 1014S mutations were present at very low frequency. Strong phenotypic resistance to pyrethroids and partial implication of MFOs indicated that additional mechanisms may be important. Widespread high intensity pyrethroid resistance may threaten the effectiveness of LLINs in Mali. However, contrary to predictions, the intensity of resistance has not increased between 2014 and 2016 despite mass LLIN distribution. IRS with pirimiphos-methyl should continue to be an important complementary vector control tool due to continued susceptibility.

### INDOOR RESIDUAL SPRAYING WITH FLUDORA FUSION (A CLOTHIANIDIN AND DELTAMETHRIN INSECTICIDE MIXTURE) PROVIDES IMPROVED CONTROL AND LONG RESIDUAL ACTIVITY AGAINST PYRETHROID RESISTANT *ANOPHELES GAMBIAE* SL IN SOUTHERN BENIN

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There is an urgent need for new insecticides for indoor residual spraying (IRS) which can provide improved and prolonged control of malaria vectors that have developed resistance to existing insecticides. The neonicotinoid, clothianidin represents a class of chemistry new to public health. Clothianidin acts as an agonist on nicotinic acetyl choline receptors. IRS with a mixture of Clothianidin and another WHO approved insecticide such as deltamethrin could provide improved control of insecticide resistant malaria vector populations and serve as a tool for insecticide resistance management. The efficacy and residual activity of Fludora Fusion, a novel IRS mixture of deltamethrin and clothianidin was evaluated against wild pyrethroid resistant *An. gambiae* sl in semi-field experimental hut trials in Cote d'Ivoire, Benin. Comparison was made with clothianidin and deltamethrin applied alone. To assess the residual efficacy of the treatments on different local wall substrates, the inner walls of the experimental huts were covered with either cement or mud. Clothianidin demonstrated a clear delayed expression in mortality of wild pyrethroid resistant *An. gambiae* sl in the experimental huts which reached its full effect 120 hours after exposure. Overall mortality over the 12-month hut trial was 4% in the control hut and 12-16% in the deltamethrin-treated huts. Fludora induced high overall hut mortality rates of wild free-flying pyrethroid resistant malaria vectors that entered the experimental huts (72% on mud walls, 70% on cement walls) largely due to the clothianidin component and high hut exiting rates (55-60%) mostly due to the deltamethrin component. Mortality rates remained >60% for 5-7 months on mud and cement walls. The residual activity trend was confirmed by results from cone bioassays with laboratory-maintained resistant and susceptible *An. gambiae* strains. IRS campaigns with the mixture of clothianidin plus deltamethrin have the potential to provide prolonged control of malaria transmitted by pyrethroid resistant mosquito populations.

### MECHANISMS OF PYRETHROID RESISTANCE IN *AEDES AEGYPTI* FROM DENGUE ENDEMIC AREAS OF SAUDI ARABIA: A PRIMARY ROLE FOR TARGET SITE MUTATIONS

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Control of *Aedes aegypti* using insecticides is the main method to reduce the prevalence of dengue in the disease-foci cities of Makkah and Jeddah. Despite potential impacts on control, little is known about insecticide resistance in the Middle Eastern Region, and nothing about underlying mechanisms. We characterized insecticide resistance in *Ae. aegypti* from Jeddah and Makkah and investigated the role of the two main resistance mechanisms, target site mutations and metabolic detoxification. WHO bioassays were performed on adult *Ae. aegypti* collected in Jeddah and Makkah. Portions of the voltage-gated sodium channel (*Vgsc*) gene were sequenced to identify target site mutations. Microarray analyses were performed to identify transcriptome variation potentially linked to deltamethrin resistance and qPCR of candidate genes applied to investigate mechanistic cross-resistance in lines selected for other insecticides. Adults bioassays revealed resistance/suspected resistance to all insecticides tested in *Ae. aegypti* with significantly higher levels of deltamethrin resistance in Makkah compared to a reference resistant Cayman strain. Three *kdr* mutations (S989P, V1016G, F1534C)

were detected for the first time in the Middle Eastern region, with S989P and V1016G markers in perfect linkage disequilibrium (LD) and strongly predicting deltamethrin resistance. The synergist PBO, which is applied to block the action of P450 enzymes, caused a moderate increase in deltamethrin mortality in Jeddah but had a non-significant effect on Makkah mortality. Transcriptional analysis identified multiple differentially expressed genes, numerically dominated by P450s, most more highly expressed in Jeddah than Makkah, consistent with the synergist test results. *Ae. aegypti* mosquitoes are highly resistant to pyrethroids in the two regions. *Vgsc* mutations are strongly associated with deltamethrin resistance and, whilst P450 enzymes appear to be the dominant metabolic mechanism, our data suggest a greater importance of target site mutations; however further investigation is required to determine the importance of other resistance mechanisms.

### COMMERCIAL AEROSOLIZED INSECTICIDES CAN SERVE AS A STRONG SELECTION FORCE FOR PYRETHROID-RESISTANCE IN *AEDES AEGYPTI*

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Long term use of pyrethroids, poor efficacy and sub-optimal insecticide doses are considered the main drivers of the high prevalence of insecticide resistance currently observed among *Ae. aegypti* populations in the Americas. Commercial aerosolized insecticides may be an additional, yet unproven, source of pyrethroid resistance. This study experimentally quantified the biological efficacy of household products commonly used among households in Mérida, Mexico against pyrethroid-resistant (field strain) and pyrethroid-susceptible (New Orleans strain) *Ae. aegypti*. The bioefficacy and residual action of the two most commonly used pyrethroid aerosols, designated P1 (tetramethrin, allethrin, and d-phenothrin) and P2 (imiprothrin and cypermethrin), were tested against the two *Ae. aegypti* strains. Field strain mosquitoes originated from eggs collected in three sites outside of Mérida known for high levels of pyrethroid resistance (and treated as resistant colonies, labeled C1, C2, and C3) and use of P1 and P2 insecticides. Mortality among female, 2-5 day-old *Ae. aegypti* was measured after exposure to P1 and P2 as an aerial space spray and a residual surface sprayer and surface sprays. Detection of the I1016 allele through PCR was used as an indicator of resistance. GLMM modeling showed that all three resistant colonies had lower mortality rates than the control (C1 OR: 0.04, C2 OR: 0.11, C3 OR: 0.34). Similarly, I1016 homozygous mutant genotype conferred the greatest resistance to both insecticides (OR: 0.06, 95CI: 0.03, 0.12). While I1016 allele frequency was associated with increased survivorship in the aerial spray trials (all p-values <0.05), no such association was made in the residual spray trials. Our results suggest that household insecticides act as a strong force of pyrethroid selection pressure and that household use of insecticides can contribute to observed increasing trends in pyrethroid resistance in *Ae. aegypti*.

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### CONTRIBUTION OF TWO SYMPATRIC SIBLING SPECIES, *ANOPHELES COLUZZII* AND *AN. GAMBIAE*, TO MALARIA TRANSMISSION IN NORTH BENIN

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*Anopheles gambiae* s.l. is an 8-species taxonomic complex. These include *An. gambiae* s.s. Giles and *An. coluzzii* Coetzee & Wilkerson, formerly referred to as the S form and the M form, respectively. The members of the complex display different bio-ecological characteristics, for example: feeding on humans versus cattle, or resting indoors versus outdoors. Additionally, there are differences in the suitability of each for development of *Plasmodium falciparum* sporozoites. The Departments of Alibori and Donga, Benin, have been targeted by the National Malaria Control Program for indoor residual spraying (IRS) in 2017. Pre-IRS vector assessments indicated that two species of the *An. gambiae* complex are present: *An. coluzzii* and *An. gambiae* s.s. The goal of this study was to measure and compare IRS-related entomological measures of transmission for each species. Human landing capture (HLC), inside and outside households, and pyrethrum spray capture (PSC) were used to sample vector populations in 6 districts targeted for IRS. Captured *An. gambiae* s.l. mosquitoes were dissected to assess parity; tested by enzyme-linked immunosorbent assay (ELISA) to determine circumsporozoite antigen positivity; and identified to species by polymerase chain reaction (PCR). In addition, PCR was used to estimate the human blood index and the frequency of the *Kdr L1014F* mutation in the sodium channel which has been associated with resistance to pyrethroid insecticides. There were no significant differences between the two species in the rates of anthropophily, blood meal indices, indoor and outdoor biting rates, or sporozoite rates ( $p > 0.05$  all comparisons). However, monitoring data revealed two differences that could affect the impact of IRS: *An. coluzzii* is characterized by a lower *Kdr L1014F* frequency than *An. gambiae* s.s., and the ratio of *An. coluzzi* to *An. gambiae* s.s. was significantly greater in Donga than in Alibori ( $p < 0.05$ ). These results suggest that the presence of more *An. coluzzii*, with a lower frequency of *Kdr L1014F* (compared with *An. gambiae*), could result in better control of malaria by IRS in Alibori compared to Donga.

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### TRANSCRIPTOME ANALYSIS OF GENES ASSOCIATED WITH PYRETHROID RESISTANCE IN SOUTH AND CENTRAL AMERICAN *ANOPHELES ALBIMANUS*

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Decades of unmanaged insecticide use and routine exposure to agrochemicals have left many populations of malaria vectors in the Americas resistant to multiple classes of insecticides, including pyrethroids. Using a transcriptome-wide approach, we characterized the mechanisms of deltamethrin and alpha-cypermethrin resistance in *Anopheles albimanus* from sites in Peru and Guatemala. Field collected *An. albimanus* were phenotyped as either deltamethrin or alpha-

cypermethrin resistant using the CDC bottle bioassay. RNA from 1) field-collected resistant, 2) field-collected unexposed, and 3) a susceptible laboratory strain of *An. albimanus* was sequenced and analyzed using RNA-Seq. The expression profiles of the three groups were compared based on the current annotation of *An. albimanus* reference genome sequence. Several candidate genes associated with pyrethroid resistance in other malaria vectors were found to be over-expressed in resistant *An. albimanus* mosquitoes. The cytochrome P450 monooxygenase CYP9K1 was overexpressed in both Guatemala and Peru, relative to a susceptible laboratory colony (Sanarate), but to a much greater extent in Peru (14x) than Guatemala (2x). CYP6P5 was over-expressed in Peru (68x) but not in Guatemala. The results suggest different mechanisms may mediate pyrethroid resistance in different populations of *An. albimanus*. Differences were also noted in the voltage-gated sodium channel between Peruvian alpha-cypermethrin-resistant and deltamethrin-resistant samples. In deltamethrin resistant samples, the knockdown resistance mutation (*kdr*) variant alleles at position 1014 were rare, with approximately 5% frequency but in the alpha-cypermethrin resistant samples the frequency of these alleles was approximately 15-30%. Validation of select candidate genes and the *kdr* mutation as a resistance marker for alpha-cypermethrin will provide key information for the development of mechanism-specific assays.

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### COI BARCODING OF *ANOPHELES COLUZZII* AND INSECTICIDE RESISTANCE MONITORING IN DEMOCRATIC REPUBLIC OF SAO TOME AND PRINCIPE

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Democratic Republic of Sao Tome and Principe (DRSTP) is an island nation located in the central west Africa. The malaria vector in DRSTP is *Anopheles coluzzii* which was formerly named *Anopheles gambiae* M form. This study sequenced and analyzed the gene of mitochondrial cytochrome oxidase subunit I (COI) of *Anopheles coluzzii* from different locations and time slots in DRSTP. Results showed that the COI sequences of *Anopheles coluzzii* were similar in DRSTP, but different from the voucher specimen (Accession No. KT382819) with an identity cut-off of 99.4%. On the other hand, there were differences in knockdown resistance (*kdr*) between *Anopheles coluzzii* (M form) and *Anopheles gambiae* Giles (S form). *Anopheles coluzzii* in DRSTP was found no *kdr* resistant type before 2004. However, intensive anti-vector interventions including indoor residual spraying (IRS) and long-lasting insecticidal nets (LLINs) has conducted from 2004 in DRSTP. Therefore, this study performed a monitoring on *kdr* 1014 locus associated with pyrethroid resistance from 2010 to 2015. Results showed that after 2-3 years of yearly IRS and LLINs with pyrethroid insecticides in DRSTP, the *kdr* L1014F allele frequency was detected in 2010 (6.39%), and had dramatically increased to 73.5% in 2013. The province Agua Grande with the highest population had no *kdr* L1014F frequency in 2010, but increased to 73.85% in 2013. Followed by the change of IRS insecticide from alphacypermethrin to bendiocarb at the end of 2013, the *kdr* L1014F frequency had decreased to 38.1% in 2015. *Ace-1* G119S resistant type to carbamate had not been detected until 2015.

865

### MALARIA VECTORS IN ASIA: A COMPOSITE SET OF APPROACHES FOR IMPROVING THEIR CONTROL

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The current goals of WHO to achieve the 2020 milestones of a 40% reduction in case incidence and mortality, and malaria elimination by

2030, rely for a large part on vector control interventions. Then, updated knowledge on *Anopheles* vectors is important to obtain for developing malaria surveillance plan and risk assessment. In Asia, the biodiversity of *Anopheles* mosquitoes is particularly rich compared to the other continents. The vector system is composite as each malaria vector belongs to a species complex with morphologically indistinguishable sibling species that may present different trophic behaviors and vectorial capacities. Recent advances in molecular systematics have allowed reliable species identification for their precise study. Another specific trait in Asia is that most malaria endemic areas occur in foci distributed along forested international borders with population movement maintaining the endemicity. The main aim of this work is to obtain information on the human-vector system, which is of primordial importance for applying appropriate vector control programs targeting the correct vector species and protecting the human population. Several approaches are being developed, in collaboration with Asian partners (Cambodia, China, Thailand, Vietnam), for answering crucial questions and improving vector control strategies. Do we know all the vectors, even secondary ones that may play a role in transmission? What is the role of the mosquito microbiota in its vectorial capacity? Can we evaluate human antibody responses to *Anopheles* saliva to evaluate the efficacy of vector control methods? Are there alternatives to synthetic insecticides capable of protecting the human population and less harmful to the environment? These joint studies are providing reliable data on *Anopheles* mosquitoes throughout Asia highlighting also the gaps to fill up. Today, there is an urgent need to extend this network at the regional scale as malaria vectors do not know boundaries and their control will be a lot more efficient if an international taskforce is identified which will increase the chance to meet the goal of malaria elimination by 2030.

## 866

### INSECTICIDE-TREATED COW-BAITED TENTS AS A TOOL TO CONTROL OUTDOOR BITING MALARIA VECTORS

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Insecticide-treated cow-baited tents were tested in Cambodia over one year as an outdoor-biting mosquito control and monitoring tool. Insecticide-treated cow-baited tents exploit the animal feeding tendencies of outdoor vectors and could potentially target and eliminate large numbers of different anopheline species that contribute to outdoor, or residual, malaria transmission. The potential success of this strategy is that anophelines with a successful bloodmeal may linger on treated tent walls, increasing their rate of contact with insecticide treated or impregnated netting. Even if the mosquitoes are not knocked down directly, this exposure could drastically reduce the number of surviving females that are able to transmit, thus reducing malaria transmission. Since many outdoor biting mosquitoes carry other diseases, this type of approach could target not only vectors of residual malaria, but also those of filariasis and other zoonoses. Large tents fitted with deltamethrin-impregnated panels with a cow in the center were compared to untreated control tents every three months during 2016 in Pursat and Preah Vihear, Cambodia, provinces with emerging artemisinin-resistant malaria parasite populations. Mosquitoes were collected hourly from 6pm to 6am and were tested for knockdown effects exactly two hours after collection. The same diverse set of more than 25 *Anopheles* species were captured in treated versus untreated tents, hundreds of anophelines were entering treated tents every night, and some of those mosquitoes were carrying malaria parasites with Kelch-13 mutations, a marker that is linked to artemisinin-resistance. These results show that insecticide-treated cow-baited tents could be a valuable tool for targeting outdoor vector species.

### CROSS-RESISTANCE INVOLVING THREE CLASSES OF INSECTICIDES AND TWO MAJOR MALARIA VECTOR SPECIES IMPACTING MALARIA CONTROL IN NCHELENGE, A HIGH MALARIA TRANSMISSION REGION IN NORTHERN ZAMBIA

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For over a decade insecticide-treated bed nets (ITNs) and indoor residual spraying (IRS) have been the vector control tools in Nchelenge District, northern Zambia, although prevalence of malaria remains high. Some areas, particularly the islands, have only received ITNs. Studies carried out in the district provided insight into the problem of malaria on the mainland. We included islands in Lake Mweru with no history of IRS and determined the status of insecticide resistance and the intensity of this resistance using the World Health Organization (WHO) standard assays. We tested field-collected and F1 mosquitoes on five insecticides representing four classes between 2014 and 2016. Ninety-seven percent of the collections were *Anopheles funestus* group and 3% *An. gambiae sensu lato*. *An. funestus* from all the sites were resistant to pyrethroids and carbamates but susceptible to organophosphates and DDT. *An. gambiae* was resistant to pyrethroids and DDT but susceptible to organophosphates and bendiocarb. The intensity of resistance in *An. funestus* from all collection sites was high against bendiocarb and deltamethrin. On the mainland there was a significant increase in resistance intensity to bendiocarb between 2014 and 2016 (ANOVA F = 93, p-value < 0.001). We recorded 10% mortality in *An. gambiae* to DDT, an insecticide that has never been used before in the district. We recorded cross-resistance between pyrethroids and carbamates in *An. funestus* and between pyrethroids and DDT in *An. gambiae*. We detected cytochrome p450 resistance mechanism in *An. funestus* and knockdown resistance (kdr) in *An. gambiae*. Insecticide resistance may explain why control efforts have not made an appreciable impact on the transmission of malaria in Nchelenge. Well thought out integrated vector control and resistance management strategies are required, as options for vector control in Nchelenge are very limited. The use of new innovations such as ITNs incorporated with an insecticide synergist on the islands may be helpful. Alternative insecticides are needed in this area and Nchelenge could provide a good field site for evaluating efficacy of new vector control tools.

## 868

### PYRETHROID RESISTANCE INTENSITY AND MECHANISMS OF INSECTICIDE RESISTANCE IN THE MALARIA VECTOR *ANOPHELES GAMBIAE* S.L. IN SELECTED DISTRICTS IN NORTHERN GHANA

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Insecticide susceptibility tests and resistance mechanism assays are conducted annually across selected sentinel sites for the President's Malaria Initiative (PMI) supported indoor residual spraying (IRS) Ghana project, to guide the selection of insecticides for the spray campaign. In 2016, WHO tube tests were conducted on the malaria vector *Anopheles gambiae* s.l. using selected insecticides recommended for IRS. Follow-up resistance intensity tests were also conducted to determine the pyrethroid resistance intensity among *Anopheles gambiae* s.l. in two IRS sites. Synergist assays with detoxification enzyme inhibitors (PBO and DEF), and biochemical tests were conducted to determine the presence and level of metabolic resistance. The insecticide susceptibility test results indicated that *An. gambiae* s.l. from all the sites tested are resistant to pyrethroids (alphacypermethrin 0.5% and deltamethrin 0.05%). The *An. gambiae* s.l. from Gbullung and Kumbungu showed high frequency of insecticide resistance to deltamethrin (0-5% mortality). However, the frequency of phenotypic resistance to deltamethrin is mainly of low intensity, because the vector was only resistant to the 1x and 2x diagnostic doses of deltamethrin, but susceptible to the 5x and 10x doses. The vectors from Gbullung were also resistant to bendiocarb (89% mortality). In contrast, vectors from all the IRS communities were susceptible to pirimiphos-methyl (98% -100% mortality). Synergist assays with PBO resulted in a significant increase in vector susceptibility to deltamethrin (from 67% to 95%;  $p < 0.0001$ ), assays with DEF increased the vector susceptibility only marginally, from 66% to 71% ( $p = 0.447$ ), suggesting that mono-oxygenases play a significant role in the resistance detected in Gbullung. Confirmatory biochemical assays showed significantly elevated activities of cytochrome P450 mono-oxygenases, and  $\alpha$  and  $\beta$ -esterases in samples collected from Gbullung ( $p < 0.0001$ ). Organophosphates remain the only insecticide to which no confirmed resistance has been reported. Insecticide resistance monitoring is crucial in maintaining the efficacy of IRS in the area.

## 869

### LANDSCAPE STRUCTURE AND *ANOPHELES* (DIPTERA: CULICIDAE) COMMUNITIES IN THE URABÁ AND BAJO CAUCA, COLOMBIA

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Landscape structure and composition greatly influences inhabitant insect communities and it is an aspect of importance for understanding malaria vector ecology. This work aimed to evaluate the influence of landscape coverages on the composition and diversity of *Anopheles* mosquitoes collected in localities of two Colombian malaria endemic regions, the Urabá and Bajo Cauca. Land cover types were characterized using orthorectified aerial photographs, in areas within 1.5 km radius of the collection site. In general, eight main coverage types were detected; forest, water bodies, grass, stubble, bare soil, crops, wetland and crop mosaics with natural areas; but the predominant coverages were forest, bare soil and crops. A total of 2,543 mosquitoes were collected corresponding to 13 species, being the most abundant, *Anopheles braziliensis* (33%), *Anopheles nuneztovari* (24%) and *Anopheles darlingi* (20%). *Anopheles darlingi* and *An. nuneztovari* showed higher densities in forest predominant landscapes, and *An. braziliensis* in grass and bare soil landscapes. There was no relationship between landscape diversity and anopheline community diversity. Studies on landscape structure

and anopheline communities for important malaria endemic regions of Colombia help to elucidate the malaria vector species spatial distribution which constitutes useful information for vector control interventions.

## 870

### DECIPHERING THE IMPACT OF *PLASMODIUM* AND *TRYPANOSOMA* COINFECTIONS ON THE VECTORIAL CAPACITY OF *ANOPHELES* MOSQUITOES

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Malaria and African trypanosomiasis (AT) are two parasitic diseases prevalent in African tropics, transmitted to humans and other mammals by the bite of competent female *Anopheles* mosquitoes for the malaria parasite *Plasmodium*, and male and female tsetse flies (*Glossina*) for *Trypanosoma*. In some sub-Saharan Africa areas, these two parasites are sympatric, thus competent *Anopheles* or *Glossina* vectors could feed directly on a co-infected host, or more likely, on two successive hosts respectively infected with one parasite and the other. This study aims at understanding how the ingestion of *Trypanosoma* could impact the development and the transmission of *Plasmodium* by *Anopheles*. Both concomitant co-infection and successive co-infection scenarios were assessed on *Anopheles coluzzi* using *Trypanosoma brucei brucei* and *Plasmodium spp* (*yoelii* or *falciparum*). In both concomitant and successive co-infection scenarios, our results showed that mosquitoes co-infected with *T. b. brucei* bloodstream forms are more susceptible to *P. yoelii* or *P. falciparum* as compared to the batch of mosquitoes mono-infected with *Plasmodium*. A transcriptomic approach will help us at identifying mosquito factors that could impact on *Plasmodium* development in co-infected *Anopheles* mosquitoes. These results emphasize the need for considering co-endemic areas for designing vector control strategies.

## 871

### A MOSQUITO ASSOCIATED *CHROMOBACTERIUM* CAUSES LETHALITY IN *ANOPHELES GAMBIAE* LARVAE THROUGH PRODUCTION OF HYDROGEN CYANIDE

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Malaria and other vector borne diseases account for hundreds of millions of disease cases each year and hundreds of thousands of deaths, and novel vector control strategies are a critical component of preventing disease transmission. In previous work, we have shown that a soil-derived bacterium *Chromobacterium species Panama* (*Csp\_P*), has insecticidal properties against *Anopheles gambiae* mosquitoes at both the larval and adult stages of development. In the current work, we dissected the mechanism of larval killing by *Csp\_P* when the bacterium is present in suspension in the breeding water. We determined that *Csp\_P* produces a larvicidal factor that persists after removal of live bacteria via filtration and that larval killing activity increases during log phase of bacterial growth, peaking near the end of log phase. Further, we determined that the larvicidal factor is a heat-stable and volatile small molecule (< 3 kDa). Upon sequencing the genome of *Csp\_P*, we discovered that the bacterium's genome encodes a hydrogen cyanide (HCN) synthase, as is the case for other species of *Chromobacterium*. We then determined that *Csp\_P* produces hydrogen cyanide when present in larval rearing water, reaching a concentration of approximately 0.51 mg/L, which is sufficient to kill the larvae. The HCN persisted in the larval water for less than 24 hours. We also determined that HCN production by *Csp\_P* occurs during the log phase of bacteria growth, and peaks at the end of log phase, mirroring the pattern of *Csp\_P*-mediated larval killing. Finally, we successfully eliminated larval mortality through treatment of the larval rearing water with

hydroxocobalamin, an HCN antidote. Taken together, these data strongly suggest that *Csp\_P*'s larvicidal activity is derived from its production of HCN in the larval breeding water.

## 872

### ANALYSIS OF CELLULAR INTERACTIONS BETWEEN *PLASMODIUM* PARASITES AND *ANOPHELES* SALIVARY GLANDS

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*Plasmodium* parasites are the causal agent of malaria, a mosquito-borne disease still with hundreds of millions of infections and around 500,000 deaths each year. Parasites are acquired by female mosquitoes that blood feed on an infected individual. *Plasmodium* undergoes reproduction and development in and on the midgut. Sporozoites are subsequently released into the mosquito hemocoel, where they locate and specifically invade the salivary glands (SGs). During subsequent blood meals, the SGs provide the gateway for *Plasmodium* transmission to a new host. We sought to characterize interactions between sporozoites and SG cells using confocal immunofluorescence microscopy to better understand this stage of malaria transmission. *Plasmodium berghei* parasite- (either ANKA or GFP-tagged ANKA) infected *Anopheles stephensi* SGs were dissected at time points between 18 and 30 days post-infection and stained for features including SG secreted saliva proteins, cytoskeletal markers, organelle markers, dyes (lipids, chitin/O-GlcNAcylation), and antibodies to parasite-expressed GFP or *P. berghei* circumsporozoite protein (CSP). Using this strategy on a large sample size, we confirmed many of the described features of invaded SGs, including SG distal lateral lobe-focused invasion, SG cell stress and/or death, possible SG basal membrane repair after invasion, parasite bundling, and the presence of transient parasitophorous vacuoles. We have also uncovered novel preliminary information regarding SG infections, such as sites of subcellular SG localization of parasite CSP, very few parasites in the salivary duct (in the absence of a second blood meal), emergence from the parasitophorous vacuole, parasite maturation in the SG, cell traversal strategies, and the effects of infection on SG structure/function. Interactions between SG cells and the parasites are likely to be critical factors on parasite transmission rates. A better understanding of these interactions will inform future strategies to block parasite transmission at the SGs.

## 873

### COMPETITIVE MATING CHALLENGES OF TRANSGENIC *Aedes Aegypti* AGAINST WILD-TYPE STRAINS REARED UNDER LABORATORY AND SIMULATED FIELD CONDITIONS

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Population replacement and suppression as a means of mosquito control is a paradigm heavily reliant on the release of genetically altered strains into the wild. The ability of these transgenic mosquitoes to competitively interbreed with wild populations is critical to the success of such strategies, but has not been well studied. Here we performed mating challenges of transgenic and recent Trinidadian field isolates of *Aedes aegypti*. We subjected larvae to either standard optimum laboratory regimes or conditions simulating environmental stress found in the field, and determined that larval crowding and nutritional deprivation negatively influenced the competitiveness and reproductive success of Trinidad males. Specifically, in individual head-to-head challenges against optimally reared transgenic males, Trinidad males raised under stressed conditions were less likely to successfully inseminate females. Further, females were more likely to mate with multiple partners in challenges that included stress-reared Trinidad males. Here we provide evidence that upon release, transgenic *A. aegypti* males raised under optimal laboratory conditions are likely to have a competitive mating advantage over wild males in the field.

## 874

### BLOOD MEAL PREFERENCE OF MAIN MALARIA PARASITE VECTOR SPECIES AFTER AN INTENSIVE USE OF INSECTICIDE ON MALARIA VECTOR CONTROL IN MADAGASCAR

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The detection of a high proportion of human blood meals in the vector population increases the risk of the spread of malaria parasite through the community. Since 2010, the National Malaria Control Programme of Madagascar has undertaken an intensive use of insecticides inside homes through the use of insecticide impregnated Nets or the indoor residual insecticide spraying. This study aimed to identify the origin of blood meals taken by the main malaria parasite vectors in Madagascar. Blood fed mosquito population tested were collected using pyrethrum spray catch technique conducted from 2010 to 2016 in 9 villages. All mosquito collected was morphologically identified and then abdomen of blood fed of vector species were crushed on a filter paper for blood meal analysis using a direct ELISA assay. Overall, 760 *Anopheles* blood fed were tested. Morphological identification showed that of 760 blood fed *Anopheles* vectors collected: 196 are *Anopheles funestus*, 548 *An. gambiae* s.l. and 15 *An. mascarensis*. The blood meal analysis revealed that 82.8% of *An. gambiae* s.l. fed on bovine and 7.7 % on human. However, 40.6% of blood meals from *An. funestus* were taken on human and 40.1% on bovine. As for blood meals of *An. mascarensis*, 20% were taken on human and 60% on bovine. Also, mixed blood meals (human and bovine) and blood meals from other domestic animals were identified. This study showed that *An. gambiae* s.l. and *An. mascarensis* are highly zoophilic, while *An. funestus* is both anthropophilic and zoophilic. The result of this study is one of important information needed for an effective vector control measures to be implemented in the country.

## 875

### INFLITE\*: A BEHAVIORAL SIMULATION PACKAGE FOR THE RAPID EVALUATION OF LLINS, CHEMISTRIES AND OTHER VECTOR CONTROL TOOLS (\*INDIVIDUAL FLYING INSECT TESTING ENVIRONMENT)

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Whether to combat insecticide resistance, exploit new knowledge of vector biology or to accommodate changes in abiotic conditions, disease vector control methods are under constant pressure to innovate, iterate and improve. Novel tools are constantly sought in order to increase cost-effectiveness and to improve community acceptance and sustainability of vector control programs. However, testing new tools is a highly risky, expensive and time-consuming process, facing numerous challenges and tasks ranging from the technical to the research ethics review process. Recent advancements in video-tracking and behavioral analysis research conducted by the authors, has enabled the accurate recording and quantification of multiple parameters associated with mosquito flight, host-seeking, resting and other behaviors. This enables the development of Individual (agent) Based Models operating at much finer spatial and temporal scales than was previously possible. The behavioral parameters of *Anopheles gambiae* and other mosquitoes interacting with bed nets during host-seeking have now been exploited to create a novel virtual testing simulation, allowing a wide range of innovative vector control ideas, both actual and theoretical, to be rapidly and easily assessed at marginal cost. The resulting model being presented here, represents an

easy to use computer software package, capable of simulating vector behavior. It allows the end user to compare, quantitatively and visually, alternative designs of vector control tools or systems, individually or in combination. The model parameters and validation presented are based on data collected during laboratory and field tests with *An. gambiae* and *An. arabiensis* interacting with different bed net designs, with or without human hosts. However, this *in silico* virtual testing model has been designed from the ground up to incorporate flexibility and ease of use to allow its adaptation for a wide range of control tools or vector families.

## 876

### SOCIAL-ECOLOGICAL FACTORS INFLUENCING RECEPTIVITY TO ZIKA VIRUS AND THE EFFICACY OF INTERVENTIONS IN COMMUNITIES ALONG THE TEXAS-MEXICO BORDER

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The emergence and re-emergence of mosquito-borne diseases such as Zika, chikungunya and dengue fever remains a global public health challenge that threatens many communities in the continental United States. We are conducting studies along the Texas-Mexico border assessing the environmental receptivity to these mosquito borne viruses and evaluating different forms of vector control interventions. From September, 2016 to present, we have been gathering data on the abundance of the three primary mosquito vector species: *Aedes aegypti*, *Aedes albopictus*, and *Culex quinquefasciatus* in multiple communities that represent variable socio-economic conditions. We are using the CDC Sentinel Autocidal Gravid Ovitrap (SAGO traps) to estimate the relative abundance of vector species indoors and outdoors. The mosquito communities in these neighborhoods in South Texas are dominated by *Ae. aegypti* and *Cx. quinquefasciatus* with only a few *Ae. albopictus* specimens recorded. Our preliminary results show the mean number of female *Ae. aegypti* per SAGO trap per week to be  $0.3952 \pm 0.05742$  ( $n=377$ ) indoor and  $1.774 \pm 0.1262$  ( $n=384$ ) outdoor. The outdoor abundance of *Ae. aegypti* was higher in low income communities both indoor and outdoor. Ongoing surveys of the households with traps aims to identify risk factors for increased human-mosquito contact indoors. The testing of these mosquitoes for Zika virus has not revealed any positives although the pan-Flavivirus assays are documenting infection with insect-specific viruses. These baseline mosquito data are the foundation for a large-scale mosquito control intervention using CDC AGO traps that will begin in the summer of 2017.

## 877

### RESOLVING TEMPERATURE-DRIVEN MALARIA TRANSMISSION MODELS

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Predicting current and future vector-borne disease (VBD) transmission is a significant challenge. While many factors influence VBD, recent work in a variety of transmission systems highlights the importance of climatic factors in shaping vector-borne disease transmission. In particular, temperature has strong effects on many aspects of mosquito biology involved in transmission including bite rate, mortality rate, fecundity, as well as, the rate of pathogen development within the mosquito vector. These trait by temperature relationships are non-linear, unimodal, and asymmetric. However, the resolution of these trait by temperature relationships is poor as few studies have investigated the variation in these life history traits across the full thermal breadth for a single mosquito species, or how age and temperature integrate to influence trait performance. Transmission models which incorporate these poorly

defined relationships with temperature and age can have significant impacts on disease risk predictions, the implementation of intervention strategies, and the evaluation of interventions. To address these gaps, we conducted a study which observed individual *Anopheles stephensi* mosquitoes, the primary malaria vector in India, across six temperature treatments (16°C, 20°C, 24°C, 28°C, 32°C, 36°C) over the duration of their lifespan. We provided a bloodmeal daily and directly measured bite rate, mortality rate, and fecundity for each individually-housed mosquito across our temperature treatments. Temperature effects were observed for each trait, with mosquito age affecting a subset of traits. We also observed interactions between temperature and age for some of these traits, suggesting that warming temperature can accelerate the effects of mosquito age on these traits. These data were used to parameterize a temperature-dependent R0 and compared to malaria models generated from coarser mix-species data. This study highlights that mosquito thermal performance is likely to vary with mosquito species, temperature, and age suggesting that current transmission models likely fail to capture important variation.

## 878

### SPATIAL PATTERNING AND FINE-SCALE HETEROGENEITY OF MALARIA RISK ALONG AN URBAN-RURAL CONTINUUM IN BLANTYRE, MALAWI

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Malaria risk in urban and peri-urban environments is of increasing concern in highly-endemic settings undergoing urbanization. Understanding drivers of urban transmission is hampered by inconsistencies in how "urban" contexts are defined. A dichotomized "urban-rural" approach may misclassify environments or fail to capture fine-scale heterogeneity across time and space. We analyzed household-level *Anopheles* abundance patterns in and around Malawi's commercial capital of Blantyre (~1.1M pop.). Mosquito densities and distribution were measured inside houses using CDC light traps during rainy and dry seasons of 2015 and 2016. Clusters (N=64) of five houses each located at 2.5km intervals along eight transects radiating out from Blantyre city center were sampled. Multivariate mixed models for negative binomial distributions were used to evaluate abundances of *Anopheles funestus* (N= 3,367) and *An. arabiensis* (N= 1,177) separately. *Anopheles funestus* and *An. arabiensis* females were significantly more abundant in "rural" vs. "urban" Blantyre, as defined by governmental city boundary, increasing with distance from the urban center. Abundances were higher and more varied during rainy seasons. At the neighborhood level, lower elevation and lower household density significantly predicted higher *Anopheles* abundance. At the household level, peri-domestic agriculture, few rooms, few or no ITNs, and unscreened windows were significantly positively associated with *An. funestus* and *An. arabiensis* abundances. Among houses with peri-domestic agriculture, *Anopheles* were more abundant than among houses lacking crops, even after adjusting for potential confounders (e.g. house construction, nearby household density, elevation). Significant interactions of agriculture and unscreened windows with household density suggested that peri-domestic agriculture and house structure influence *Anopheles* abundance differently based on degree of urbanicity. A closer look at household- and peri-domestic-level factors should improve understanding and prevention of malaria transmission in urban and peri-urban settings such as Blantyre.

## THE LANDSCAPE OF METAGENOMES IN WILD POPULATIONS OF ANOPHELES GAMBIAE, AN. SINENSIS, AEDES ALBOPICTUS AND AE. AEGYPTI

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Mosquito associated microbiome contributes to various life traits of host mosquitoes. 16S rDNA based profiling has revealed bacterial composition and community structure in major vector species. To further understand the microbial genetic repertoire and their functional impact on host physiology, we developed a pipeline to characterize microbiome using NGS shotgun sequencing data. Genomic DNA of wild caught mosquito specimens of *Anopheles sinensis* from Shandong, China, *Aedes albopictus* from Hainan, China and Florida, USA were sequenced using Illumina paired end platform. The clean sequence reads were mapped against corresponding mosquito genomes to separate mosquito reads from non-mosquito reads. Non-mosquito reads were *de novo* assembled into contigs. The contigs in size of 5kb-889.8kb were annotated. The taxonomic assignment was based on Blastn against available complete bacterial genomes. The functions of predicted genes were assigned using subsystems approach. The pipeline was also used for data mining from the Ag1000G project. Sequencing data from 150 *An. gambiae* individuals from Burkina Faso and 75 *An. gambiae* individuals from Gabon were analyzed. The assembled bacterial metagenome was 77.4Mb in *Ae. albopictus* from China, 16.7Mb in *Ae. albopictus* from Florida, 2.7Mb in *Ae. aegypti* from Florida, 4.1Mb in *An. sinensis* from China, 45.7Mb in *Ag. gambiae* from Burkina Faso and 9.4Mb in *Ag. gambiae* from Gabon. More than 100 genera/families were identified. Proteobacteria are predominant in the microbiome of all mosquito species, represented largely by taxa in Enterobacteriales. *Wolbachia pipientis* wAlbA and wAlbB strains were identified in *Ae. albopictus* from both Florida and China. The draft genomes of both *Wolbachia* strains was assembled. The *Wolbachia* contigs were present in *Ae. aegypti* from Florida, which is consistent with the report by Coon et al. that *Ae. aegypti* has natural infection of *Wolbachia* in the same region. Gene catalogues were compiled from the metagenomes. Characterization of fungal contigs is underway. The metagenome reference will facilitate to study interactions of mosquitoes and microbial symbionts.

## FIVE YEARS OF MALARIA PARASITE VECTOR SPECIES SURVEILLANCE IN MADAGASCAR

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Understanding the species composition of malaria parasite vector species is a component key for an effective malaria vector control in an endemic area. Since 2012 to 2016, an entomological survey was conducted in 10 sentinel sites belonging to different Malagasy malaria epidemiological "facies" by the entomological team of the National Malaria Control Program of Madagascar. Adult mosquito collection inside and outside homes was performed using human landing catch. Then collected mosquitoes were morphologically and molecularly identified. Results of the five years of entomological survey showed that 18 189 potential malaria vector species were collected: 21.8% *An. funestus*, 49.9% *An. arabiensis*, 13.6% *An.gambiae* s.s., 13.5% *An. mascarensis* and 1.2% *An. coustani*. The data collected revealed the changing on the behavior of *An. funestus* and *An. arabiensis*. Also, collected data demonstrated the spreading of *An. gambiae* s.s. to the high altitude of Central Highlands of Madagascar.

The results of the current entomological survey in Madagascar confirm the necessity of an intensive survey of the malaria vector species in an endemic country.

## MOSQUITO-MICROBE INTERACTIONS IN CONTAINER HABITATS: EFFECTS OF DETRITUS CONDITION ON MOSQUITO PRODUCTION AND MICROBIAL COMMUNITIES

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Many medically important mosquito species use natural or artificial containers as larval habitats. Factors controlling production of adult mosquitoes from these habitats are important to population dynamics of these disease vectors. Food webs in these habitats, forming the basis for larval growth and adult mosquito production, are driven by carbon and nutrient subsidies to tree holes, such as senesced plant parts, animal carcasses, or organic matter dissolved in stemflow water. These subsidies support the production of heterotrophic microbes which are consumed by mosquito larvae. Microbes colonizing detrital surfaces are an important component of the diets of *Aedes* species. Larvae have access to detritus in various stages of microbial conditioning, ranging from poorly conditioned, fresh detritus to detritus with well-established communities in later stages of development. Reciprocally, microbial communities in various stages of succession are exposed to larval predation. We simulated water-filled tree holes in the laboratory to explore the effects of poorly and well-conditioned detritus on *Aedes triseriatus* production and the reciprocal effects of larval predation on microbial communities. We used a 2 x 2 x 3 full factorial design with poorly or well-conditioned detritus, low or high detritus ration, and larval density as experimental factors. Detrital condition was an important driver of ration-density interactions. Larvae fed poorly conditioned leaves showed evidence of density-dependent resource limitation, with emerged female adults over 6x heavier in high ration, low density treatments than in low ration, high density treatments. Well-conditioned detritus alleviated this limitation: adult female mosquito mass was similar across density and ration treatments when larvae were provided well-conditioned leaves. Larval feeding affected bacterial and fungal communities differently, constraining bacterial diversity while promoting fungal diversity associated with the detritus. Overall, our results demonstrate that detrital conditioning affects mosquito production.

## THE PRESENCE OF CIBARIAL ARMATURE IN MOSQUITOES AND IMPACT ON THE TRANSMISSION OF LYMPHATIC FILARIASIS IN GHANA

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The success of MDA programmes in eliminating LF depends on density dependent processes exhibited by existing and novel vectors. This may be attributed to role played by their cibarial armatures. This study investigated mosquito composition from Western and Upper East regions of Ghana, and vectorial competencies of mosquito species based on their cibarial armatures. Samples were collected from Mpohor, Ahanta West (AW), Kassena Nankana West (KNW) and Bongo districts with a total of 22,584, 4,784, 2,191 and 1,505 mosquitoes respectively. *An. gambiae* (25,548), *An. pharoensis* (54), *An. coustani* (22) *Aedes* (22), *Mansonia* (2,531) and *Culex* (2,807) were sampled from all districts. *An. coluzzii* and S form identification from subsamples of *An. gambiae* s.s revealed respectively:

Mpohor (125:60), AW (9:3), KNW (124:24) and Bongo (90:46). Observed teeth numbers for mounted mosquitoes was significantly different between *An. pharoensis/Culex*, *An. gambiae s.s./An. pharoensis*, *Culex/An. gambiae s.s.*, *An. melas/An. pharoensis*, *An. melas/Culex*, *An. arabiensis/Culex* ( $P<0.001$ ) and between *An. melas/An. gambiae s.s.*, *An. pharoensis/An. arabiensis* ( $P=0.001$ ) but not *An. arabiensis/An. gambiae s.s.* ( $P>0.05$ ) and *An. arabiensis/An. melas* ( $P=0.162$ ). *Aedes* and *Mansonia* had no cibarial teeth. Mean number of teeth was: *An. pharoensis* (21.00), *Culex* (25.69), *An. gambiae s.s.* (15.82), *An. melas* (13.25) and *An. arabiensis* (16.00). From Western and Upper East Regions, there was no significant differences in teeth numbers among *Culex* ( $P=0.079$ ) as in *An. gambiae* ( $P=0.726$ ). Cibarial teeth are sharp, pointed and long in *An. gambiae s.l.* though few in *An. melas*. However, it was less developed in *Culex* and absent in *Aedes/Mansonia*. *An. melas* was found only in AW district. Transmission of LF in Ghana might be due to few or no teeth seen in mosquito species coupled with their refractoriness. Where vectors, *An. melas* and *Mansonia* exhibit limitation, MDA must be complemented with vector control. *Culex* with less developed cibarial armature not being vectors in Ghana indicate other possible mechanisms preventing their capability to transmit LF in this area.

## 883

### IMMUNOMODULATORY ROLE OF ARYL-HYDROCARBON RECEPTOR IN *ANOPHELES GAMBIAE*

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Mosquitoes have developed an efficient immune system during the evolutionary interactions with microbes, which includes Toll, Imd, JAK/STAT and RNAi pathways to defend against various microbial pathogens. The immune homeostasis is well maintained by different negative feedback mechanisms. In vertebrates, the aryl hydrocarbon receptor (AhR) pathway has been shown to operate xenobiotic detoxification and immunosuppression functions upon sensing endogenous and exogenous ligands. Upon activation, AhR is translocated into nucleus and transcribe genes for immune regulation or detoxification. In this study, we examined the role of mosquito AhR signaling in immune regulation. Kynurenine is an endogenous ligand of AhR, which is generated during tryptophan oxidation, mediated by tryptophan-2,3-dioxygenase (TDO) in mosquitoes. We used the TDO inhibitor 680C91 to reduce the production of kynurenine, which would decrease AhR activation. We also used two AhR antagonists, CH233191 and StemRegenin 1 (SR1), which inhibit AhR translocation and DNA binding. After feeding with these compounds for three days, the mosquitoes were challenged with a systemic infection by injection with a midgut bacterium *Serratia* sp. S1. The inhibition of AhR significantly increased the survival from 49.8% in the naïve control to 80.6% in the treated mosquitoes ( $P<0.01$ ). Furthermore, the interrogation of transcriptome of before and after infections in AhR manipulated mosquitoes revealed various genes that may be involved in AhR mediated immune regulation and xenobiotic metabolism. The data indicate that mosquito AhR may be a part of immune regulatory networks.

## 884

### INTERACTIVE VOICE BASED MOBILE PHONE TECHNOLOGY IN ANTENATAL AND INFANT MONITORING (AIM): A PROOF OF CONCEPT STUDY

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Vaccine safety is integral to immunization program acceptance and success. Current Adverse Events Following Immunization (AEFI) reporting in India has been found suboptimal. With more than one billion

subscribers, mobile technology offers opportunities for mobile Health (mHealth) services overcoming literacy and geographical barriers. The automated interactive voice technology used in AIM provided antenatal and postnatal support and monitoring for 837 mothers (62% urban, 37% rural) through 130 interactive voice messages over 19 months (till the end of primary infant immunization) while capturing AEFIs directly from mothers. It also documented antenatal visits, Iron and Folic Acid (IFA) compliance, danger signs, high-risk mothers, maternal (Tetanus toxoid) and infant (Pentavalent) immunization and AEFIs at 24 hrs and 7 days post-immunization by converting verbal responses to data and sending alerts to health staff in real-time. Of the 837, 746 (89.1%) mothers completed the 19 month follow-up, 83% (619) reported timely antenatal visits and 5% (139) danger signs (reduced fetal movements, headaches and associated vomiting) while 10.6% (80) reported being diagnosed as hypertensive and 19% missed timely maternal immunization. AIM documented timely infant immunization as 86%, 72.8% and 56.8% for Penta 1, 2 and 3 respectively of 329 AEFIs reported 254 (77.2%) were validated to be true, of those reported all but 2 were non-serious. Few instances of wrong reports were due to voice data capture and data transfer, continuous modifications enabled the AIM system to capture a majority of data and its flexibility to choose time of message, changing registered phone numbers, re-listening health messages, speak to health supervisors, identified danger signs, immunization reminders while identifying those missing immunization schedules and confirming those reporting AEFI made it convenient, effective and acceptable. The AIM system was found to be an ideal to support antenatal and immunization program monitoring by grass-root level health workers, medical officers and program managers while capturing AEFI from mothers directly.

## 885

### STRENGTHENING CASE MANAGEMENT SKILLS OF FACILITY-BASED HEALTH PROVIDERS FOR THE MANAGEMENT OF CHILDHOOD DIARRHEA AND PNEUMONIA IN UTTAR PRADESH: KEY TO REDUCING UNDER-5 MORTALITY

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In India, diarrhea and pneumonia contribute to 12% and 23% of all under-5 deaths respectively. Most of these deaths are preventable with correct diagnoses and treatment. To inform an intervention focused on the appropriate management of childhood diarrhea and pneumonia, we conducted a survey to assess adherence of facility providers to World Health Organization guidelines for facility based integrated management of neonatal and childhood illnesses (F-IMNCI). We assessed 251 suspected diarrhea and 141 suspected pneumonia under-5 cases in 15 health facilities of Uttar Pradesh. While trained observers assessed provider skills, their knowledge was tested using case studies on diarrhea and pneumonia. Of 251 suspected diarrhea children, 101 were diagnosed with diarrhea, 1 with some and 3 with severe dehydration by the providers, and of 141 children with suspected pneumonia, 9 were diagnosed with pneumonia. While assessing adherence to F-IMNCI guidelines, observers concluded that providers asked about diarrhea in 16% of suspected diarrhea cases; 51% were asked about frequency; 38% about stool consistency; 29% about duration; 4% for blood in stools; and 28% were administered skin pinch test, to assess severity of dehydration. Of all suspected pneumonia cases, 26% were asked about cough; 13% about difficulty in breathing; 20% were assessed for chest in-drawing; and 9% each were assessed for fast breathing, peripheral and central cyanosis. Only 7 of the diagnosed diarrhea cases were recommended both ORS and zinc, while 4 of the diagnosed pneumonia cases were recommended Amoxicillin. None of the severe dehydration cases received intravenous drip. Mean score on diarrhea and pneumonia test was 1.6 (maximum

score: 11, SD:2.9) and 1.4 (maximum score: 14, SD:2.5) respectively. Fidelity to evidence based clinical practice was low with facility providers displaying low knowledge and practice skills for the diagnosis and management of childhood diarrhea and pneumonia. Future programs should promote the use of F-IMNCI based clinical algorithms to distinguish non-severe from severe illnesses and strengthen case management skills of facility providers.

## 886

### A SLAUGHTER OF THE INNOCENTS: PEDIATRIC MORTALITY AMONG BOER CIVILIANS IN SOUTH AFRICAN CONCENTRATION CAMPS, 1901-1902

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The Second Boer War began in the fall of 1899 and ended in the spring of 1902. (The first had been fought two decades earlier.) Utilizing guerrilla tactics, Boer forces fought a bloody and costly campaign—particularly for the British—that lasted roughly two and half years. By the end of hostilities in May 1902, the conflict had cost the British some £200000 and roughly 55,000 deaths and casualties—nearly the number of men who were killed or wounded on the first day of the Somme (July 1915) or total American dead during the Vietnam War a half-century later.

The British commander, Kitchener, countered the Boer tactics in a brutal attempt to subdue the Boer population, combatants or not. As farms smoldered in the wake of this scorched-earth strategy, however, thousands of Boer civilians were sent to British-run “concentration camps.” (Similarly squalid camps had been established by the Spanish during the 1890s in an attempt to quell a grassroots independence movement.)

Camp-based infectious diseases rates among children quickly skyrocketed due to chronic malnutrition and, most of all, an ongoing lack of proper sanitation and potable water. This presentation will offer an analysis of concentration camp mortality among children and adolescents between 1901 and 1902, focusing particular attention on the wide differences across more than 40 camps in which Boer civilians were held.

## 887

### INDIVIDUAL CONSENT PROCESS IN CLINICAL RESEARCH IN SUB-SAHARA AFRICA, BAMAKO, MALI

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Individual consent process is important to carried-out a vaccine trial in the world. This process should be understood by the study subjects to follow and complete all the study procedures. In Mali, only 33.5% of the population is literate, to facilitate understanding of the consent process, the center for vaccine development of Mali make a translation of the inform consent into the national language and records on the audiotape. Individual inform consent procedure and obtaining are carried out by a trained CVD-Mali doctor with mature adult parent or gardien with witness as needed and respecting confidentiality. For non-literate parent or participant we translate the consent form in local language and play on audio tape and use an impartial witness from the community. From 2003 to 2017 we have conducted 16 clinical studies at CVD-Mali and administered individual consent to 19842 participants aged from 9 months to 85 years old including pregnant women. Among them 18861 have been vaccinated 95%. The most common questions and comments

were made by participant parents or gardien. Understanding of certain participants in relation to the technical terms used during obtaining consent, vaccine test or trial, Antibody; Randomization; Placebo; Why should blood be taken before and after vaccination, some participants are impatient, which leads them to want to sign a consent form without understanding its content, signing a consent form would be an act that could have other intentions beyond the scope of clinical research such as prosecution this is why they often ask the investigator to sign in his place, the participants did not understand why a signature? Informed consent is a complex process and can be an element of protection for those involved in research studies. A good understanding of parents is a better partnership and may generate less anxiety for parents or investigators.

## 888

### CONDUCTING CLINICAL TRIALS IN CRISIS SETTINGS, 2012 MILITARY COUP IN MALI AND THE EBOLA VIRUS OUTBREAK IN 2014 IN WEST AFRICA

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Clinical research in epidemic, political and economic crises is a complex challenge in countries with limited resources including Mali. In a crisis, an experienced and pragmatic approach to implementing clinical trials is essential. Mali experienced a military coup and a counter coup in 2012 and the Ebola Outbreak in 2014. Challenges reported by investigators, field workers, community members and study participants were recorded in different study documents including individual case report forms and field registers. We have gathered and analyzed these challenges. During the period of military political crisis, following the curfew in Mali in 2012, the priority in conducting clinical trials was the safety of CVD Mali staff and participants, recruitment of participants and their follow-up at home. Obtaining community leaders commitment, training community relays on the protocol for adverse events and serious adverse events detection, involving clinics and health centers in the area for receiving and guiding participants, home visits where conditions could permit and the alternative of using mobile phones allowed CVD staff ethical concerns around research methods, and field operational challenges such as cold chain management and effective communication with those affected. During the 2014 Ebola Virus Disease outbreak caused panic in the general population, many rumors and negative attitudes towards health activities and Response Teams. The development of a strong communication plan focusing on positive behavior changes, Mobilization of community leaders, religious and traditions healers allowed the continuation of activities in accordance with the rules of ethics and to reassure the health workers and the general population. Clinical studies are difficult to perform and follow during crisis. Team spirit and performance of the staff in the conduct of clinical trials allow obtaining good results. However, researchers, sponsors and authorities must work together to better manage these crisis in order to achieve good results.

## 889

### COLLABORATION IS CRUCIAL; DELIVERING RESEARCH SKILLS TRAINING TO THOSE WHO NEED IT THE MOST

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Everyone working in global health research should have access to effective and appropriate research skills training and this is particularly important in resource-poor settings where the need for locally applicable research findings is so important. There are initiatives providing research skills

training in resource-poor settings; but the majority of this training is face to face, cannot be accessed widely enough and often focus on a specific protocol or disease area. The Global Health Network has collaborated with multiple organisations and institutions to share training materials and produce 24 high-quality, peer reviewed online training courses across a wide range of research topic areas which are free, easily accessible and available to all. Courses range from individual module short courses that can take up to 2 hours to complete to much more in-depth multi-module courses that can take anywhere up to 8 hours to complete. These courses are hosted within the Global Health Training Centre, which is a trusted and well-regarded centralised training hub within the Network, and each course is designed to be accessible from areas with low bandwidth internet connection, which can often be a barrier to accessing online training from resource-poor settings. By the end of March 2017, over 214,000 modules on the Training Centre had been taken by over 32,000 individuals. In 2016, on average 1644 modules were taken per week by an average of 226 individuals. This equates to an average of 32 individuals taking a course daily. Over 2500 individuals provided feedback on the courses they had taken and over 96% stated they would recommend the course to a friend or colleague. The majority of individuals accessing the courses are from Africa. There is a clear demand for free, easily accessible, high quality online research skills training. Increased collaboration and sharing of training materials between organisations and institutions is required to produce further online training resources to support research staff and develop capacity to conduct research in some of the world's poorest areas.

## 890

### APPLICATION OF EVENT-BASED SURVEILLANCE FOR EMERGING INFECTIOUS DISEASE PREPAREDNESS IN U.S. EUROPEAN COMMAND HEADQUARTERS

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Event-based surveillance can be a reliable tool to fill gaps in surveillance information from traditional programs. For vector-borne diseases (VBD) and zoonoses, event-based surveillance permits monitoring of outbreaks, invasive vector species, reservoir hosts and imported disease cases. Collation of disparate information sources grants a comprehensive view of emerging VBD diseases and informs mitigation strategies in areas at risk. The US European Command (EUCOM) of the Department of Defense utilizes the Counter Biothreats Cell (CBC) for monitoring of infectious diseases of operational concern. The CBC conducts event-based biosurveillance in the EUCOM area of operation to mitigate risks associated with disease and ensure force health protection. By applying conceptual epidemiology principles for biopreparedness and bioresponsiveness, the CBC can discern disease trends, conduct rapid risk assessments and provide recommendations that inform decision-makers about potential risks and impacts of VBD emergence or outbreaks. Three examples of CBC assessments for diseases with differing epidemiological risk include Hantavirus, Crimean-Congo Hemorrhagic Fever (CCHF) and Zika virus. The CBC program successfully monitored the potential spread and impact of the Zika epidemic in 2015-16, emergence of CCHF in Western Europe and increased seasonal Hantavirus risk in Germany. Rapid analytics highlighted the timeliness and broad scope of biosurveillance when compared with traditional surveillance programs and allows for prompt information dissemination on prevention protocols and risk mitigation strategies. The CBC poses a unique opportunity to interpret disease surveillance data and determine operational impact. Biosurveillance programs can also provide documentation of risk likelihood for disease introduction and movement in lieu of traditional surveillance where resources are limited or overwhelmed. Application of surveillance information to military operations helps increase prevention efforts to ensure EUCOM is maintaining strategies for risk mitigation in disease movement.

## 891

### IMPACT OF COMMUNITY PERMISSION MEETING IN A LOW LITERACY SETTING IN SUB-SAHARAN AFRICA

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Clinical trials are recent activities in Malian culture. Community permission to implementation in research study search as epidemiological studies or vaccine trials in low income, low literacy settings (sub-Saharan Africa) play a key role in an understanding the study protocol in the study population. In developing countries like Mali where populations do not have much knowledge about vaccine research, community consent meeting is a prerequisite for the success of a research study. As an attempt to ensure an accurate understanding of the study risks and benefits, The National Translation Office translates the approved individual information consent and assent forms into the local languages and have it recorded on audiotapes. Community meetings are organized in collaboration with local religious and socio-cultural leaders and the community members. In addition to the public criers, invitations letters are distributed widely in the community. During the meeting, the audiotape is listened first by the audience; then the study investigators explain all study procedures, risks, benefits, and compensation. Community members are encouraged to ask questions. The community permission is obtained upon community satisfaction. The community permission allows to initiate and facilitate the process of individual informed consent/assent. From January 2006 to December 2017, we were able to observe the impact of community permission on screening in 10 clinical trials that CVD Mali has successfully conducted in the Malian communities, specifically in Bamako. A total of 25924 were registered among those 18094 were screening and 17163 were enrolled and vaccinated, with a retention rate of 94.9%. The success of a study depends on the degree of understanding of the community. For the population's comprehension of research studies, it is necessary to hold community permission meetings. Given the low literacy rate in the communities, it is important to develop strategies allowing them to better understand various aspects of the studies, and this can only be done by holding of community meetings before, during and after clinical studies.

## 892

### HIGHLIGHTING THE SUCCESSES AND CHALLENGES OF INTEGRATION OF SELF-CARE FOR PEOPLE AFFECTED BY FILARIAL LYMPHOEDEMA INTO EXISTING COMMUNITY LEPROSY SELF-HELP GROUPS IN NEPAL

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Lymphatic filariasis (LF) and leprosy are neglected tropical diseases endemic in Nepal. LF infection can lead to lymphoedema and hydrocele, while secondary effects of leprosy infection include impairments to hands, eyes and feet. The disabling effects of both conditions can be managed through self-care and the supportive effects of self-help groups (SHG). A network of SHGs exists for people affected by leprosy (PAL) in four districts in Nepal's Central Development Region, however no such service was available for people affected by LF (PALF). This study assessed the

success of integrating PALF into SHGs for PAL in this area, and documents challenges to achieving integration. A questionnaire was conducted pre-integration and six months post-integration with selected PALF and PAL to elicit information and changes on: (i) participant characteristics; disease burden; uptake of integrated services; (ii) participant knowledge of self-care for their condition; access to services; (iii) participant knowledge and perception of the alternate condition (PALFs' knowledge of leprosy and vice versa); attitudes towards integration. Community supervisors and SHG facilitators were interviewed to document experiences and improvements to integration. Comparison of 52 PALF and 53 PAL pre-integration showed that PAL had greater knowledge and practice of self-care compared to PALF ( $p < 0.001$ ). Perceived stigma of both PALF and PAL towards the other condition was high, but did not differ significantly between groups ( $p = 0.47$ ). Six months into the integrated programme, 18% of PALF surveyed reported joining a SHG, and 38% had received self-care training in a SHG. The stigma of both PAL and PALF towards the other condition reduced significantly ( $p < 0.005$ ), however stigma was reported by supervisors as a challenge. A lack of trust in the effectiveness of self-care by PALF was also cited as a reason for slow uptake of SHGs. Facilitators reported making home visits to PALF to encourage attendance, but also cited self-stigma of PALF as a challenge. Peer-support and community education were frequently suggested ways to improve uptake. Full results will be presented.

## 893

### DETERMINATION OF CAUSES OF DEATH IN STILLBORN BABIES AND NEONATES. VALIDITY OF A MINIMALLY INVASIVE AUTOPSY METHOD: AN OBSERVATIONAL STUDY

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Over 5 million stillbirths and neonatal deaths occur annually. Limited and imprecise information on the cause of these deaths hampers progress in achieving global health targets. Complete diagnostic autopsies (CDA) -gold standard for cause of death determination-, are difficult to perform in most high burden settings. Therefore, validation of simpler and more feasible methods is needed. We aimed to compare the performance of a standardized minimally invasive autopsy (MIA) approach with the CDA in a series of stillbirths and neonatal deaths at Maputo Central Hospital in Mozambique. In this observational study the validity of a MIA in determining the cause of death was assessed in 18 stillbirths and 41 neonatal deaths by comparing the results of the MIA with those of the CDA. Concordance between the categories of diseases obtained by the two methods was assessed by the Kappa statistic and the sensitivity, specificity, positive and negative predictive values of the MIA diagnoses were calculated. A cause of death was identified in 89% and 83% stillborn babies in the CDA and the MIA, respectively. Fetal growth restriction accounted for 39%, infectious diseases for 22%, intrapartum hypoxia for 17% and intrauterine hypoxia for 11% of stillborn babies. Overall, the MIA showed in this group a substantial concordance with the CDA [Kappa=0.78, 95%CI (0.56, 0.99)]. A cause of death was identified in all (CDA) and 85% (MIA) of neonatal deaths. In this group, the majority of deaths were due to infectious diseases (66%). The overall concordance of the MIA with the CDA in neonates was moderate [Kappa=0.40, 95%CI (0.18, 0.63)]. A high percentage of accuracy was observed for the MIA in all the diagnostic categories in both stillbirths and neonates (>75%). The MIA could be a useful tool for cause of death determination in stillbirths and neonatal deaths. These findings may help to accelerate progress

towards meeting global health targets by obtaining more accurate information on the causes of death in these age groups, which is essential in guiding the design of new interventions and increasing the effectiveness of those already implemented.

## 894

### VALIDITY OF A MINIMALLY INVASIVE AUTOPSY TOOL FOR CAUSE OF DEATH DETERMINATION IN MATERNAL DEATHS FROM MOZAMBIQUE

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Despite global health efforts to reduce maternal mortality, rates continue unacceptably high in large parts of the world. A reliable knowledge of the causes of maternal deaths is a condition to reduce them through adequate health planning. Accurate and feasible tools for cause of death determination are needed for decision-making on interventions to reduce this burden. The validity of a minimally invasive autopsy method (MIA) in determining the cause of death in 57 maternal deaths was assessed in an observational study by comparing the results of the MIA with those of the complete diagnostic autopsy (CDA). Concordance between the categories of diseases obtained by the two methods was assessed by the Kappa statistic and the sensitivity, specificity, positive and negative predictive values of the MIA diagnoses were calculated. Most deaths (82.5%) occurred postpartum. A cause of death was identified in the CDA in 98.2% (56/57) of cases, with indirect obstetric conditions accounting for 56.1% of deaths while direct obstetric complications accounted for 42.1% of deaths. Infectious diseases (68.8%) and obstetric hemorrhage (50.0%) were the commonest causes of death among indirect and direct obstetric conditions, respectively. The MIA identified the cause of death in 84.2% of cases. The overall concordance of the MIA with the CDA was moderate (Kappa= 0.521, p-value<0.0001) and both methods agreed in 70.2% of the diagnostic categories. The agreement was higher for indirect (90.6%) than for direct obstetric causes (41.7%). Thirty-six women were HIV-positive and HIV-related conditions accounted for 28% of deaths. Cerebral malaria accounted for 7% of deaths being all cases identified both in the CDA and the MIA. The MIA procedure could be a valuable tool to determine the cause of maternal deaths especially in those due to indirect obstetric conditions most of which are infectious diseases. This information is required for decision-making on health planning and prioritisation of interventions to reduce maternal mortality, and for monitoring progress towards achieving global health targets.

## 895

### UTILITY OF SPATIAL INTERPOLATION FOR GENERATING DHS INDICATORS AT SUB-NATIONAL ADMINISTRATIVE LEVELS

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Improved understanding of geographic variation and inequity in health status, wealth, and access to resources within countries is central to meeting sustainable development goals (SDGs). Furthermore, as national governments decentralize and policy decisions are made at the local level, there is a growing need to utilize existing data to accurately target, monitor, and evaluate the impact of programs in smaller geographic areas. The Demographic and Health Surveys (DHS) Program's spatially

interpolated maps for 15 standard development indicators contribute to this need of the development community without increasing survey sample size, cost, or time. This analysis used the Kenya 2014 DHS survey data to focus on the utility of these spatially interpolated map products in generating indicator estimates at lower sub-national administrative levels. The Kenya survey has a sampling density substantially higher than usual, which allows for directly estimating indicators at the county level. Multiple artificially thinned survey samples were created from the original sample to replicate a more traditional DHS sampling density. Geospatial modelling of the original sample and the artificially-thinned samples were used to generate new interpolated surfaces using conditional simulation to allow for county-level indicator estimates and uncertainty intervals. Systematic comparisons were performed on the county-level indicator estimate accuracy among the (1) full survey sample, (2) thinned survey samples, and (3) the final DHS report data estimate (gold standard). Results show that the geospatial methods perform well in predicting the “true” county-level estimate for many indicators. Variations in predictive ability depended on both specific indicators as well as density of sampled locations. This work contributes to a better understanding of the ability of spatial interpolation to estimate DHS indicators at sub-national administrative levels and also provides some basic guidance on how interpolation methods, as well as DHS surveys, can be adapted to improve modeling of development indicators.

## 896

### ASSESSMENT OF QUALITY INDICATORS OF INTENSIVE CARE UNIT IN A TERTIARY CARE HOSPITAL IN NORTH INDIA

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Several countries have developed quality indicators (QI) for intensive care units (ICU) for the auditing the performance of ICU. The Indian Society of Critical Care Medicine identified QIs for ICUs in India to help them judge performance levels and compare with the national data base. Very common parameters mainly focusing on the outcome, process, infection, communication, human resource and safety were selected. We did an objective audit at Post graduate institute of medical education and research (PGIMER), a tertiary care academic centre in North India as per these parameters. After the approval of institute ethics committee (NK/2762/study/922), data was collected from May 2015 to October 2015. All the patients admitted to ICU were included which is a 12 bedded mixed ICU with a patient staff nurse ratio of 2:3. Out of total of 241 patients in ICU (129 males and 112 females) 36 patients were less than 18 years of age, 187 patients were between 19-65 years of age and 18 more than 65 years old. The average length of stay in ICU was 10.14 days and the average duration of mechanical ventilation was 8.63 days. The incidence of acute kidney injury was 11.2%. The incidence of iatrogenic pneumothorax was 12.4% and that of decubitus ulcer was also same. The incidence of patient falling off the bed was 0.40%. The reintubation rate was 7.14% and the readmission rate was 3.32. The incidence of medication error was 1.63. The incidence of ventilator-associated pneumonia was 14.43 per 1000 ventilator days and incidence of central line associated blood stream infection was 14.06 per 1000 catheter days. The incidence of catheter associated urinary tract infection was 3.96 per 1000 catheter days. A total of 38 patients died in six months and the observed mortality rate was 32.49. APACHE II within 24 hours of admission was recorded, 38 was the highest score and 5 minimum. 49 patients admitted to ICU had a 24 hour admission APACHE II score of 15. The formulation of set of parameters as QI for Indian ICUs is an attempt to judge performance of ICUs in resource constrained settings. Acceptability and utility of these parameters in the Indian scenario will have to be assessed over a period of time.

## 897

### HEALTH RISKS FROM EXPOSURE TO UNTREATED WASTEWATER USED FOR IRRIGATION IN THE MEZQUITAL VALLEY, MEXICO: A 25-YEAR UPDATE

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Wastewater reuse for agriculture is a common practice worldwide. Wastewater treatment, however, is rare in many countries, leading to high possibility for exposure to harmful pathogens. The Mexico City-Mezquital Valley reuse system is one of the largest wastewater reuse systems worldwide and was the site of key epidemiologic studies on wastewater conducted in the 1990s. We conducted a cross-sectional study of diarrheal disease and wastewater contamination in the Mezquital Valley to provide an updated assessment of the health risks associated with wastewater and to inform an upcoming update of the 2006 World Health Organization guidelines on safe wastewater reuse. We surveyed 314 households among communities that use wastewater for irrigation and communities that irrigate with well water to compare the prevalence of self-reported diarrheal disease in children under five years old. Wastewater, well water, household environmental samples, and stool samples were collected and analyzed for the presence of potential pathogens. Communities exposed to wastewater had a higher one-week prevalence of diarrhea (10%) compared to unexposed communities (5%). This association remained in an adjusted modified Poisson regression model (PR = 2.31, 95% CI 1.00, 5.31), but the association was not as strong when limited to households engaged in agriculture. Water quality indicators document differences between irrigation water from the two community groups. While overall diarrheal prevalence has declined when compared to studies conducted over 25 years ago in the same region, the association between diarrheal disease and wastewater exposure has remained and possibly increased. With rising urbanization worldwide, attention to the health risks of wastewater reuse and the impact of treatment is becoming increasingly important.

## 898

### CONTRIBUTION OF THE CLINICAL INFORMATION TO THE MINIMALLY INVASIVE AUTOPSY IN DEATHS FROM SUB-SAHARAN AFRICA

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There is a need to refine the existing tools of cause of death attribution in low- and middle-income regions. The minimally invasive autopsy (MIA), a simple method based on needle sampling of key organs followed by histological and microbiological analysis has shown an acceptable performance for cause of death identification in all age groups in Mozambique, when compared with complete diagnostic autopsy (CDA). This study aims to determine whether the addition of clinical data can improve the performance of MIA. Coupled MIAs and CDAs, the latter

considered as the gold standard, were performed blindly to 264 deaths (112 adults, 57 maternal deaths, 54 children and 41 neonates) conducted in a referral hospital of Mozambique. Kappa value was used to compare the agreement between the gold standard and the MIA diagnosis blind to any clinical data and with MIA enriched by clinical information. The addition of clinical data improved the results of MIA in all age groups. The improvement was particularly high in maternal deaths (kappa value increasing from 0.521, moderate agreement, to 0.838, almost perfect agreement,  $p < 0.001$ ), in neonates (kappa value increasing from 0.404, moderate agreement, to 0.618, substantial agreement,  $p = 0.025$ ), and in adults (kappa value increasing from 0.732, substantial agreement, to 0.813, almost perfect agreement,  $p = 0.021$ ). The improvement was also evident in children (kappa value increase from 0.704, substantial agreement to 0.802, almost agreement), although did not reach statistical significance ( $p = 0.173$ ). The addition of clinical data to the pathological and microbiological analyses performed in the MIA significantly improves its diagnostic performance. The improvement is higher in the maternal deaths, neonates and other adults. Clinical data or, in its absence, verbal autopsy data may improve the cause of death diagnosis by minimally invasive autopsy.

## 899

### INFORMED CONSENT ISSUES IN CLINICAL TRIALS INVOLVING CHILDREN WITH MINOR PARENTS IN SUB-SAHARAN AFRICA: A SYSTEMATIC REVIEW

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Before participating in clinical trials (CT), international guidelines recommend potential research subjects to provide a fully informed and voluntary consent. The validity of informed consent (IC) depends on a subject's legal competence and decision-making capacity (DMC). In CT involving children, IC has to be provided by legally acceptable representatives. Additionally, children can assent in accordance with their DMC (i.e. intellectual maturity). In certain cases, however, adolescents can be considered mature or emancipated and provide autonomous consent. Emancipation status is defined by statute or case law and may include pregnant or underage parents. International guidelines remain vague about these aspects or refer to national legislation. Large variability in handling this topic in different countries complicates the implementation of IC procedures. Challenges arise in CT conducted with children in sub-Saharan Africa (SSA) where a higher proportion of CT involves children compared to European countries. In SAA, the population under 24 years is almost twice as high as in Europe and the mean age of mothers at first birth is 20 versus nearly 29 in Europe. Considering these facts, it is probable for children involved in clinical research in SSA to have minor parents and, therefore, guidance on the determination of DMC and legal competence is needed. We are currently conducting a literature search following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement for evidence and appropriate solutions to this sensitive issue. The following databases: PubMed, Embase, Medline, and Google Scholar, will be mined to identify information about CT with child participants whose parents are minors and examine IC procedures and issues, child subject protection measures and ethical review. This study has the potential to contribute to an improvement in quality and efficiency of CT by laying the foundation for recommendations for guidance on clinical research conducted in children in resource-limited settings.

## 900

### USING MHEALTH TO PROMOTE HAND WASHING WITH SOAP: HOW DO TANZANIAN YOUTH PERCEIVE TEXT MESSAGE INTERVENTIONS FOR HAND HYGIENE?

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Over 1 million deaths a year could be prevented if effective hand hygiene was practiced throughout the world. With the application of mobile technology in health, there are now new opportunities to trigger motive based behaviour change through a Text Message Intervention (TMI) to enable cost effective, efficient and repeatable interventions, delivered at an individual level. This study produced formative research describing the preferences of youth towards an intervention using text messages, to increase hand washing with soap in rural Tanzania. Three main areas were investigated: 1. the text content to optimise behaviour change to increase hand hygiene, 2. the acceptability of text messages for health promotion, and 3. the preferences on the communication style to convey health promotion messages. A cross-sectional sample design was used and both a self-administered survey and focus group discussions were undertaken in a sample of 60 youth (18-25 years) from Mbeya region. A comparison of proportions and analysed weighted rankings were used for statistical analysis. For qualitative data a Thematic Analysis approach was used. In the inductive phase of analysis, a coding framework enabled themes to emerge then the deductive phase aligned these with identified motives. The results showed that text messages is a popular method of communication with 91.7% of the study sample both sending and receiving texts. There was a 100% willingness to receive health promotion text messages and the optimum time to receive them was found to be directly before or after school/work hours. The themes that emerged from the study on preferred motives were safety, protection and comfort that drive this youth group to increase hand hygiene. The study found that the messages should be clear, specific and action based, adopting the preferred language of the target population rather than losing clarity to accommodate humour or story telling communication styles.

## 901

### DATA-DRIVEN DECISION-MAKING FOR MALARIA ELIMINATION IN NAMIBIA: DESIGN AND IMPLEMENTATION OF CUSTOMIZED DASHBOARDS IN DHIS2

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While Namibia has reduced malaria incidence by 97% since 2004, its 2020 national elimination goal has required re-orientation of surveillance systems to rapidly identify areas of continuing transmission and make data-driven decisions about targeting interventions. Paper-based case-reporting forms were simplified and moved to the DHIS2 Tracker system in early 2017, allowing for real-time capture of and access to decision-critical information. The DHIS2 platform also serves as an integrated central database, collating case-based and aggregate data. The integrated electronic information system has allowed for the creation of customized dashboards for decision makers. Each dashboard provides overviews of malaria cases and deaths, socio-demographic profiles of cases, and case management performance at each level of the health system, based on user interest in surveillance data. Key informant interviews and interactive sessions with national, regional, district, and health facility users during surveillance trainings informed the dashboard designs. These sessions provided overviews of current data review and analyses, priorities for indicators, and visualization preferences. Further reviews of surveillance

data, users' technical capacity, and existing digital infrastructure contributed to the design process. At health facilities, the dashboard facilitates immediate corrective action in testing and case management, and to improve quality and timeliness of data reporting. District-level users may use the dashboard to identify underperforming facilities and guide case investigations. Senior managers and users at the National Vector-Borne Diseases Control Programme plan to use the dashboard to design targeted interventions and generate evidence for policy change. The dashboards are undergoing testing with select users at each level for feedback on ease of access, appropriateness of visuals and data, and use in decision making, with the intention of developing a culture of data use. They will be rolled out to all users in April 2017 and closely monitored for use and further feedback.

## 902

### UNDER-FIVE MORTALITY REPORTING FOLLOWING THE EBOLA VIRUS DISEASE EPIDEMIC — BOMBALI DISTRICT, SIERRA LEONE, 2015-2016

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Sierra Leone has an estimated under-five (U5) child mortality rate (120 per 1,000 live births) that ranks among the highest globally. National mortality surveillance and vital registration are limited. The 2014-2015 Ebola Virus Disease (EVD) epidemic response rapidly established death reporting mechanisms that have continued post-outbreak, including a community-based toll-free phone alert system. We assessed under-5 (U5) death reporting in Sierra Leone to prepare for implementation of Child Health and Mortality Prevention Surveillance (CHAMPS) - a network aimed at determining child mortality etiology to guide prevention. Documented U5 deaths between January 2015 and November 2016 were retrospectively reviewed in Bombali Seborra chiefdom, Bombali District, Sierra Leone. The catchment area included approximately 161,383 persons with 1,269 expected U5 deaths calculated from published estimates. Data were abstracted into Excel spreadsheets from three sources: records from eight health facilities, district vital records, and a phone alert system established during the EVD epidemic. Data sources were consolidated and de-duplicated. Overall, 930 unique U5 deaths (73% of expected) were documented. Data sources were minimally overlapping; 65% of deaths were reported by telephone alert only, 20% by health facilities only, 5% by vital records only, and 9% through >1 source. Reporting patterns changed over time. Documented deaths were 92% of expected in 2015, compared to 53% in 2016, and the proportion of cases reported by telephone alert declined from 81% to 65% from 2015-2016. Periods of incomplete and/or inconsistent documentation were evident in all sources. Health facilities and vital records incompletely captured U5 mortality in Bombali Seborra. The phone alert system captured community-based deaths not recorded through other sources and can be used to improve completeness of mortality surveillance in Bombali Seborra; however, strategies are needed to promote post-EVD community participation and ensure the sustainability of the phone alert system.

## 903

### A COMPARISON OF THREE STATISTICAL THRESHOLDS TO TRIGGER A PUBLIC HEALTH RESPONSE TO MONKEYPOX — DEMOCRATIC REPUBLIC OF THE CONGO, 2011-13

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Endemic to the Democratic Republic of the Congo (DRC), monkeypox is a zoonotic disease that causes smallpox-like illness in humans. Observed fluctuations in the number of reported cases over time raises questions about when it is appropriate to mount a public health response, and what specific actions should be taken. Here, we evaluate three different thresholds to differentiate between baseline and heightened incidence of disease, and propose a tiered approach to public health action. Both suspected and confirmed monkeypox cases occurring in Tshuapa Province from 2011-13 were used to calculate three different statistical thresholds (Cullen, C-sum, and a World Health Organization method) based on monthly case counts. When the observed cases exceeded the threshold for a given month, that month was considered to be an 'aberrant' month. The number of aberrant months detected was summed by year for each approach. There was notable variation in the number of aberrant months for each method. The Cullen approach, based on the mean incidence + 2\*standard deviation, did not detect any aberrant signals over the period of consideration (0/36 months). The C-sum method, calculated as the ratio of past to present cases, was the most sensitive, resulting in 18/36 aberrant months. The WHO method, involving the upper 3rd quartile of data from past cases, resulted in 11/36 aberrant months. A single threshold for triggering public health action may be insufficient for monkeypox and other diseases that are endemic yet relatively rare. We propose instead that multiple thresholds be considered to allow responses of varying intensity: 1) an alert threshold prompting further investigation/intervention beyond routine surveillance, and 2) an epidemic threshold, which may entail contact tracing and community education.

## 904

### #GLOBALHEALTH ON TWITTER: ANALYSIS OF TWEETS ON #MALARIA, #HIV, #TB, #NCDS AND #NTDS

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Social media enables users to connect globally and engage in conversations on global health issues. Global health advocates use the hashtag #GlobalHealth on Twitter to draw users' attention to prominent themes on global health, to harness their support, and to advocate for change. Through analyzing five subcorpora within a #GlobalHealth Twitter corpus, we aim to summarize the Twitter global health conversations pertinent to 5 high burden diseases or groups of diseases into 4 major themes. Tweets containing the hashtag #GlobalHealth (n=157,951) from January 1, 2014, to April 30, 2015 were purchased from GNIP Inc. We extracted 5 sub-corpora of tweets, each with one of the top 5 co-occurring disease-specific hashtags (#Malaria, #HIV, #TB, #NCDS, and #NTDS) for further analysis. Unsupervised machine learning (Latent Dirichlet Allocation) was applied to each sub-corpus to categorize the tweets by their underlying topics and obtain the representative tweets of each topic. The topics were grouped into 4 themes (advocacy; epidemiological information; prevention, control and treatment; societal impact) and miscellaneous. In the entire #GlobalHealth corpus (N=157,951), there were 40,266 unique users, 85,168 retweets, and 13,107 unique co-occurring hashtags. Of the 13087 tweets across the 5 subcorpora with co-occurring hashtag #malaria (n=3640), #HIV (n=3557), #NCDS (n=2373), #TB (n=1781) and #NTDS (n=1736), the most prevalent theme was prevention, control and treatment (4339, 33.16%), followed by advocacy (3706, 28.32%), epidemiological information (1803, 13.78%), and societal impact (1617, 12.36%). The rest were miscellaneous (1622, 12.39%). This study highlighted the specific #GlobalHealth Twitter conversation pertinent to malaria, HIV, tuberculosis, non-communicable diseases, and neglected tropical diseases. These

conversations reflect the priorities of advocates, funders, policy-makers and practitioners of global health on these high burden diseases as they presented their views and information on Twitter to their followers.

## 905

### USING NOVEL ESOURCE EDC SYSTEM FOR CLINICAL RESEARCH STUDY IN MULTIPLE COUNTRIES WITH LIMITED INTERNET CONNECTIVITY

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A community-based mass drug administration study for filariasis required collection of drug safety data from some 24,000 participants in 4 countries. The electronic data collection system (EDC) needed to collect data securely (per ICH and CDASH standards) in resource-poor areas with limited internet availability. After evaluating multiple options, we selected an eSource system developed by CliniOps. eSource is a next generation electronic data capture approach where data is entered directly into a tablet via a mobile application ("App"). Electronic case report forms for the study were designed to ensure compliance with country regulations and industry standards and to be used by ground teams with varying levels of technology expertise. The CliniOps eSource technology allowed near real-time data visibility that allowed safety review and oversight by multiple stakeholders around the world. Over a period of five months, 165 users using 4 different languages entered data from approximately 190,000 visits for 24,000 participants generating more than 4,500,000 data points in a database with 3,000 variables. Data was synced regularly through a secured AWS cloud server either using 3G local Enterprise internet providers (DigiCel in Haiti; Airtail in India) or satellite wifi (IndoSat in Indonesia; Northern Axxess in PNG). Strengths included remote monitoring of data management, high quality data with edit checks and query management built into the system, real time data access for all participating groups, minimal paper, offline capability, and guided workflow based data collection, which minimized errors. Limitations are that the system requires at least intermittent wifi for syncing and a cost that is higher than REDCap. In summary, this eSource EDC system allowed supported data collection with real-time analytics and enhanced data quality. This leapfrogging of technology for data collection in research trials provided increased data quality and remote oversight in parts of the world previously considered to be off the beaten digital track.

## 906

### ETHICS OF PREVENTIVE CHEMOTHERAPY FOR NEGLECTED TROPICAL DISEASES

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Preventive chemotherapy (PC) - periodic single-dose drug treatment of all eligible persons in endemic areas regardless of individual infection status - is a key strategy to control or eliminate seven neglected tropical diseases (NTDs). The World Health Organization estimates that ~1 billion persons received PC for NTDs in 2015. Advocates regard PC as an ethical mandate to alleviate immense NTD-related suffering of neglected populations. Critics have raised ethical concerns around informed consent, safety of drug combinations, and lack of benefit for uninfected individuals. However, comprehensive, systematic analyses of the ethics of PC are lacking. I analyzed the ethics of PC using standard principles of bioethics (beneficence, non-maleficence, autonomy, justice), public health ethics principles (proportionality, reciprocity, least infringement, transparency), and the social justice approach of Bailey et al., which considers impact on 'clusters of disadvantage.' In general, PC programs provide massive benefits to disadvantaged populations, minimize maleficence, and adequately respect autonomy; they also serve to dismantle 'clusters

of disadvantage' and promote social justice. However, patterns vary. For example, community-directed treatment with ivermectin (CDTI) for onchocerciasis control likely provided greater autonomy to communities than do some global NTD programs currently focused on interrupting transmission. Factors affecting the ethical dimensions of PC include: the target NTD; the drug or drugs used; frequency and severity of adverse reactions (AEs); the degree to which PC benefits individuals with clinical disease; the intent or goal (control or elimination); evidence of effectiveness; and degree to which programs adhere to public health ethics principles. Further attention is warranted regarding consent for minors ('opt-in' vs. 'opt-out'); AE prevention; outcome measurement; ensuring that PC reaches those at highest risk; and the role of apology. Systematic ethical analyses can help inform global health policy and improve program adherence to ethical principles.

## 907

### FOLLOW-UP OF TRICHIASIS SURGEONS TRAINED ON THE HEAD START SURGICAL SIMULATION DEVICE IN NIGER: WERE SURGEONS ABLE TO MAINTAIN THEIR COMPETENCY ONE YEAR FOLLOWING THE TRAINING?

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Trachoma is targeted for elimination as a public health problem by the year 2020. Providing quality surgical services for trichiasis (TT) patients is necessary to reach the elimination targets, and maintaining surgeons' skills of quality surgery is crucial. In Niger, a study was carried out from September 2015 to February 2016 to determine whether a surgical simulation device, HEAD START, can help experienced surgeons improve their skills. Twenty-three surgeons from the Maradi region took part in this study, 22 completed. Results indicated that a half-day training on HEAD START improved these surgeons' skills, particularly placing traction sutures, suture spacing and correct incisions. In January 2017, we re-evaluated these surgeons on the same skills to determine whether their competency had been maintained. Each surgeon operated on one TT patient under observation of a HEAD START trainer, who evaluated the surgeon on 11 skills on a scale of 1 (poor) to 5 (excellent). If the surgeon had a grade of  $\leq 3$  on one skill, the trainer provided refresher training on HEAD START for that skill; if the surgeon had a grade of  $\leq 3$  on 2 or more skills, then the trainer provided a complete refresher training on HEAD START. In the follow-up, 20 of the 22 surgeons were re-evaluated (two had moved). Nineteen of these surgeons had continued to operate TT patients; their output varied from 7 to 155 cases in the last year. Sixteen of the surgeons received grades of 3 or above on all skills and did not require further refresher training. In almost all skills, the mean composite change for all skills was -0.26 points; individually, all skills were slightly lower at follow-up. Individual surgeons lost a mean of 0.24 points from their score one year ago. There was no correlation between number of surgeries conducted over the past year and score at one year following the training. These results indicate that to maintain skills, ongoing training and supportive supervision is necessary.

## 908

### ACADEMIC HEALTH SYSTEM PARTNERSHIPS MOUNTCREST UNIVERSITY (GHANA), EASTERN REGIONAL HOSPITAL (GHANA) AND PENN STATE COLLEGE OF MEDICINE (USA)

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Founded in 2008 and accredited in 2011, Mountcrest University College (MCUC) is based in Accra, Ghana. MCUC signed a Memorandum of Understanding in July 2014 with the leadership at Penn State College of Medicine (PSCOM) based in Hershey, PA. This collaboration is aimed at opening doors of opportunity for the two campuses to collaborate and support one another as they train the next generation of physicians in their respective countries. The founders of MCUC value higher education and are establishing a new medical school in rural Larteh. This will be the first rural medical school in Ghana and will begin accepting students in September 2017. The multi institutional collaboration has 3 overlapping realms of interaction: an international health systems collaboration between MCUC, PSCOM and Eastern Regional Medical Center (ERMC, the academic center for training interns and residents); global health education for residents in training and medical students at PSCOM and ERMC; and global health research/innovation for faculty at MCUC, ERMC and MCUC. A multitude of endeavors have been initiated between the 3 institutions. Research efforts include external grant funding applications and groundwork to develop a Population Health Research Institute, quality improvement projects, and medical students' community health research projects. On an educational front, a simulation (sim) center at MCUC is being explored through a grant proposal at USAID's Office of American Schools and Hospitals Abroad (USAID/ASHA) to be managed by a MCUCU faculty who was hosted for a 6 month fellowship at PSCOM sim center. On a clinical front, medical students and residents in training at PSCOM (pediatrics, family medicine, med- peds) work in both the inpatient and outpatient clinics alongside residents in training at ERMC. This enhances their ability at maneuvering and adapting medicine learned in a resource abundant environment to work efficiently in a resource limited setting. Additionally the PSCOM residents enhance their fund of knowledge as it pertains to infectious diseases and illness not commonly encountered in central PA.

## 909

### REPORT FROM THE WORLD HEALTH ORGANIZATION'S ADVISORY COMMITTEES ON INNOVATIVE PERSONAL PROTECTIVE EQUIPMENT FOR FRONT LINE HEALTH WORKERS

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The West Africa epidemic of Ebola virus disease (EVD) resulted in 28,646 cases with 11,323 recorded fatalities. The World Health Organization (WHO) documented 815 confirmed and probable cases of Ebola amongst the frontline health workers (FHW). This estimate represents 21 to 32 times greater than the general adult population in the affected West African countries. Preliminary findings of a WHO report found it very difficult to identify the precise risk factors and situations in which FHW were infected. Protection of the health worker was paramount to ensure a safe and conducive working environment. The use of personal protective equipment (PPE), infection prevention control practices and environment and administrative engineering were critical to reduce Ebola infections. In hot, humid settings this was challenging with the use of a full cover PPE which allowed for an average wear time of 45 minutes. Problems with PPE were identified as the key likely contributor to acquiring EVD in FHW. WHO during the height of the Ebola epidemic, reviewed PPE standards, design, material and social acceptance in order to find science-based evidence and innovative ideas to overcome the problems with inconsistent guidance, types of PPE and training. There are no international standards for PPE that protect health workers in the frontline for Ebola and very few systematic designed studies to show PPE use and protection for the health worker. Since October 2014, WHO has convened 4 consultations and workshops and set up 4 Advisory Committees for Innovative PPE to

define the technical and preferred characteristics of PPE for the future. These characteristics would incorporate existing published evidence on PPE effectiveness, the technical specifications, innovative designs, new materials, test standards and cultural aspects to create a PPE for FHW treating high consequence pathogens like Ebola and other filoviruses. We will report on the deliberative process, findings and recommendations of the WHO Advisory Committees.

## 910

### DO PERFORMANCE-BASED MONETARY INCENTIVES FOR REFERRALS BY TRADITIONAL BIRTH ATTENDANTS INCREASE POSTNATAL CARE USE? EVIDENCE FROM A NIGERIAN FIELD EXPERIMENT

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In Nigeria, only 16 percent of mothers who deliver outside facilities receive postnatal care within the 48 hours of delivery. These mothers have a higher risk of mortality from preventable delivery complications. Qualitative evaluations suggest that monetary incentives for performance may motivate referrals of mothers to skilled providers by traditional birth attendants or TBAs. Using data collected through a field experiment in Ebonyi State, Nigeria, this study estimates the impact of monetary incentives for referrals by TBAs to maternal postnatal care within 48 hours of delivery. We recruited 207 TBAs and conducted an individually randomized controlled trial between August and December 2016 in Ebonyi State, South-Eastern Nigeria. TBAs were randomly assigned with 50-50 probability either to receive \$2.00 for every maternal client that attended postnatal care within 48 hours of delivery (treatment group) or to receive no monetary incentive for referrals (control group). We found that the intervention increased the proportion of maternal clients that attended postnatal care within 48 hours of delivery by 15.4 percentage points, and the proportion of neonatal clients that attended postnatal care within 48 hours of delivery by 12.6 percentage points. However, surveys of mothers revealed that in many cases health care providers did not address the issues that may have led to postnatal complications during their consultations. To our knowledge, this is the first field experiment examining the impact of performance-based monetary incentives on referrals by TBAs for maternal and neonatal postnatal care attendance in Africa. We show that motivating TBAs using monetary incentives can increase postnatal care demand among their clients, who have a higher risk of maternal and neonatal complications because of their exposure to unskilled birth attendance. Policy aimed at improving postnatal care uptake using performance-based monetary incentives directed at TBAs should also address improvements in the quality of postnatal care provision so that increases in postnatal care demand can translate to maternal and neonatal health gains.

## 911

### INTEGRATION OF MALARIA ROUTINE AND SURVEILLANCE INFORMATION SYSTEMS IN MALI'S HEALTH MANAGEMENT INFORMATION SYSTEM: BEST PRACTICES AND LESSONS LEARNED

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Until 2010, the platform for Mali's health management information system (HMIS) was Microsoft Access. Due to several challenges with that platform, MEASURE Evaluation helped the National Malaria Control Program (NMCP) to develop and implement an application for routine malaria data reporting using mobile phones in selected regions. However, Mali's interest in an integrated HMIS that could better collect and analyze all health data for quick action, strategic planning, and policy development led the country to adoption of District Health Information Software 2 (DHIS 2). Integration of Mali's HMIS using DHIS 2 started in 2015. The process involved consensus building among national and international partners, development of a DHIS 2 implementation road map, and creation of a budget aligned with development partners. The system was developed in a participative manner with direct involvement of users. Implementation began with the revision of indicators and data collection tools, followed by development of data entry screens and dashboards, and finally, cascade training for users across the health pyramid. To date, the collaborative effort between MEASURE Evaluation and key stakeholders to integrate HMIS has achieved impressive milestones. All 65 districts and 90% of the 1,340 *centres de santé communautaire* (CSCoM) have been equipped with laptop computers and Internet access, and a few CSCoM are equipped with a power source. Additionally, all data managers and malaria focal persons have been trained to use DHIS 2. Successful rollout and deployment of DHIS 2 in Mali relied on oversight committees, good coordination, and trust among partners. The commitment of health facilities to support Internet fees and to use the system was also key. Among the challenges encountered were initial reluctance of national counterparts to switch to DHIS 2, coordinating heterogeneous partners, harmonization of data collection tools, and poor Internet coverage. Ultimately, integration on a web-based platform has empowered the NMCP, as it now has full access to its own data while benefiting from data from other programs.

## 912

### HOW RUMORS OF PLACENTA SELLERS LED TO THE DECLINE OF A MALARIA IN PREGNANCY TRIAL IN BENIN: AN ETHNOGRAPHIC STUDY

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A multi-country community-based trial on scheduled screening and treatment (SST) for malaria in pregnancy (MiP) was conducted in Benin, The Gambia and Burkina Faso. Despite similar design and procedures, trial participation proved to be problematic in Benin where the study became the subject of "rumours". Ethnographic research was carried out in Benin, Burkina Faso and the Gambia on the effectiveness of SST for MiP with a specific emphasis in Benin on factors leading to the deterioration of the trial. Data from group discussions, semi-structured interviews, and participant observation were triangulated and analyzed with NVivo 10 qualitative analysis software. After rumours started of placentas being sold by the trial research team, community members refused to participate or continue in the trial. In Benin, the placenta is considered sacred and is object of several rituals that aim at assuring the new-born's general well-being later on in life. Cultural conceptions on the placenta, however, were similar in all three trial countries and could therefore not be a sufficient condition to have generated trial refusal and drop-out rates. Instead, the

rumors were set in motion by a confluence of factors initiating after a trial-related adverse event and caused by the historical distrust in governmental organizations, socio-economic inequality, sociocultural beliefs in the sacred nature of the afterbirth, and challenges in communication during the informed consent procedures. An improved understanding of study participants' concerns and of historical and geo-political factors can be decisive for a trial's efficacy.

## 913

### ENHANCING CORE COMPETENCIES AND IMPROVING MIDWIFERY QUALITY OF CARE IN LAKE ZONE, TANZANIA

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In sub-Saharan Africa, maternal mortality is unacceptably high accounting for 56% of all maternal deaths. Tanzania is not different. It is estimated that five in every 100 children die before their first birthdays and that four women out of 1000 live births die due to pregnancy related causes. With prompt recognition and timely intervention most maternal and neonatal deaths can be avoided. Access to skilled care at these critical times save lives. A new initiative is working to improve midwifery care by building capacity of training institutions to prepare highly skilled nurse-midwives to enhance on job live saving skills. In 2014 A collaboration between the Tanzania Ministry of Health, Jhpiego through Maternal child survival program (MCSP), conducted a baseline assessment to assess the quality of midwifery pre-service education to adequately prepare students with the clinical skills to provide competent nursing and midwifery care. Four nursing and midwifery schools from two regions of Lake Zone were assessed to identify issues affecting the schools' ability to produce clinically competent graduates in nursing and midwifery. Among things, the assessment focused on tutors and recent graduates, with findings showing critical deficit on content/skill competencies. To address these challenges, midwifery tutors from 9 schools (100%) were updated in high impact midwifery interventions through trainings and supportive supervision including coaching and mentorship. Skills labs were also equipped with all mannequins necessary for midwifery training. The program is in the third year of implementation with tremendous improvement in midwifery training as evidenced by students final examination results as well as tutors' and students' testimonies. Experience of MCSP approach to strengthen competencies of graduates has contributed to improve midwifery quality of care to reduce maternal deaths in Tanzania.

## 914

### CENTERS OF EXCELLENCE IN MONITORING AND EVALUATION: AN APPROACH TO IMPROVING DATA QUALITY FOR EFFECTIVE DECISION MAKING IN THE DEMOCRATIC REPUBLIC OF THE CONGO

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Malaria remains a major public health problem in the Democratic Republic of the Congo (DRC). High-quality data are important to effectively allocate resources, measure achievement, and ultimately, improve malaria control efforts. Data quality, however, remains a challenge in health facilities. The USAID-funded MEASURE Evaluation project supported the Ministry of Health in DRC to set up centers of excellence (COE) to document best practices and improve data quality and use at the health facility level. The establishment of COE included selection of health facilities in targeted health zones, collection of baseline data, and training of providers in fundamentals of monitoring and evaluation (M&E). The training curriculum reviewed concepts related to health information systems and tools for

data collection and transmission. It also reviewed routine supervision and the implementation of data quality assessments. In the original four health facilities selected in 2015, healthcare workers were equipped with data-management tools for health information systems, and they received technical support through supervision visits. Preliminary assessment findings show that data analysis meetings are held regularly, data are validated by the COE team, dashboards are well displayed, and indicators are documented. The findings also indicate that data completeness and timeliness have improved. Data use, however, remains limited, so a data demand and use plan is under development, to increase engagement of healthcare workers. Among this approach's requirements are increasing M&E skills among healthcare workers, improving Internet connection, boosting technical support, and ensuring adequate funding. The COE model has now been scaled up to 22 health facilities, with a further expansion planned to all health facilities in the targeted health zones by 2018. COE are contributing to improvements in data quality and evidence-informed decision making to reduce malaria burden in the DRC.

## 915

### COVERAGE GAPS IN EARLY INITIATION OF BREASTFEEDING AMONG NEWBORNS, SUB-SAHARAN AFRICA, 2010 - 2015

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The neonatal mortality rate in sub-Saharan Africa is estimated at 28 deaths per 1000 live births. Early initiation of breastfeeding is associated with a reduced risk of newborn mortality. We examined data from recent Demographic and Health Surveys (DHS) in sub-Saharan Africa to evaluate early initiation of breastfeeding (breastfeeding within the first hour after birth), as reported by mothers of children < 5 years old. We assessed differences in early initiation of breastfeeding by delivery at home vs at a health facility. In sub-Saharan Africa, 31 countries conducted DHS during 2010 to 2015. Delivery of the neonate at health facilities was reported in greater than 50% of births in 26 of the 31 countries. The median prevalence of reported early breastfeeding was 53% (IQR 37% - 62%). The lowest reported prevalence (16.6%) was observed in Guinea and the highest (81%) in Rwanda. In Ethiopia, 90% of births during the 5 years preceding survey took place at home; no difference was observed in early breastfeeding based on place of birth (52% of births in facilities and at home). Home deliveries were reported in 63% of births in Nigeria; early breastfeeding was reported in a minority of births (29% of home births and 40% of facility births). The three countries with the highest reported prevalences of early breastfeeding (Namibia, 71%; Malawi, 77%; Rwanda, 81%) all had facility delivery rates exceeding 80%. However, other countries with > 80% of births occurring in health facilities had low - moderate prevalence of early breastfeeding (Congo, 24%; Gabon, 32%; Democratic Republic of Congo, 54%). Despite its importance for neonatal mortality and morbidity prevention, early initiation of breastfeeding is not being offered to sizable proportions of newborns in sub-Saharan Africa. Coverage gaps exist even among newborns born in health facilities in many countries, underscoring the pressing need to improve the quality of care during delivery and in the postnatal period in order to achieve the Sustainable Development goal target of reducing neonatal mortality to as low as 12 per 1,000 live births by 2030.

## 916

### READINESS OF PUBLIC HEALTH FACILITIES TO TREAT UNDER-5 DIARRHEA AND PNEUMONIA: OBSERVATION OF PRIMARY HEALTH CARE FACILITIES IN UTTAR PRADESH

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Although the Integrated Management of Neonatal and Childhood Illness (IMNCI) strategy was introduced in India in 2005, in Uttar Pradesh, one of the states with high under-5 mortality rates, the number of children seeking care at government health facilities for pneumonia and diarrhea remains low. This indicates a strong need of improving the quality of care at lower-level public health facilities that may lead to better outcomes, including increased care seeking at government facilities and reduced under-5 mortality. We conducted a survey of 15 government block facilities with multiple different methods to assess the quality of care delivered to sick children: direct observation of the health provider's examination of the child; exit interview with the caregiver; inventory of equipment and supplies; a self-administered assessment of health providers' knowledge and attitudes; a follow-up interview at the sick child's home 15 days after the facility visit; and a follow-up telephone interview with the caregiver after 60 days since the facility visit for a subset of children. Though care seeking for diarrhea and pneumonia is high among the caregivers, majority of the cases end up consulting rural medical practitioners not the government block facilities. The basic equipment and supplies are available but not used at these facilities. The providers at the block level lack knowledge and assessment skills to manage childhood diarrhea and pneumonia. Treatment of severe cases at these facilities is poor with practically no inpatient admission.

## 917

### USING TASK SHIFTING TO EASE THE MENTAL HEALTH BURDEN OF NON-COMMUNICABLE DISEASES

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Depression is the leading cause of ill health and disability worldwide. According to the latest estimates from WHO more than 300 million people are now living with depression. However, robust mental healthcare is nearly non-existent in most emerging markets. Depression is often comorbid with other chronic diseases and can worsen their associated health outcomes. The largest treatment gap for mental disorders is found in sub-Saharan Africa where the ratio of psychiatrists to population is estimated at 1: 1 million. Even when they are available they are often expensive and there is a lot of stigma associated with seeking mental care. Yet depression and anxiety disorders can be chronic, severely impair quality of life, increase mortality and societal costs. In Kenya, mild-moderate depression rates in primary care have been estimated at between 11-42%. Evidence from large RCTs in primary care across low resource settings show that ordinary people with limited training on a specific form of cognitive behavioral therapy (CBT) - problem solving therapy (PST) can deliver effective therapy for people with common mental disorders. PST is universally applicable, avoids stigma and effective for a wide range of issues. CBT therapy via computer has also been shown to be as effective as regular face to face treatment with medication and increases access to care as anonymous access allows to circumvent stigma. Inuka (Swahili for "rise up") is an online coaching chat platform that is used to deliver PST through ordinary lay people trained as coaches. Preliminary testing of the Inuka prototype through interviews, online tests, focus group discussions and two pilots (n=13 and n=12) resulted in positive feedback that provided sufficient evidence to proceed with additional development. A pilot study aimed at assessing whether lay workers can be trained to provide counselling via chat and the associated clinical outcomes on clients is ongoing. These results will contribute to the evidence on whether

depression can effectively be managed by task-shifting in order to reduce the disease burden and disability and improve the overall health of populations.

## 918

### FROM NEW BIOMARKERS TO DIAGNOSTIC TOOLS FOR THE MANAGEMENT OF FEVER IN LOW- AND MIDDLE-INCOME COUNTRIES: AN OVERVIEW OF THE CHALLENGES

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During the past two decades, the incidence of malaria has been decreasing in low- and middle-income countries (LMICs) but acute febrile illnesses are still a major cause of mortality and morbidity. A lack of simple, cheap and rapid tests for febrile illnesses other than malaria is resulting in overtreatment with antibiotics for those who test negative for malaria, contributing to the global rise in antimicrobial resistance. New tests for the detection of host biomarkers provide promising tools to differentiate bacterial from non-bacterial infections in febrile patients. However, most biomarker tests are not designed for use in LMICs and do not exist in rapid test format. Moreover, very few evaluation studies have been performed in the overall non-severe febrile population of LMICs, resulting in poor knowledge on the performance of these biomarker tests in a context of high prevalence of infectious and poverty-related diseases such as malaria, HIV, malnutrition, intestinal parasites. To address the public health challenge of fever management in LMICs, it is important to view the problem holistically, with the goal of an approved test in mind. This review presents some of the many challenges faced during the process of getting to an approved test, including difficulties in selecting the most appropriate fever biomarkers, and suitable study design and sites for test evaluations; lack of reference tests available to evaluate the performance of new tests; lack of interest from *in vitro* diagnostic companies to invest in products with uncertain markets; and lack of clear national and international guidelines for regulatory pathways to introduce such tests. Understanding the difficulties on the product development pathway will enable partners working in this area to address them ahead of time and in collaboration with national and international stakeholders. Improved fever management will have direct impact on patient care but will also influence public and global health as well as outbreak response activities. Product development is a key piece of the puzzle.

## 919

### GIVES: A COLLABORATIVE EFFORT FOR GIS CAPACITY BUILDING IN VECTOR SURVEILLANCE

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The 2016 Zika virus outbreak highlighted challenges inherent in the surveillance and control of its primary vector, *Aedes aegypti*, particularly with respect to the use of Geographic Information Systems (GIS). The capacity to use GIS platforms for *Aedes aegypti* surveillance/control data greatly increases vector control program efficiency. To address these gaps, Emory University's Center for Humanitarian Emergencies, the Liverpool School of Tropical Medicine, and the Integrated Vector Management Team of the U.S. Centers for Disease Control and Prevention developed and implemented the Geographic Information for Vector Surveillance (GIveS) program. GIveS undertook a needs assessment of 37 countries in the Americas, determining individual learning needs and system capacity. Most countries had partially or fully functioning vector control systems; high-level map making skills were not present in the region. The GIveS curriculum is a five-day training utilizing free QGIS mapping software,

available in both Spanish/English. It introduces GIS concepts, guides participants on using tools from mapping basics through the creation of maps using their own spatial data. National/regional-level entomologists and key decision makers engaged in four GIveS workshops. Monitoring and evaluation activities consisted of pre/posttests, daily skills-based assessments, quantitative course evaluation, and two focus group discussions (FGD) per training. Key FGD themes included a perceived need for GIS mapping tools with real-time data; the need for data quality improvement and advanced training. By course-end, participants produced quality maps from their own vector surveillance data. Evaluation data will identify further learning needs and systemic challenges for improved vector surveillance.

## 920

### KNOWLEDGE IS POWER, BY INVESTING IN TRAINING WE ARE SECURING A STRONG AND POWERFUL NATION AND DEVELOPING FUTURE LEADERS

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The Manhica Health Research Center-“Centro de Investigação em Saúde da Manhica - CISM” aims to promote and conduct biomedical research in health priority areas to safeguard the health of the most vulnerable populations in Mozambique. CISM is located in Manhica Village, in southern Mozambique, approximately 80 km north Maputo province. The Village is located in the western margin of Inkomati River. Manhica district has a total area of 2,500 km<sup>2</sup> with near 178,000 people. Since the creation of the CISM in 1996, the training of researchers has been one of its strategic areas, while retaining talent has been a key aspect. The center invests in a training fellowship program, which has been one of the most successful training programs of the Centre in recent years. Based on this program, Mozambican and Southern African candidates with a licentiate or honours degree, with the potential to develop a professional career as researchers are trained in health-related areas. The program ensures that the participants acquire direct experience in research methodology focussed on research projects within the Centre and within the scope of post-graduate training at Master and/or PhD level at internationally recognized universities, and it allows the opportunity to participate in research stays at international universities, research centers and organisations. At the end of this program, researchers are prepared to apply for competitive grants at international level, therefore boost research capacity by the design and implementation of research projects. The training fellowship program contributes to scientific development and to the creation of a critical mass of personnel qualified to assume the management and leadership of research centers in Mozambique and around the world. The centre has trained almost 40 people, 15 of which are women. Almost all of these are now in management and leadership positions at the center or other institutions, such as dean of the school of medicine, national program directors, principal investigators; members of national scientific committees, or members of international boards.

## 921

### ECONOMIC COSTS AND BENEFITS OF MORBIDITY MANAGEMENT AND DISABILITY PREVENTION FOR LYMPHATIC FILARIASIS IN INDIA

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Lymphatic filariasis afflicts 68 million people in 73 countries, with 17 million persons living with chronic lymphedema. The two objectives of the Global Programme to Eliminate Lymphatic Filariasis (GPELF) are to stop new infections and to prevent disability in persons already affected, but morbidity management and disability prevention (MMDP) programs have been initiated in only 24 endemic countries. India is the country

with the most people affected by lymphatic filariasis. We examined the economic costs and benefits of alleviating chronic lymphedema and its effects in India through a simple limb-care program at the community level that includes instruction in limb washing and provision of soap, topical antibiotics, and antifungals. Such a program can reduce ADL and slow progression of lymphedema, attenuating disability and productivity loss. Modeling costs and benefits of this community-based program in the Khurda district of Odisha state in India, we found that such a program would reduce economic costs of lymphedema and ADLA over the lifetimes of affected persons by 55%. Per-person savings are more than 130 times the per-person cost of the program. Building on the study in Khurda, we scaled up the analysis to the level of India as a whole. Most of the parameter values in the Khurda study are specific to that district. Scaling up the community-based program to all-India requires determining new values for the country as a whole, including average daily wage rates used to measure the opportunity cost of lost productivity, lifetime out-of-pocket medical costs for ADLA treatment, and lifetime costs for treatment of chronic lymphedema. The new study demonstrates the lifetime savings from simple limb-care management for reducing disability in all-India through community-based programs. This strengthens the economic rationale for MMDP, which has an ethical mandate as the second pillar of GPELF.

## 922

### USING SHORT MESSAGE SERVICE (SMS) TO REMIND PREGNANT WOMEN OF ANTENATAL APPOINTMENTS IN GUINEA

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The Millennium Development Goals (MDG) 4 and 5 adopted in 2000 aim to reduce child mortality and improve maternal health. The targets for these aims are 1) the reduction of under-five mortality rate by two-thirds, 2) the reduction of maternal mortality by three quarters and 3) universal access to reproductive health. Great strides have been made towards reaching these goals. However, Sub-Saharan Africa continues to report the highest child and maternal mortality rates in the world. The World Health Organization (WHO) recommends four antenatal care (ANC) visits where women receive identification and management of complications, preventive treatment for malaria, identification and management of infection, information to promote the use of skilled attendance at birth and education on healthy behaviors postnatal. 82% of pregnant women in Guinea attend a minimum of one ANC visit while only 49% attend the recommended four visits. Increasing the percent of women attending four ANC visits will improve maternal and infant health in Guinea. The USAID/ Guinea StopPalu project will pilot test the use of short message service (SMS) using Frontline as an alerting system to remind patients of upcoming ANC appointments. RTI International will conduct a formative evaluation that aims to determine if pregnant women who receive SMS reminders are more likely to attend follow-up ANC appointments than those who do not receive SMS reminders and to determine the perception of receiving SMS reminders among patients and the cost benefit of using SMS reminder program. As a result of analyzing the StopPalu's intervention outcomes, RTI International proposed to integrate technology to accelerate the development outcomes of the project. The integration of technology into a project intervention is a new area that is being explored across USAID and other donor-funded projects as the rapid spread of mobile technologies to developing countries continues. The findings of this evaluation can serve as a base for similar project interventions.

## 923

### TIME SERIES ANALYSIS OF THE INTEGRATED DISEASE SURVEILLANCE AS AN INTEGRATED ACTIVITY IN THE DRC

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Classic disease surveillance programs have generally focused on a single disease or group of symptoms. More recently, national programs and organizations have worked to develop integrated platforms where information on multiple diseases and symptoms are collected in parallel to improve the quality of data, especially in limited resource areas. In the Democratic Republic of Congo (DRC), the Integrated Disease Surveillance and Response (IDSR) unit has been implemented to collect information on multiple diseases. Using polio as a case study, we studied existing passive surveillance and laboratory test confirmation data to explore the association between these two components of the integrated system. Using available case counts from the IDSR and lab data from the National Institute of Biomedical Research (INRB) from 2008 to 2013, we explored factors that could be associated with disease reporting. We performed a time series analysis to determine if there are trends in confirmed polio cases and reporting for passively reported diseases as a result of increased activities in zones after confirmation of a case. During the study period, confirmed polio cases in a health zone had a negative association with MPX, measles and tetanus reporting, but had a positive association with acute flaccid paralysis (AFP) reporting. Additionally, health zones with confirmed cases of MPX had a positive association with MPX reporting, but a negative association with AFP, measles and tetanus reporting. The IDSR was implemented to integrate disease identification and confirmation, and improve the overall detection of all reportable diseases. The analyses indicate that the specific individual disease components may currently only work to strengthen individual disease detection. Further research should be done to learn how the system could be better integrated for improved utilization of already limited resources.

## 924

### FUNCTIONAL AND MECHANISTIC CHARACTERIZATION OF O-FUCOSYLATION IN MALARIA PARASITES

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Protein glycosylation is one of the largest and most diverse classes of post-translational modifications (PTM). Of these modifications, O-fucosylation represents a relatively small but important glycosylation modality that plays a role in protein trafficking, cell movement/adhesion, and protein secretion. In Eukaryotes, the enzyme O-fucosyltransferase 2 (PoFUT2) is responsible for the specific O-fucosylation of a serine/threonine residue on thrombospondin type I repeat (TSR) protein domains. The TSR domains of several key proteins of the malarial parasite facilitate attachment to mammalian and mosquito host cells, and it was recently shown that *Plasmodium* sporozoite surface proteins containing TSR domains are

fucosylated. We hypothesize that *Plasmodium* modifies these domains via the putatively identified parasite PoFUT2 homolog and that this PTM influences ookinete and sporozoite invasion of mosquito and human host cells. We describe biochemically the activity of PfPoFUT2 in modifying TSR-domain containing proteins, such as the circumsporozoite protein (CSP), and evaluate its biological significance during parasite transmission through the mosquito. We characterized by mass spectrometry (MS) the TSR domain of CSP, as well as full length CSP, and show that both can be fucosylated by human and *Plasmodium* PoFUT2 enzymes. We generated a PoFUT2 null mutant of *P. falciparum* and *P. berghei* and compared the growth and survival of parasites throughout the life cycle. We observed that the PoFUT2 null mutant is viable, without any notable defects during development in blood-stage culture and mosquitoes; but *P. falciparum* salivary gland sporozoites are compromised in their motility in the null mutant compared to wild-type, potentially affecting hepatocyte invasion. Our MS data indicate that CSP lacks this fucose residue in the PoFUT2 null mutant sporozoites. We further compared introduction of null-mutant and wild-type sporozoites to mice by IV inoculation and mosquito bite by monitoring liver and blood stage infection. Finally, we examined the immunogenicity of O-fucosylation on full length PfCSP in mouse immunization studies.

## 925

### ANALYSIS PIPELINE FOR PFEMP1S IN PARASITES ISOLATED FROM CHILDREN PRESENTING WITH MALARIA

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One of the major challenges in *Plasmodium falciparum* proteomics studies is identifying PfEMP1s at the protein level due to antigenic variation. To overcome this technical limitation, we developed a pipeline that combines high resolution mass spectrometry with bioinformatics tools including RNAseq on a subset of parasite isolates, *de novo* sequencing of proteins and sequence alignment to identify PfEMP1 expressed by clinical parasite isolates. Parasites from thirty-five *P. falciparum* infected Malian children were grown in culture for 14 to 20 hours in culture, membrane proteins were extracted and electrophoresed followed by in-gel digestion with trypsin. Peptides were analyzed by LC-MS/MS using the LTQ Orbitrap Velos. Acquired spectra were analyzed with PEAKS Studio using a combined database which includes RNAseq constructs. The peptides were then analyzed with the in-house Local Peptide Alignment (LAX) algorithm to match to a protein. The PfEMP1-matched peptides were placed in matrices to understand the relationship amongst the PfEMP1 protein matches. Peptides were then assigned to PfEMP1s with the most coverage and highest match score. Using this strategy, we doubled the total number of peptides that can be assigned to PfEMP1s. We will present the analysis pipeline for PfEMP1 proteins.

## 926

### SEEKING CARE AT A DRUG SHOP AS THE INITIAL RESPONSE TO ILLNESS WAS A RISK FACTOR FOR SEVERE MALARIA IN UGANDAN CHILDREN

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We studied associations between delayed care seeking, demographic, socioeconomic and geographic factors, and risk of severe malaria in Ugandan children. A case-control study was based at Jinja Hospital, Uganda. We enrolled 325 severe malaria cases and 325 uncomplicated malaria controls matched by age and residence. Patient details, an itinerary of events in response to illness, household information and location of participants' residences were captured. Conditional logistic regression was used to determine risk factors for severe malaria and delayed care seeking. Delayed care seeking (> 24 hours after fever onset; OR 5.88; 95%CI 2.75, 12.5), seeking care at a drug shop as the initial response to illness (OR 3.37; 95% CI 1.66, 6.83) and increasing distance from place of residence to the nearest health center (OR 1.52; 95% CI 1.21, 1.91) were independent risk factors for severe malaria. On sub-group analysis, delayed care seeking was a significant risk factor in children with severe malaria attributable to severe anemia (OR 11.5; 95%CI 2.78, 48.2), but not unconsciousness (OR 1.12; 95%CI 0.29, 4.19). Seeking care at a drug shop (OR 3.85, 95% CI 1.19, 12.3) and increasing distance to the nearest health center (OR 1.93, 95%CI 1.21, 3.08) were independent risk factors for delayed care seeking. Delayed care seeking and seeking care at a drug shop were risk factors for severe malaria. Seeking care at a drug shop was also a predictor of delayed care seeking. The role of drug shops in contributing to delayed care and risk of severe malaria requires further study.

## 927

### ALTERED GUT MICROBIOTA COMPOSITION IN PLASMODIUM FALCIPARUM PATIENTS IN UGANDA

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Malaria caused by protozoa of the genus *Plasmodium* is the most prevalent infectious disease in tropical and subtropical regions. Gastrointestinal symptoms such as abdominal pain, vomiting and diarrhea are frequently observed in malaria patients in addition to the malarial triad of fever, anemia, and splenomegaly. However, the association between intestinal microbiota and malaria pathology is poorly understood. We previously reported that mice infected with *P. berghei* ANKA suffer from intestinal pathology associated with remarkable changes in microbiota. In this study, we investigated the influence of falciparum malaria on microbiota in Ugandan patients. We collected stool samples from *P. falciparum* patients who were recruited from outpatients and inpatients in hospital during illness and convalescence. 16S rRNA metagenome analyses of stool samples revealed that patients have a different microbiota composition compared with healthy children, and the recovery from infection leads to different changes in gut microbiota composition between inpatients and outpatients. We also found that fecal microbiota of Ugandan children could be clustered into two enterotypes, *Bacteroides*/*UC\_Lachnospiraceae* and *Prevotella*, and the main changes in microbiota structure during illness was associated with a shift in enterotypes. Further investigations of the relationship between intestinal microbiota and malarial pathogenesis will add to our understanding of the mechanisms involved in intestinal pathology in malaria patients.

928

### PYGM75, A PROTEIN IN OSMIOPHILIC BODIES, IS DISPENSABLE FOR EGRESS OF MALE GAMETOCYTES BUT IMPORTANT FOR EXFLAGELLATION OF MICROGAMETES

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*Plasmodium* transmission via mosquitoes requires sexual stage parasite development and fertilization in mosquito midguts. Once gametocytes are taken up by mosquitoes, gametogenesis is initiated by the temperature shift and chemical factors in mosquito midguts. Parasite egress from erythrocytes is one of the essential steps during gametogenesis, which requires lysis of parasitophorous vacuole membrane (PVM) and erythrocytic membrane (EM). Gametocytes retain unique vesicle structure, named osmiophilic body (OB), which contains several secretory proteins such as GEST, MDV1/Peg3, involved in PVM break down. Previously, we identified a novel male specific OB protein PyGM75 required for the transmission in *Plasmodium yoelii*. Here, we examined in which steps, PyGM75 has an important role during transmission by using *pygm75* gene disrupted parasites. To demonstrate whether PyGM75 is involved in egress of male gametocytes, *pygm75* disrupted gametocytes were activated to initiate gametogenesis for 15 min, and then incubated with  $\alpha$ tubulin antibodies and TER-119 antibodies to stain male parasites surrounding EM. Approximately 70% of *pygm75* disrupted male parasites did not retain the EM, which was comparable to wild type. In addition to EM breakdown, loss of PVM was observed by electron microscopy. On the other hand, although axoneme were formed in *pygm75* disrupted male parasites, release of flagella was affected. These results indicate that PyGM75 is dispensable for parasite egress from erythrocytes and flagella formation, but it plays a role in the process of exflagellation.

929

### DEVELOPMENT OF RELIABLE MOSQUITO INFECTIONS WITH NON-3D7 PLASMODIUM FALCIPARUM STRAINS FOR HETEROLOGOUS CONTROLLED HUMAN MALARIA INFECTION (CHMI)

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Since 1985, the Walter Reed Army Institute of Research has produced *Plasmodium falciparum*-infected mosquitoes for use in clinical trials to evaluate anti-malarial vaccines, drugs, and immunity. The vast majority of those > 110 experimental malaria challenges used Pf3D7 and its parent strain, PfnF54, but the recent progression of candidate anti-malarial products has necessitated development of *P. falciparum* strains that are heterologous to Pf3D7. However, attaining strains that are culture-adapted and produce reliable, robust sporozoite infections in mosquitoes is difficult and laborious. Here, we describe the optimization of an existing clonal strain (Pf7G8) from Brazil and the development of new strains from Kenyan clinical isolates. For Pf7G8, we used parallel membrane feeding assays to evaluate the ability of three different *Anopheles* species to sustain CHMI-worthy sporozoite infections. These species differentially give low, medium, and high infection rates, indicating the vector species is a primary determinate of Pf7G8 success. For development of new strains, we received clonal parasite lines derived from parasitemic patient collections at hospitals or centers within the USAMRU-K Surveillance Network. We processed these lines for adaptation to our blood-stage culture methods, optimization for gametocyte production, and ability to

infect mosquitoes. We are currently evaluating CHMI suitability of 4 lines that were prioritized based on circumsporozoite protein (CSP) sequencing data; over 30 additional lines could be subsequently evaluated.

930

### FUNCTIONAL CHARACTERIZATION OF A PUTATIVE SEX SPECIFIC BIOMARKER IN PLASMODIUM FALCIPARUM

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*Plasmodium falciparum* is an obligate parasite that completes its asexual and sexual life cycles in vertebrate and invertebrate hosts. Mature gametocytes are the sexual stages of the parasite present in the vertebrate microvasculature that are ingested by *Anopheles* mosquitoes during blood meal. The mature gametocytes are responsible for parasite transmission to the *Anopheles* vector where gametogenesis, fertilization, and reproduction occur. While the male and female gametocytes are morphologically distinguishable in Giemsa-stained thin blood smears, little is known about the mechanism and key players of sexual dimorphism in *P. falciparum*. Identification and functional characterization of sex-specific biomarkers would aid in exploring their biology and help differentiate the parasite "gender". Our previous systematic subtractive bioinformatic analyses compared the sex partitioned proteins in *P. berghei* and *P. falciparum*. This study led to the identification of conserved putative female and male specific gametocyte protein biomarkers, but the function of these putative sex specific proteins remains unknown. This study focuses on the functional characterization of a putative conserved protein that may serve as a biomarker for mature female gametocytes and play a role in sex partitioning in *P. falciparum*. This putative female-specific gene was cloned and expressed using a heterologous bacterial expression system. The spatio-temporal expression profile of this gene over a 12 day development period was determined by immunofluorescence, real time PCR and quantitative Single Reaction Monitoring Mass Spectrometry. Furthermore, antibodies against this sex-specific protein were assessed for their potential to block the parasite transmission to the mosquitoes by standard membrane feeding assays. Our body of work has shed light on a novel gene in the context of gametocyte development biology and hints at the utility of such a target for transmission-blocking interventions.

931

### A MECHANISTIC APPROACH TO UNDERSTANDING THE EFFECTS OF HIV-RELATED INFLAMMATION ON PLASMODIUM FALCIPARUM GAMETOCYTE DEVELOPMENT

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Co-infection with HIV-1 infection and *Plasmodium falciparum* has been associated with more frequent and severe episodes of clinical malaria. The effect of co-infection on malaria transmission is less well understood, although a previous study of SIV and *Plasmodium fragile* in rhesus macaques showed increased transmission potential due to much higher numbers of gametocytes in co-infected animals. We therefore hypothesize that people who are infected with both diseases may be carrying higher numbers of gametocytes, even during asymptomatic infections. We further hypothesize that an increase in inflammatory cytokines due to HIV infection is contributing to an increase in soluble adhesion molecules, including VCAMs and ICAMs, which are then binding to parasitized red blood cells (iRBCs), reducing the ability of iRBCs to sequester in the vasculature. It is possible that an inability to sequester may, in turn, serve as a signal for gametocytogenesis as the parasite attempts to move to a more suitable host environment. In humans, this is particularly difficult to study due to both the natural variations in gametocyte levels over time in asymptotically infected individuals, and the presence of potential

confounders that can dampen or interfere with the effects of increased inflammatory cytokines. To better understand the mechanism of this interaction, we are taking a multifaceted approach to analyze the effects of inflammatory cytokines on gametocyte adhesion and sequestration in a more simplified *in vitro* system of *P. falciparum* and endothelial cell co-culture. We look at the effects of cytokines on several outcomes, including the production of soluble adhesion molecules, *P. falciparum* gametocyte gene expression, and the ability of parasitized red blood cells to adhere to the vasculature. We hope to recapitulate the results of these *in vitro* studies using serum from HIV positive individuals. A better understanding of the specific triggers of gametocyte formation will help reduce transmission and aid in the movement to eradicate malaria worldwide.

### 932

#### EXPLOITING MECHANISMS OF GLYCOLYTIC REGULATION IN MALARIA PARASITES

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Malaria is estimated to be responsible for over half of all deaths throughout human history. Pregnant mothers, young children, and the immune compromised are at the highest risk for infection. Unfortunately, the treatment and prevention of malaria remains an up-hill battle despite major advances in modern healthcare. Resistance has developed to every available antimalarial highlighting the importance of early stage drug target discovery in addition to late stage drug development. To address this problem, we investigate novel metabolic control mechanisms that are common in malaria parasites but distinct from the human host. In humans and malaria parasites glycolysis serves as a central point for controlling carbon for energy production and essential biomolecules such as lipids, nucleotides, and amino acids. A long history of studying glycolysis in model organisms has shown that phosphofructokinases (PFKs) serve an important role in overall metabolism via strong glycolytic control. Interestingly, malaria parasites contain a biochemically and phylogenetically unique PFK, which differs from the PFK found in the human host. Understanding the unique biochemical, structural, and regulatory functions of the malaria parasite PFK will provide a strong foundation for rational drug development strategies for the next generation of antimalarials. Additionally, the unique phylogeny of the malaria PFK matches other apicomplexan parasites and will likely translate our understanding to other important pathogens including *Cryptosporidium* spp.

### 933

#### PROTEOMIC CHARACTERIZATION OF *PLASMODIUM BERGHEI* LIVER STAGE MEROZOITES

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Malaria parasites must replicate in the liver before initiating the symptomatic blood stage of infection. Liver stage replication yields tens of thousands of liver stage merozoites, which are released into the bloodstream in packets called 'merosomes' that are enclosed within a host-derived membrane. Liver stage merozoites have structural and functional overlap with blood stage merozoites as both invade red blood cells, but data suggest they also have unique biology. However, little is known about the molecular events involved in merozoite formation, liver stage merozoite release, and red blood cell invasion. In this study, we use the *Plasmodium berghei* rodent malaria model to perform the first proteomic characterization of mature liver stage merozoites. We optimized large-scale *in vitro* culture of *P. berghei* liver stages and use high-resolution

Orbitrap mass spectrometry to perform untargeted proteomic analysis of isolated merosomes. We identify 1188 liver stage merozoite proteins with high confidence, including proteins involved in motility, egress and invasion, metabolism and housekeeping processes. Furthermore, we identify N-terminally processed peptides from several proteins that contain PEXEL protein export sequences, indicating these proteins are exported into the host liver cell during merozoite formation. This study reveals new insight into the biology of this elusive life cycle stage and identifies intriguing candidates for future study.

### 934

#### EVALUATING NEW METHODS FOR *PLASMODIUM VIVAX* IN VITRO CULTURE FROM FROZEN SAMPLES

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Historically, *Plasmodium vivax* (Pv) cultures have encountered significant challenges that have limited the ability to perform laboratory-based studies on this important human malaria parasite species. A growing number of approaches have now begun to report conditions that support short-term Pv *in vitro* cultures, however, little progress has been made toward developing long-term culture. Past studies have used standard medias supplemented with human AB serum and a variety of additives with varying degrees of success. The majority of these studies made further efforts to supplement cultures with reticulocytes, given the well-known Pv reference for these cells. Here we have compared long-term growth and propagation of the Pv monkey-adapted strain, Vietnam IV/ Palo Alto in *Saimiri* blood in published culture conditions as well as the newly developed AIM V media. All data shown are the results from flow cytometry which counted the number of DNA positive cells from triplicate culture conditions, 144 hrs after thawing. We demonstrated that AIM V had double the number of parasites compared to other medias (AIM V +10% human AB serum= 16,687 (± 92) parasitized (pRBC)/uL, RPMI 1640 + Waymouths +15% serum= 8,487 (±54) pRBC/uL, McCoys 5A + 25% serum = 8,137 (±19) pRBC/uL, and RPMI 1640 + 25% serum = 2,900 (±6) pRBC/uL). We further improved AIM V media by supplementing with between 10% up to 25% human AB Serum. With 10% Serum yielding 25,350 (±18,793) pRBC/uL, 15% = 28,133 (±21,418) pRBC/uL, 20%= 18,117 (±553) pRBC/uL, and 25%=24,875 (±672) pRBC/uL. Finally, we compared how variation in the atmospheric conditions influenced Pv *in vitro* propagation in AIM V with 10% AB Serum by varying the CO<sub>2</sub> concentration from 5% to 10%. In 5% CO<sub>2</sub> there were 12,683 (±769) pRBC/uL but when increasing the CO<sub>2</sub> to 10% we saw a marked increase in the number of parasites up to 51,767 (±6,804) pRBC/uL. Through this work, we identified AIM V with 10% AB Serum in 10% CO<sub>2</sub> as the conditions that best supported Pv *in vitro* culture and with this we were able to show 4 expansion cycles of the Vietnam IV strain of Pv over 40 days in *Saimiri* blood with parasites continuously visible by light microscopy using Giemsa stain.

### 935

#### EFFICACY OF ARTEMETHER LUMEFANTRINE AND DIHYDROARTEMISININ PIPERAQUIN FOR THE TREATMENT OF UNCOMPLICATED MALARIA IN KISUMU, WESTERN KENYA

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*Plasmodium falciparum* (Pf) resistance to artemisinin is prevalent in Southeast Asia (SEA) and is a threat to malaria control efforts. Africa is currently spared, but this observation is evocative of the emergence of

chloroquine and sulphadoxine-pyrimethamine resistance that was first observed in SEA and later in Africa. More comprehensive monitoring is required in malaria endemic areas. In 2014-2015, we conducted an efficacy study of artemether lumefantrine (AL) and dihydroartemisinin piperazine (DHA-PPQ) for the treatment of uncomplicated *Pf* malaria in Kisumu, western Kenya - an area with high malaria transmission. A total of 118 subjects were randomized in a 1:1 ratio to receive either AL or DHA-PPQ. Treatment was directly observed. Blood draws for malaria tests were performed at hours 0, 4, 8, 12, 18, 24 and 6 hourly thereafter until 2 consecutive negative malaria blood films (MBFs) were obtained. Blood samples for MBFs were also collected during weekly follow-up visits from day 7 to 42. Hour 0 samples were tested for *ex vivo* sensitivity to antimalarial drugs, as well as for the presence of genetic markers of drug resistance. The geometric mean parasitemia at presentation was 36928 parasites/ $\mu$ L and 38653 parasites/ $\mu$ L for the AL and DHA-PPQ arms respectively. There were no cases of early treatment failure. After PCR correction, 100% of the subjects in both arms had 28 and 42 day ACPR. The median parasite clearance slope half-life was 2.3 hours (IQR 2.0 - 2.9) and 2.2 hours (interquartile range (IQR) 1.9 - 2.5) for the AL and DHA-PPQ arms respectively. The time taken to clear 99% of parasites was 20.1 hours (IQR 17.5 - 22.1) and 16.8 hours (IQR 14.6 - 19.5) for the AL and DHA-PPQ arms respectively. At least 20% of the parasites had the wild type genotype in respect to K13. Hour 0 drug sensitivity IC50 median values for artemether, dihydroartemisinin and lumefantrine were all within the threshold for sensitivity. AL efficacy for the treatment of uncomplicated malaria in Kisumu is still high. None of the K13 mutations reported in SEA. This study provides baseline malaria parasite clearance profiles that must continuously be monitored.

### 936

#### INTERMITTENT PREVENTIVE TREATMENT WITH DIHYDROARTEMISININ-PIPERAZINE IN YOUNG UGANDAN CHILDREN IN THE SETTING OF INDOOR RESIDUAL SPRAYING OF INSECTICIDE

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Intermittent preventive treatment (IPT) is a promising strategy for the prevention of malaria in young children living in Africa. Dihydroartemisinin-piperazine (DP) is safe and highly efficacious when used for the treatment of malaria and is an excellent candidate for IPT because of its prolonged post-treatment prophylaxis. However, the optimal dosing strategy for DP as IPT in young children is unclear. We are conducting a double blinded randomized controlled trial (NCT02163447) to evaluate different IPT dosing strategies using DP in a birth cohort of children born to HIV uninfected pregnant women in Tororo district. Tororo is an area with historically high transmission intensity with an incidence of malaria of ~ 5 episodes per year in children 6-24 months of age based on previous cohort studies. In December 2014, indoor residual spraying of insecticide (IRS) was implemented in Tororo and ~ 90% of children from this study were born after IRS was implemented. Pregnant women were randomized to receive DP every 4 or 8 weeks during pregnancy and children were randomized to DP every 4 or 12 weeks between 8 and 104 weeks of age. Children are followed for all their health care needs and malaria diagnosed when a child presents with fever and the detection of malaria parasites by microscopy. Routine blood samples are collected every 4 weeks to estimate parasite prevalence by microscopy and loop mediated isothermal amplification (LAMP). Of 191 live births, 183 have reached 8 weeks of age and were initiated on study drugs. As of March 2017, 149 had reached 104 weeks of age, 17 had been withdrawn from the study and 17 were

still taking study drugs. There have been 65 episodes of malaria during 324.3 person years of follow-up giving an overall incidence of 0.20 episodes per person years. During routine visits, the prevalence of malaria parasitemia, as determined by microscopy and LAMP, has been 1.6% and 2.2% respectively. It is anticipated that the final un-blinded results stratified by children's IPT regimen will be available before November 2017. Compared to historical controls, the burden of malaria has been very low in this birth cohort in the setting of IRS and IPT.

### 937

#### ACTIVE MONITORING OF ARTEMISININ COMBINATIONS THERAPY ACT USE FOR TREATMENT OF UNCOMPLICATED MALARIA AMONG PREGNANT WOMEN IN SENEGAL

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Since 2010, the World Health Organization (WHO) recommends the use of ACTs in pregnant women for treatment of uncomplicated malaria from the second trimester of pregnancy. In May 2013, the NMCP in Senegal revised its case management guidelines to incorporate this recommendation. ACT has the singularity of having a rapid action, but its large scale use in pregnant women has to be supported by active pharmacovigilance in order to monitor its safety and tolerance. Accordingly, 12 health facilities of the districts of Mbao, Pikine, Guediawaye and Kedougou. were purposively selected for the implementation of this approach, taking into account their capacity for recruiting cases of malaria in pregnant women. From January to December 2016, the NMCP recorded 7044 cases of malaria in pregnant women nationwide and a single malaria-associated death, according to routine data. In the same period, a total of 210 cases of uncomplicated malaria were confirmed among pregnant women in their 2nd or 3rd trimester of pregnancy at the 12 monitoring sites. Among these 208 women, 208 (100.0%) were treated and cured using ACTs. No cases of therapeutic failure were identified among pregnant women treated with ACT during the monitoring period. 21 (10.1%) of these women were followed at 4 health centers and had laboratory testing (diagnostic microscopy and blood count) performed on days 0 and 28 to monitor their response to treatment, which confirmed their full recovery. Of all the 208 cases treated with ACT, only 2 pregnant women had adverse effects identified thru active follow-up. The first woman presented minors effects like vomiting, asthenia and heavy legs on Day 3, and the second had side effects of tinnitus, Gastralgia and paleness on Day 7. Nonetheless, these two cases suffering of minor adverse effects were treated and cured of malaria after 3 days of ACT treatment. In conclusion, ACTs are effective and well tolerated in pregnant women, but the biggest limitation is the contraindication of usage during the first trimester of pregnancy due to the medication's teratogenic effects.

### 938

#### IMPROVING HEALTH CARE WORKER PERFORMANCE IN ADHERENCE TO TESTING AND TEST RESULTS FOR MALARIA IN EIGHT SUB-SAHARAN AFRICAN COUNTRIES

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Adherence to malaria diagnostic test results is crucial to ensure effective treatment of malaria and other febrile conditions, and to decrease irrational use of artemisinin-combination therapies (ACT). Reasons for lack of adherence may include lack of knowledge of guidelines, lack of

confidence in the diagnostic test or laboratory, stock-outs of ACTs or other drugs, and satisfying patient demands for treatment. The MalariaCare project has worked with National Malaria Control Programs to train a cadre of laboratory and clinical experts as on-site supervisors. They then work with local staff at health facility level to perform on-site outreach training and supportive supervision (OTSS). During OTSS, adherence is measured in two ways. First, is through direct observation of clinical encounters, where adherence is recorded as 'correct prescription per test result and diagnosis'. In 4,451 clinical observations in 7 sub-Saharan African countries, 93% were adherent with this measure. The second method, a register review, evaluates 3 measures: identifies 10 patients who received ACTs and records if they received a test prior to treatment (TPT), and starting from the diagnostic side identifies 10 tested patients (5 RDT, 5 microscopy) each with negative and positive test results (NTR & PTR) and recording if they received an ACT. The project strives for 90% adherence for these measures: TPT, withholding ACTs for NTR and treating with ACTs for PTR. The proportion of facilities meeting this standard for all 3 indicators in 8 countries during the most recent OTSS visit was 81%. Steady improvement in performance for each measure in facilities receiving at least 3 OTSS visits has been seen by 13, 18, and 10 percentage points respectively for TPT, NTR and PTR. No improvement or a decrease in performance for PTR was seen in 3 countries and were associated with ACT stock-outs and/or ongoing use of quinine. Findings indicate that OTSS may improve test adherence in areas where diagnostic and ACT stock-outs are minimal and national guidelines are in line with global standards.

### 939

#### PREVALENCE OF MDR1 AND K13 POLYMORPHISMS IN PLASMODIUM FALCIPARUM AFTER A DECADE OF USING ARTEMISININ-BASED COMBINATION THERAPY IN MAINLAND TANZANIA

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In 2016, a therapeutic efficacy study (TES) was conducted at 4 sentinel sites (Kibaha, Mkuzi, Mlimba and Ujiji) in Tanzania. This single-arm prospective study evaluated artemether-lumefantrine (AL) for the treatment of uncomplicated malaria in children aged six months to 10 years. Follow-up was done for 28 days with scheduled and unscheduled visits (if symptoms reoccurred) for clinical and laboratory assessments. A total of 344 patients were enrolled in the study and 332 completed follow-up or had a pre-defined study outcome. Of these, 67(20.2%) demonstrated asexual parasitaemia after day 3 and none had early treatment failure. After PCR correction to distinguish recrudescence from new infections, only one patient from Mkuzi had late clinical failure

with a recrudescence infection on day 28, yielding a corrected adequate clinical and parasitological response rate of >99%. Sequencing of *k13* gene was successful for 395 samples (327 and 68 samples from day zero and recurrent infections, respectively) and revealed non-synonymous mutations in 6 samples: I416V, E433D, R471R, and A578S each in 1 sample; Q613E in 2 samples. However, none of these *k13* mutations have been associated with artemisinin resistance. For the *Pfmdr1* gene, 409 were successfully sequenced (334 and 75 samples from day zero and recurrent infections, respectively) and 178 (44.1%) samples possessed non-synonymous mutations: 2 with N86Y, 1 with N86I, 168 with Y184F, 1 with Y184N, 2 with S1050F, 1 with D1111N, and 3 with D1246Y. All samples analysed for *Pfmdr1* copy number (n=184; 149 from patients with recurrent parasitemia during follow up and 35 additional day zero samples) had a single copy of the gene. The one patient with recrudescence infection had wild type *k13* and *Pfmdr1* genes in both the initial and recrudescence samples. In summary, we observed no *k13* mutations associated with artemisinin resistance and high efficacy of AL treatment. Continued monitoring of molecular markers of resistance to artemisinin-based combination therapies is critical in supporting TESS and providing evidence-based malaria treatment policies.

### 940

#### COMPLEXITY OF INFECTION AND PARASITE RELATEDNESS OF PLASMODIUM FALCIPARUM PARASITE POPULATIONS IN PATIENTS ADMINISTERED ARTEMETHER-LUMEFANTRINE (AL) IN KENYA

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Molecular barcode of twenty-four unlinked single nucleotide polymorphisms (SNPs) have been used to characterize *Plasmodium falciparum* parasite. Complexity of infection (COI) is the average number of infections within human host that influences malaria clinical outcome and transmission of resistant strains. Owing to the wide range of disease endemicities, data on molecular barcode characterization of Kenyan parasites from all transmission zones is still inconclusive. This study sought to identify and track specific *Plasmodium falciparum* parasites in patients administered artemether-lumefantrine (AL) in different malaria endemic regions in Kenya. DNA from 71 blood samples collected from the various malaria transmission zones in Kenya in the year 2014 were isolated prior to target amplification and high resolution melting for SNP discrimination. Each sample was genotyped using a molecular barcode of 24 SNPs distributed across *P. falciparum* genome which have a high minor allele frequency (average MAF > 35%). Clustering was done in comparison with Asian, South American and African isolates. The highest COI value was 2, reported in 8% of all samples which represented presence of poly-genomic infections while the rest had COI of 1 representing isolate with mono-genomic infections. Poly-genomic infections showed evidence of high genetic diversity and malaria transmission among Kenyan parasites. All isolates from Asia, South America and Africa clustered geographically. More specific, clustering of Kenyan parasites showed presence of distinct (similar-genotype) parasite profile. Eight samples (11%) with posterior probability value of < 0.95 were eliminated from subsequent network analysis. These findings are important in evaluating reduced response to anti-malarial therapy which its findings pose a threat of artemisinin resistance emergence thus hampering malaria control and elimination efforts. Studies clustering COI and Poly-genomic samples with treatment outcome are warranted for detecting effect of current treatment in infection composition and parasite evolution.

## 941

### MOLECULAR CHARACTERIZATION OF IMPORTED MALARIA PARASITES DIAGNOSED IN THE UNITED STATES BETWEEN 2014 AND 2016

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Malaria continues to be a significant disease in returning travelers from endemic countries. In 2012, the Centers for Disease Control and Prevention (CDC) expanded its malaria surveillance activities to detect molecular markers of antimalarial drug resistance in malaria parasites imported into the United States. Approximately 1,500 cases of imported malaria are reported in the United States annually and, blood samples are sent to the CDC Malaria Branch Laboratory for about 10%-15% of these. Between 2014 and 2016, a total of 683 samples were received. The majority of these specimens were from patients who had travelled to Africa and the bulk of the infections were caused by *Plasmodium falciparum* (70.5%). Infections with the other species were less frequent: 11.0% *Plasmodium vivax*, 9.3% *P. ovale* and 3% *P. malariae*. The *P. falciparum* positive specimens were evaluated by sequencing for known molecular markers of antimalarial drug resistance in *Pfcr*, *Pfdhfr*, *Pfdhps*, *Pfmdr-1* and *PfK13* genes. Copy number variation (CNV) in the *Pfmdr-1* gene was also evaluated. Mutations in the *PfK13* gene were observed in 4 of 439 successfully sequenced samples (all acquired in Africa): two had the 578S mutation, commonly observed in Africa and two had a synonymous mutation at codon 493 (TAC to TAT). Of the 391 samples tested for *Pfmdr-1* CNV, 12 (3.1%) had more than one copy of the *pfmdr-1* gene, associated with mefloquine resistance. Mutations in all the genes investigated reflected patterns similar to what is commonly observed in parasites from the countries where the infections were acquired. CDC will continue to track drug resistance markers in malaria samples and these data will contribute to chemoprophylaxis guidelines for United States travelers to malaria endemic countries and appropriate treatment guidelines for malaria in the United States.

## 942

### PREVALENCE OF K13 MUTATION AND DAY-3 POSITIVE PARASITEMIA IN AN ARTEMISININ-RESISTANT MALARIA ENDEMIC AREA OF CAMBODIA: A CROSS-SECTIONAL STUDY

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Artemisinin-resistant malaria was confirmed in western Cambodia in 2009. In 2013, mutations in the propeller domain of the kelch protein K13 "K13-propeller" was found to be associated with artemisinin resistance. From June-July 2014, a cross-sectional study was conducted at one private clinic to determine the prevalence of Day-3 parasitemia (no further follow up was conducted), estimate the frequency of K13 molecular marker and assess their relationship. Blood smears and filter paper blood spots were collected from febrile patients in Kravanh District, Pursat Province. The blood smears were examined by microscopy, and blood spots by a K13 mutation assay. Data from 92 patients were analyzed. Only one was positive for Day-3 parasitemia. Results of the K13 assay were interpretable for 76 of the 92 samples. All positive patients were treated as per 2012 national treatment guidelines with DHA-PPQ. The results showed that 9 (12%) were wild type, 64 (84%) were C580Y, and 3(4%) were Y493H. Therefore, despite the high prevalence of K13 mutants (67/76 - 88%), only one of the 92 patients remained blood smear positive for *P. falciparum* on

Day-3. There was weak correlation between presence of K13 mutations and delayed parasite clearance times on Day 3 in the sample. Further investigation is needed in order to confirm if the results are generalizable.

## 943

### DRUG COMBINATION THERAPY FOR ARTEMISININ RESISTANT *PLASMODIUM FALCIPARUM*

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Resistance to artemisinin combination therapy (ACT) is a growing problem. Current ACTs were discovered empirically without determination of drug mode(s) of action or drug-drug interactions. New ACTs that circumvent evolving ACT resistance mechanisms are needed. Recently our group found that while some malarial parasites are cross-resistant to many different artemisinin-like compounds, the synthetic, endoperoxide-containing ozonide, OZ439, is equally potent against both artemisinin resistant and sensitive *P. falciparum* strains. Other work from our group in collaboration with scientists at the National Center for Advancing Translational Sciences suggests that phosphatidylinositol 3'-kinase (PI3K) inhibitors are quite potent antimalarial compounds, including against drug resistant strains. Perhaps not coincidentally then, we found that *P. falciparum* parasites exhibit an autophagy-like pathway triggered in response to cytotoxic (or cell-kill) drug treatment. Additionally, class III PI3K enzymes are known to be important regulators of autophagy, which could potentially serve as a novel drug target. For these reasons, we have investigated the effects of PI3K inhibitors on the parasite autophagy-like response and have explored their utility as "partner" drugs in the design of second generation ACTs. Significant drug combination synergy, at both cytostatic (growth inhibitory) and cytotoxic (cell-kill) levels of activity, was found for various drug combinations involving OZ439 and PI3K inhibitors, including impressive cytotoxic synergy at the ring-stage of parasite development. This is an important, clinically relevant finding as ACT resistance is primarily distinguished at the parasite ring-stage under cell-kill concentrations of drugs. Based on these data, we suggest a novel strategy for the development of endoperoxide-based drug combination therapies to treat ACT resistant malarial parasites.

## 944

### ACT PARTNER DRUG EROSION: EVIDENCE OF PIPERAQUINE-RESISTANT *PLASMODIUM FALCIPARUM* IN CAMBODIA

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With the emergence and spread of artemisinin (ART) resistance, partner drugs used in ART Combination Therapies (ACTs) are themselves more vulnerable to emerging drug resistance. Recent evidence indicates not only emergence of ART resistance, but also loss of partner drug efficacy, with indications that piperazine (PPQ) resistance has emerged in Southeast Asia (Cambodia, Vietnam and eastern Thailand). To assess whether ART-resistant parasites are also PPQ-resistant, we tested previously-adapted *Plasmodium falciparum* isolates from Pursat and Pailin, Cambodia, as part of the Tracking Resistance to Artemisinin Collaboration (TRAC). Among parasites with delayed clearance and high Ring-stage Survival Assay (RSA) values as evidence of ART resistance, we observed a bimodal growth response to increasing PPQ concentrations. This bimodal response was not due to the presence of mixed parasite populations within these isolates, as daughter lines retained the phenotype after subcloning. To better quantify this bimodal response, we chose the area under the curve (AUC) instead

of the conventional half-maximal effective concentration (EC50) used in drug assays. The AUC data correlated well with Piperaquine Survival Assay (PSA) values. Exposure of parasites to PPQ at consecutive 12-h intervals throughout the life cycle showed that late-stage schizonts are less susceptible than earlier-stage parasites. Sequencing data revealed that PPQ resistance is independent of ART resistance, as parasites with mutations in the *kelch13* gene showed a range of PPQ susceptibilities. We confirmed a general association of PPQ resistance with increased *plasmepsin II* copy number as recently reported. However, we found that some isolates harbor PPQ resistance in the absence of amplification or increased expression of *plasmepsin II*, suggesting additional *loci* may modulate PPQ resistance. Our results confirm the existence of PPQ-resistant parasites in Cambodia, identify PPQ-resistant parasites that lack *plasmepsin II* amplification, and provide a fast and easy method to measure PPQ resistance.

## 945

### IMPROVING HEALTH CARE WORKER PERFORMANCE IN CLINICAL CASE MANAGEMENT OF MALARIA AND OTHER FEBRILE ILLNESSES IN SEVEN SUB-SAHARAN AFRICAN COUNTRIES

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Averting morbidity and mortality from malaria and other febrile illnesses requires a clinician to be competent in performing differential diagnosis, recognizing the need for diagnostic testing, and adhering to test results with proper treatment. To build and maintain health care worker (HCW) performance in clinical case management, the President's Malaria Initiative (PMI)-funded, PATH-led MalariaCare partnership has supported national programs implement a system of quality assurance. We report here the results of over 6,000 observations of HCWs performing clinical consultations at all levels of the health care system. The power of this multi-country monitoring data is in its large numbers measuring performance in the challenges of the work place. MalariaCare works with national programs to train clinical supervisors in diagnosis, treatment, and referral of malaria and improve skills at differential diagnosis who then work with clinical staff at health facility level to perform on-site outreach training and supportive supervision (OTSS). The OTSS visits focus on skills observation using a checklist and on the spot problem solving. Data measuring performance in 7 focus countries in sub-Saharan Africa that received between 2-5 rounds of OTSS have shown an average 18 percentage point improvement in overall score. Clinical indicators measured include recognizing the need for a malaria diagnostic test, adherence to results, treating per national guidelines, recognition of severe disease, history and physical exam skills, and patient counseling. At the most recent OTSS visit in 7 countries that included 6,178 observations, the supervisor agreed with the clinician's final diagnosis and severity assessment 94% of the time, indicated the correct prescription was given per test result and diagnosis 93% of the time, and indicated the HCW checked for at least one sign of severe malaria 83% of the time. Areas in need of improvement include performing a focused physical examination to elicit other causes of fever. Findings suggest that clinical case management refresher training and OTSS can improve management of malaria and febrile illness.

## 946

### PLASMODIUM FALCIPARUM IN AFRICA: CHANGES IN DRUG EFFICACY AND THE RATIONALE FOR EXTENDED ACT REGIMENS

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Recent evidence suggests that combination therapies such as artemether-lumefantrine are less effective against *Plasmodium falciparum* of African origin than when first widely deployed on the continent. In vivo data from clinical trials, case histories from imported malaria cases in Europe and Asia and results of *in vitro* studies will be considered in producing a current "bill of health" for ACT in Africa. As one of the options for extending the useful life of current drugs, a case for extended-duration ACT regimens will be argued on parasitological and pharmacokinetic grounds. A study design for evaluation of safety and efficacy of such regimens in African children will be proposed.

## 947

### HEME ACTIVATION OF ARTEMISININ ANTIMALARIAL DRUGS

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The current standard of care for *Plasmodium falciparum* malaria is one of several currently available Artemisinin (ART) combination therapies. Artemisinin, derived from the Chinese herb *Artemisia annua*, contains a highly reactive 1,2,4 trioxane ring. In its reduced form, free ferriprotoporphyrin IX heme is known to catalyze endoperoxide cleavage for artemisinin drugs. The activated drug likely proceeds from an oxy radical form to a carbon centered radical form that is then capable of alkylating a variety of drug targets within malarial parasites. It has been proposed for some time that heme liberated upon hemoglobin catabolism is one such target, a hypothesis that is supported by direct evidence for heme-ART adducts in artemisinin-treated mice infected with *Plasmodium vinckei petteri*. Using optimized extraction procedures and mass spectrometry, we have quantified the abundance of heme, hemozoin, and artemisinin drug - heme adducts at various stages of the *P. falciparum* life cycle. These data are important for defining the molecular pharmacology of artemisinin antimalarial drugs.

## 948

### HETEROLOGOUS EXPRESSION, PURIFICATION, AND FUNCTIONAL ANALYSIS OF PLASMODIUM FALCIPARUM PHOSPHATIDYLINOSITOL 3'-KINASE

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Recent high-throughput screening efforts have identified PI3K inhibitors as very potent antimalarial drugs (Mott B.T. *et al.* (2015) *Scientific Reports* 5:13891). The *Plasmodium falciparum* malarial parasite genome appears to encode one and only one phosphatidylinositol 3'-kinase (PI3K) and sequence analysis suggests that the enzyme is a "class III" or "Vps34" type PI3K. Recent work (Mbengue A. *et al.* (2015) *Nature* 520:683-7) suggests that this enzyme may be a target for artemisinin - based antimalarial drugs, which are critical components of current antimalarial drug therapy. For these reasons, we have optimized the *PfVps34* gene for heterologous expression in yeast, have purified the protein to homogeneity, and have used a recently validated quantitative assay for PI3P production from PI to characterize enzyme activity and to quantify drug inhibition of that activity. We have investigated whether key residues in the enzyme's catalytic and "N-lobe" domains influence drug - enzyme interaction. Data show that PfVps34 is indeed a "class III" PI3K, that helical domain segments are

not essential for function, and that PfVps34 is inhibited by artemisinin and related drugs, but only under conditions that cleave the drugs' endoperoxide bridge to generate reactive alkylating agents.

## 949

### ASSESSMENT AND IMPACT OF THE NEW INTERMITTENT PREVENTIVE TREATMENT OF MALARIA IN PREGNANCY WITH SULPHADOXINE-PYRIMETHAMINE (IPTP-SP) IMPLEMENTATION STRATEGY ON MATERNAL, FETUS AND NEONATAL OUTCOME IN GHANA

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On the back of wide spread sulphadoxine-pyrimethamine resistance across Africa and its implication to malaria prevention among pregnant women and also the dose dependent beneficial effect of SP against malaria in pregnancy, WHO reviewed their guideline to a monthly SP administration. Ghana adopted this amendment and the new policy is currently being implemented. To assess the implication of the new IPTp strategy to maternal and neonatal outcomes, 1,000 pregnant women visiting health facilities from a peri-urban (Kpone-on-sea) and urban (Maamobi) setting in the Greater Accra Region, for their first antenatal care (ANC) and another 1,000 for delivery were recruited. Plasma levels of antimalarial drugs including SP and artemisinin derivatives were measured using an HPLC/MS/MS method. Parasitemia was also determined using microscopy and real-time PCR. Of the 1,000 women recruited from the first ANC visit, the mean  $\pm$  sd gestational age was 17  $\pm$  7 weeks at Kpone-on-sea and 18  $\pm$  7 weeks at Maamobi. At Kpone-on-sea 41% of participants at ANC were PCR positive compared to 39% from Maamobi. Also, 4.2% of participants at delivery were PCR positive compared to 24% from Maamobi. At delivery, 96 % of the women reported at least one SP uptake in Kpone-On-Sea while 78% did in Maamobi. These results will be discussed in detail during the congress in relation to SP plasma levels at each visit, maternal anemia, G6PD deficiency, and the condition of the newborn.

## 950

### NO EVIDENCE OF AMPLIFIED *PLASMODIUM FALCIPARUM* *PLASMEPSIN II* GENE COPY NUMBER IN AN AREA WITH ARTEMISININ-RESISTANT MALARIA ALONG THE CHINA-MYANMAR BORDER

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The emergence and spread of artemisinin resistance in *Plasmodium falciparum* poses a significant risk to malaria control and eradication goals, including China's plan to eliminate malaria within its borders by 2020. In addition, piperazine resistance has also emerged in Cambodia, compromising an important partner drug that is widely used in China in the form of dihydroartemisinin-piperazine. Mutations in a *P. falciparum* gene encoding a kelch protein on chromosome 13 (*kelch13*) are associated with artemisinin resistance and have arisen from multiple times and spread in the Great Mekong Subregion, including the China-Myanmar border. Multiple copies of the *plasmepsin III/III* genes, located on Chromosome 14, have recently been shown to be associated with piperazine resistance in Cambodia. In this study, we investigated *plasmepsin II* amplification in relation to the presence of *kelch13* mutations from samples collected from 200 clinical trial participants from field sites along the China-Myanmar border as part of therapeutic efficacy studies conducted from 2009 to 2014. Polymorphisms within *kelch13* were genotyped by capillary

sequencing of *P. falciparum* DNA extracted from dried blood spots. *Plasmepsin II* copy number was quantified by relative-quantitative real time PCR. The preliminary data showed only a single copy variants of *plasmepsin II* within samples from the China-Myanmar border, including both parasites with and without *kelch13* mutations. The therapeutic efficacy of dihydroartemisinin-piperazine was more than 90% along the China-Myanmar border, consistent with the lack of amplification of *plasmepsin II*. Continued monitoring of the parasite population using molecular markers of both artemisinin and piperazine will be important to track emergence and spread of resistance in this geographic region.

## 951

### ARTESUNATE-AMODIAQUINE: EFFICACIOUS AFTER 10 YEARS OF USE AS TREATMENT FOR UNCOMPLICATED *P. FALCIPARUM* MALARIA IN ERITREA

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Despite the dramatic decline in malaria morbidity and mortality, malaria still remains an important public health problem in many regions of Eritrea. After the first therapeutic efficacy tests showed 97% adequate clinical and parasitological response (ACPR) in 2007, Artesunate-Amodiaquine (AS-AQ) continues to remain as the first line treatment for uncomplicated *P. falciparum* malaria in Eritrea. In this study we report efficacy of AS-AQ after 10 years of continuous use. During September to December 2016, children aged  $\geq$  6 months and adults with microscopy-confirmed uncomplicated *P. falciparum* malaria (200–200,000 parasites/ $\mu$ L) and meeting other study criteria participated in a 28-day one-arm *in-vivo* efficacy trial in 4 sentinel sites of Gash Barka region: Tokombia, Goluj, Shambuko and Akordat. The study was powered to estimate overall AS-AQ efficacy of 95%. Blood was collected for malaria microscopy and molecular testing on days 0, 2, 3, 7, 14, 21, and 28. The primary outcome was the PCR-uncorrected day 28 Kaplan-Meier cumulative success rate. A total of 281 children and adults were enrolled and out of these 270 (96%) reached a study endpoint and out of these 256 responded to the treatment making the day 28 uncorrected cumulative success rate (ACPR) at 94.8% [95% CI: 91.5%, 97.1%]. There was no early treatment failure but a total of 14 late treatment failures occurred after day 7. The proportion of ACPR may change after polymerase chain reaction (PCR) correction. The findings indicate that AS-AQ still remains efficacious for the treatment of uncomplicated malaria in Eritrea after almost 10 years of use. However, continued monitoring of efficacy levels of AS-AQ is recommended to ascertain its efficacy remains well above the 90% cut-off point.

## 952

### THE EFFICACY OF ARTEMISININ COMBINATION THERAPY IN KENYA; THE STATUS AT MSABWENI, NYANDO AND BUSIA MALARIA ENDEMIC STUDY SITES

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Artemisinin-based combination therapies (ACTs) and its derivatives are the most rapidly acting of all the current antimalarial drugs and recognition of their potential role as a component of combination therapy have led to several large trials aimed at assessing different combinations of existing drugs and to the specific development of new combination drugs. The Study Objective was to evaluate the efficacy of fixed dose combinations, Artemether-lumefantrine and dihydroartemisinin-piperazine in

uncomplicated malaria patients in Kenya with an aim to inform malaria treatment policy and practice in the country. The study was a multi-site two arm blinded randomized clinical trial. In this are the preliminary results of three study sites evaluated for the efficacy of two artemisinin-based anti-malarial combination drugs, AL and DP, in Kwale, Kisumu and Busia counties in Kenya. The *in-vivo* (clinical microscopy) treatment outcome was evaluated. Parasitological response at day 28 and 42, cure ratios in the two treatment arms, Fever Clearance Time (FCT), Asexual parasite clearance time (PCT), Gametocyte carrier rates and Adverse events were evaluated. A total of 352 children in Kwale, 315 in Kisumu and 334 in Busia, aged between 6 months and 12 years were randomized and treated with either AL or DP. For Kwale and Kisumu cumulatively, in the Intention to treat (ITT) analysis, at day 42, 483 participants had ACPR, ACPR rate of 72% (95% CI 69-76%), 268 participants in arm A (ACPR rate of 77% (95% CI 72-81%)) and 215 in arm B (ACPR rate of 68% (95% CI 62-73%)); P-value 0.008. By day 28, participants with ACPR were 542, ACPR rate of 81% (95% CI 78-84%), 293 in arm A (ACPR rate of 84% (95% CI 80-88%)) and 249 in arm B (ACPR rate of 78% (73-82%)); P-value=0.06. The results from these study sites indicate a note worth trend of the ACT efficacy in the regions and calls for further monitoring on the same for appropriate policy measures.

## 953

### EX VIVO SUSCEPTIBILITY OF *PLASMODIUM FALCIPARUM* TO DIHYDROARTEMISININ AND PIPERAQUINE BY NOVEL PHENOTYPIC ASSAYS (RSA AND PSA) IN BINH PHUOC PROVINCE, VIETNAM

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Artemisinin based combination therapies (ACTs), the most effective antimalarial medicines against *Plasmodium falciparum* have contributed in the substantial reduction of malaria burden worldwide over the last decades. Like previously used antimalarial drugs, parasite resistance to artemisinin and its derivatives has emerged and spread in Southeast Asia. Moreover, the partner drug efficacy has gradually declined recently due to increasingly recrudescence cases in late treatment failure. This indicates that parasite resistance to both artemisinin and piperazine is prevalent. To provide supporting evidence of the resistant *P. falciparum*, *ex vivo* susceptibility of *P. falciparum* to dihydroartemisinin and piperazine has been investigated by ring-stage survival assay (RSA) and piperazine survival assay (PSA). In this study, 54 blood samples were collected from patients with uncomplicated falciparum malaria in Binh Phuoc province from 2016 to 2017. The parasites were exposed to drugs followed published procedure in order to assess the percentage of viable parasites as well as identify the resistant *P. falciparum*. The results showed that 98 % (53/54) of parasites was resistant to dihydroartemisinin with a median 7.88 % (IQR: 4.21 - 15.77 %) and 89 % (48/54) of parasites was resistant to piperazine with a median 52.08 % (IQR: 29.44 - 79.57 %). Additionally, RSA and PSA survival rates from recrudescence patients were higher than non-recrudescence patients (9.89% compared to 6.47% by RSA and 77.57% compared to 36% by PSA, respectively). The high survival rate of parasite exposed to antimalarial drugs could threaten to malaria elimination targets in areas where dihydroartemisinin - piperazine was used commonly. Our findings suggest that monitoring of drug resistance with phenotypic *ex vivo* / *in vitro* assays such as RSA and PSA is necessary to evaluate drug efficacy as well as useful in elucidating the molecular mechanism of resistance.

## 954

### PREVALENCE OF CYP2D6 POLYMORPHISMS IN A CAMBODIAN POPULATION AND RELATIONSHIP TO *PLASMODIUM VIVAX* RECURRENCE RATE AND HEMOLYTIC TOXICITY

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With the large burden of *Plasmodium vivax* malaria in Cambodia, elimination efforts will require widespread administration of the 2-week course of oral primaquine (PQ) for radical cure, in an environment with 13-17% G6PD deficiency. Another host genetic factor which affects primaquine efficacy is polymorphisms in the hepatic cytochrome P450 2D6 enzyme. Clinical failure of PQ has been demonstrated in people with CYP2D6 genetic polymorphisms which result in reduced or no enzyme activity (Intermediate Metabolizer [IM] and Poor Metabolizer [PM] phenotypes, respectively), and a recent pharmacokinetic study demonstrated that IMs and PMs have significantly lower production of the PQ phenolic metabolites which may be responsible for anti-hypnozoite activity. The distribution of CYP2D6 genotypes/phenotypes in the Cambodian population is not well described. Other studies conducted in Asia have shown an approximate 50% prevalence of the IM phenotype, which could translate into increased risk of PQ radical cure failure, continued relapses with ongoing community transmission, as well as potential morbidity from repeated dosing of PQ. Here we report on the CYP2D6 genotypes from a group of 106 volunteers taking part in two malaria therapeutic efficacy studies conducted during 2010 and 2013 in Anlong Veng District in Cambodia, which represents the largest collection of CYP2D6 information in this country to date. Genotypes were assigned by using the Luminex xTag® CYP2D6 Kit v3 (Austin, TX), a cytometric bead detection assay detecting 17 different CYP2D6 alleles, on archived buffy coat samples. Phenotypes were predicted based on a published 2D6 activity score (AS-A). The 2010 study included both *P. vivax* and *P. falciparum* patients, which will enable comparison of CYP2D6 genotypes to *P. vivax* relapse frequency reported by volunteers after radical cure with PQ as well as the degree of anemia post treatment. Plans are also underway to conduct a pharmacokinetic study of primaquine in cohort of volunteers from the 2013 study in order to determine the effect of CYP2D6 polymorphisms on generation of the phenolic metabolites necessary to clear hypnozoites.

## 955

### A POINT-OF-CARE ASSAY TO DETECT ANTIMALARIAL DRUGS FROM FINGER STICK BLOOD SAMPLES

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Artemisinin-based combination therapies (ACTs) have begun to fail as first-line therapies for the treatment of *Plasmodium falciparum* malaria in Southeast Asia. Preventing the spread of drug-resistant parasites to Africa is a top priority for global malaria elimination campaigns. The ability to detect small molecule antimalarial drugs in a patient's blood at the point-of-care could enable healthcare workers to identify a previously failed

treatment and adjust the patient's new treatment to improve its efficacy and thus reduce the spread of resistant parasites. A simple assay to detect these drugs in patient samples would also facilitate real-time mapping of drug use and compliance as well as provide an inexpensive tool for testing drug quality. The goal of this research is to develop a low-cost, field-based test to detect several slow-clearing ACT drug compounds from unprocessed finger stick blood samples. The test will detect drugs through the binding of target-specific aptamers and provide a colorimetric readout of drug levels. Our research is currently focused on optimizing selection protocols to identify ssDNA aptamers that bind target antimalarial drugs with improved sensitivity and specificity.

## 956

### ABSENCE OF ASSOCIATION BETWEEN *EX VIVO* SUSCEPTIBILITY TO PIPERAQUINE AND POLYMORPHISMS IN EXONUCLEASE GENE AND COPY NUMBERS IN PLASMEPSIN 2 GENE IN AFRICAN *PLASMODIUM FALCIPARUM* ISOLATES

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Since 2005, the World Health Organization (WHO) has recommended artemisinin combination therapy (ACT) as the first-line treatment against malaria. The emergence of resistance to artemisinins, manifested by delayed parasite clearance after monotherapy with artesunate or ACT, was described in Southeast Asia. Dihydroartemisinin-piperazine is the most recent ACT to be commercialized. However, multidrug resistance to dihydroartemisinin-piperazine is currently emerging in Cambodia. The duplication of the gene coding the plasmepsin 2 was associated with decreased dihydroartemisinin-piperazine efficacy in Cambodia and *in vitro* resistance to piperazine. Additionally, the mutation E415G in the exonuclease gene (*exo-E415G*) was associated with high inhibitory concentrations 50% (IC50) of piperazine in 297 isolates from Cambodia obtained from 2011 to 2013. This E415G mutation was associated with parasite recrudescence following dihydroartemisinin-piperazine treatment. We evaluated the copy number of plasmepsin 2 in 81 isolates from Dakar, Senegal and 250 African isolates. None of the African samples harbored more than one copy. None of the African isolates harbored the E415G mutation in the exonuclease gene, although three other mutations were detected in this gene, including S114C, N419H and T435A. However, the E415G mutation and more generally polymorphisms in the exonuclease gene and copy number of plasmepsin 2 were not associated with *in vitro* reduced susceptibility to piperazine in African parasites.

## 957

### EVALUATION OF NOVEL PET-PCR PRIMERS FOR THE DETECTION OF *PLASMODIUM MALARIAE* IN CLINICAL SPECIMENS *MALARIAE* IN CLINICAL SPECIMENS

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Molecular methods are useful for the detection of low density *Plasmodium* sp. infections that cannot be detected by microscopy. In reference laboratory settings, results of molecular testing complement results of microscopy and rapid diagnostic tests (RDTs). Moreover, molecular diagnostic tools allow for rapid large scale screening with high sensitivity and specificity and can be applied to control, pre-elimination and elimination settings for confirming submicroscopic infections. We have

demonstrated this capability with our previously described genus-specific photo-induced electron transfer polymerase chain reaction (PET-PCR), a relatively easy assay to implement in endemic countries and in the species-specific PET-PCR assays for *P. falciparum* and *P. ovale*, capable of detecting *P. falciparum* and *P. ovale* respectively. Here we report on the evaluation of a *P. malariae*-specific PET-PCR assay for the accurate and sensitive detection of *P. malariae*. Using a total of 138 clinical samples, the sensitivity and specificity of this assay at detecting *P. malariae* infections were both determined to be 100%, using a nested-PCR as a reference test. The limit of detection of the assay was found to be 2-8 parasites/μl. Further field evaluation of this method especially in endemic country setting will be valuable to confirm the sensitivity and specificity of this test.

## 958

### DOES THE EXPERIENCE OF MALARIA TESTING INCREASE TRUST IN THE TEST? EVIDENCE FROM WESTERN KENYA

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Malaria diagnostic testing is a key tool for malaria control efforts. However, patients who test negative are often still treated with anti-malarial medications. This practice increases the risk of parasite resistance to the drug and limits the benefit of malaria treatment subsidies. We used data from a study conducted in Western Kenya to examine how the experience of malaria testing (either microscopy or rapid diagnostic tests) influences patients' trust in the test. Between July 2014 and June 2015, 444 individuals with a malaria-like illness, who had not yet sought any testing or treatment, were surveyed at study enrollment and at a follow-up visit one week later. At both time points, we collected individuals' beliefs about the probability that their illness was malaria, as well as their perceived probabilities that a hypothetical negative and positive malaria test result were correct. We elicited these probabilities using a visual scale ranging from 0%-100%, displayed on an interactive touchscreen tablet. Approximately 62% of patients were tested for malaria in the week between the two surveys. At the follow-up visit, patients had, on average, a 71% probability of believing that a negative malaria test result was correct, and a 76% probability of believing a positive test result was correct. Compared to those who were not tested, patients who were tested for malaria had a 6.8 percentage point (95% CI [0.005 0.131], P=0.035) greater probability of believing that a hypothetical negative test result was correct. Testing also had a positive, but statistically non-significant effect, on the perceived probability that a hypothetical positive test result was correct. (β=0.042, 95% CI [-0.019 0.102], P=0.178). Results are very similar when we control for patients' baseline beliefs about their illness and baseline trust in the test. We find no evidence that adherence to the test result is associated with patients' trust at follow-up in either a positive or negative test result. Our results suggest that greater experience with malaria diagnostic testing can increase patients' trust in the test, particularly when the test result is negative.

## 959

### PREPARATION OF A UNIFORM MONOLAYER OF GIEMSA-STAINED RED BLOOD CELLS ON HYDROPHILIC-TREATED PLASTIC PLATES FOR MALARIA DIAGNOSIS

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Microscopic evaluation of Giemsa-stained thin blood smear on glass slides has been the gold standard for malaria diagnosis for more than 100 years. However, only a small area of the blood smear would provide a monolayer of red blood cells (RBCs) suitable for microscopic evaluation, thereby

limiting the number of cells that can be examined for the *Plasmodium* infection rate (parasitemia) and parasite species. This is one of the major reasons for the low parasite detection rate of this method. Herein, we established a method that allows microscopic examination of Giemsa-stained cells spread in a monolayer over the entire surface of hydrophilic-treated plastic plates. The method may enable more reliable diagnosis of malaria in patients with low parasitemia.

## 960

### COMPETENCE IN MALARIA RAPID DIAGNOSTIC TESTS (MRDTS) PERFORMANCE TWELVE MONTHS POST TRAINING: EVALUATION OF MALARIA TESTING BY UNITY HEALTH WORKERS (CHWS)

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Task shifting in malaria diagnosis is a potential strategy towards improving access to diagnostic testing in rural communities. With appropriate training and supervision, community health workers (CHWs) can bridge the access gap by offering malaria rapid diagnostic tests (mRDTs) in the community. However there are concerns about the ability of lay health workers to achieve and maintain competence in performing the mRDTs. As part of a larger study of CHWs using RDTs, we evaluated their performance in using malaria RDTs 12 months post-training. A total of 271 CHWs were trained on use of RDTs using a 3 day validated Ministry of Health curriculum. A post training evaluation test was administered to assess their grasp of the content. A practical competency assessment using a standardized 22 step checklist was also conducted. The CHWs were equipped with supplies to offer RDT testing. At 12 months post training, a sample of 102 CHWs underwent a repeat assessment using the checklist to evaluate whether their skills were maintained. The mean age of the CHWs was 43.6 years, with 68.9% being females and 82.5% married. The mean duration of CHW experience in their role was 4.1 years. About a third had previous training in malaria case management. At least 80% of CHWs completed 18 of the 22 steps correctly, with median number of steps completed correctly being 19 at baseline and 20 at 12 months (the difference was not statistically significant). The lowest average scores involved failure to check the expiry date on the cassette with the CHWs scoring 53% at baseline and 52.4% at 12 months. Using the investigators' interpretation as the gold standard, the sensitivity and specificity of CHW interpretation of mRDTs were 92.0% and 97.3% respectively, with the commonest error occurring when there were faint positive test lines. Using multivariate regression, factors associated with fewer error counts at 12 months post training were prior RDT experience (-0.74, p value 0.002) and age below 50 years (0.03, p value 0.00). CHWs can safely and accurately use mRDTs, with their competence maintained at 12 months post training.

## 961

### MALARIA DIAGNOSTIC TESTING ASSOCIATED WITH SIGNIFICANT INCREASES IN COST OF CARE FOR FAMILIES IN RURAL WESTERN KENYA

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Although malaria cases have decreased in Kenya over the past decade, there are still areas of high incidence in western Kenya. Optimum management of malaria involves prompt diagnosis and treatment. These actions have associated costs which are often borne by community members themselves rather than the government. We used a population

based household survey, conducted in 2015 in 34 community clusters in western Kenya, to examine how malaria testing is associated with overall treatment costs. Households in which a malaria like illness was reported in the past four weeks were enrolled. Adults and caregivers of children under 18 years in 2,065 households were interviewed and asked about the treatment the patient sought, whether the patient was tested for malaria, their expenditures on malaria testing, and overall expenditures on the illness. Approximately 44% of patients in the survey received a malaria diagnostic test. Both the median and mean cost of a malaria test was 50 Kenya shillings (Kshs) (approximately \$0.50) and was similar whether the patient was tested with a rapid diagnostic test (RDT) or with microscopy. The cost of a malaria test was lower for children under the age of 5 and was higher if the patient was tested in the private sector. Total treatment costs were on average Kshs 380 (median was Kshs 150). Controlling for patient age, household wealth, distance to a health facility, and location of care, malaria testing was associated with a Kes 460 (95% CI [128.8, 790.3],  $P < 0.01$ ) increase in total treatment costs. Patients who ever visited a private health facility or who were from the richest wealth quintile also had higher overall treatment costs. The difference in treatment costs between those tested and not is much higher than the cost of the test which suggests that the test cost itself may not be the major barrier to testing but that testing decisions may be affected by associated fees such as additional laboratory tests, registration and other costs that may result from seeking care in a place that offers testing.

## 962

### ASSESSMENT OF RDT PERFORMANCE IN ENDEMIC AREA IN SAKARAH, MADAGASCAR

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Malaria remains a major public health problem in Madagascar. The National Strategic Plan requires biological diagnostic confirmation before treatment of malaria cases. Rapid Diagnostic Test (RDT) is used at based health center and coupled with microscopy in hospital center. Assessment of performance of RDT is important to improve the quality diagnosis and treatment. The aim of the study is to evaluate the performance of RDT. The data was collected during an outbreak investigation in March 2016 in Antsokay, Ankida and Andoharotsy villages. All patients presenting fever or history of fever were recruited. Each patient was tested systematically with RDT (SD Bioline Malaria *Pf/Pan*), thick drip/thin smear and blood spots. All patients with positive RDT and uncomplicated malaria were treated with Artesunate - Amodiaquine (ASAQ). The slides were read at NMCP laboratory by the WHO-accredited experts. A total of 245 patients were recruited. Sex ratio (M / F) was at 0.9. Only *Plasmodium falciparum* has been identified in this study. The results showed that the RDT positivity rates in all 3 sites were 53.9%. The smear positivity rates were 32.7%. There is a difference between RDT results and microscopy. Respectively, sensibility, specificity, VPP and VPN of RDT was 97.5%, 67.3%, 59.1% and 98.2%. The geometric mean of parasitaemia was 3405,9 parasites/ $\mu$ L. In summary, SD Bioline Malaria *Pf/Pan* is sensitive but less specific diagnostic test there is. The parasite reservoir for malaria transmission was found in Sakaraha.

## LESSONS LEARNED: MALARIA DIAGNOSTIC REFRESHER TRAINING IN AFRICA FRANCOPHONE COUNTRIES

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Since 2010, WHO has recommended that testing all suspected malaria cases with a parasitological test and high quality malaria microscopy remains the diagnostic standard. The USAID-funded MalariaCare project assists program countries to improve their accuracy of malaria diagnostic testing. We report MalariaCare's experience in implementing five-day malaria diagnostic refresher training (MDRT) for laboratory supervisors and staff in five francophone countries: Burundi, the Democratic Republic of the Congo (DRC), Guinea, Madagascar and Mali. The 377 MDRT participants were selected from central and peripheral reference facilities and were trained on parasite detection (PD), species identification (ID) and parasite counting (PC); practice on slide preparation and staining; use of malaria RDTs; and improving quality assurance measures according to WHO guidelines. The knowledge and competency of participants was evaluated through written pre- and post-tests and practical skills assessments, with their final outcome scores classifying them into one of four performance levels based on a WHO grading scale. Of the total 377 participants trained on basic MDRT, 97 were in Burundi, 84 in the DRC, 20 in Guinea, 59 in Madagascar and 117 in Mali. In addition, 63 participants (30 in Burundi and 33 in the DRC) were trained in an advanced course. Overall, significant improvements were made in slide reading for PD, ID and PC with average post-test scores at 93, 64 and 37 percent, respectively, representing improvements by 16, 15 and 21 percentage points; 19.9% obtained national expert level 1 or 2 status. Among these high performers, 11 were selected for a WHO francophone external competency accreditation malaria microscopy course in Dakar, Senegal, with six receiving WHO level 1 and three level 2 accreditation. These outcomes suggest that five day MDRTs are an effective way of administering WHO's recommendation for regular refresher training of reference level laboratory staff, while at the same time highlighting that more training needs to be done to bring all reference microscopists to a quality level of performance.

## AN OFFLINE VIRTUAL MICROSCOPE-BASED MALARIA MICROSCOPY COURSE TO IMPROVE PERFORMANCE IN THE MICROSCOPIC DIAGNOSIS OF MALARIA

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We developed an in-service malaria microscopy e-learning course that utilizes virtual microscopy and is consistent with World Health Organization standard operating procedures. A key feature of the course is that it can be taken without Internet access—the entire course is stored on a USB drive, making it accessible to malaria microscopists even in remote locations. The course is based on Amref Health Africa's workshop-based course and incorporates instructional content in five modules. A large bank of detailed blood film images can be viewed and navigated on-screen with the virtual microscopy software. The course systematically guides the participant through a process of self-learning, concentrating on specific areas of expertise. Post-module tests and pre- and post-tests allow the user to assess and improve their background knowledge and skills in interpreting blood films. A final assessment is linked to certification, in which the user submits an encrypted code containing their responses to the course organizer. While the course can stand alone as an introduction to malaria microscopy, we envision its primary role as refresher training, including preparation for the external competency assessment of malaria microscopists (ECAMM). Pre-ECAMM refresher training has been shown to increase competency levels. Expanding access to such training may decrease the burden of training during ECAMM and improve outcomes. A late-2015 pilot of a partially completed course received positive and constructive feedback which was used to guide further development. A pilot of the full course was distributed to over 300 participants in 24 countries, covering a broad range of experience, in April 2017. Initial feedback indicates that the course addresses a significant gap in malaria microscopy refresher training, and we are planning to roll out of the complete course later in 2017. A summary of the course, full results of both pilots, including a summary of pre- and post-test results and participant evaluations, and the plan for roll out will be presented.

## EFFECTIVENESS OF URINE-BASED RDT AND BLOOD-BASED RDT IN MALARIA DIAGNOSIS

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Management of malaria requires prompt diagnosis by Microscopy and Rapid Diagnostic Test (RDT) kits. The aim of this study was to compare the performance of Blood-based RDT and Urine-based RDT with Microscopy results in malaria diagnosis. This study was conducted using blood and urine specimens collected from 100 human subjects that attended the Community Health center Amansea, Awka-North Local Government Area. Giemsa stained thick and thin films were used for microscopic

examination. Care-start® Blood-based RDT Cassette and Fyodor® Urine Malaria test kit were used for rapid diagnosis of malaria. The prevalence of the malaria as recorded by microscopy was 37%. This was significantly higher than 21% prevalence obtained with blood based RDT and 5% prevalence obtained with urine based RDT. The intensity of malaria and 14% recorded by microscopy was 23% for one plus (+) of malaria and 14% for two pluses (++) of malaria. Malaria intensity as recorded by blood based RDT was 4% for faint line and 17% for thick line. Malaria intensity as recorded by urine based RDT was 3% for faint line and 2% for thick line. Blood-based RDT recorded 52.2% of malaria cases that had one plus of malaria parasite and 57.1% of cases that had two pluses (++) of malaria. Urine based RDT recorded 4.4% of malaria cases that had one plus (+) of malaria and 21.4% of malaria cases that had two pluses (++) of malaria. There was only one positive case of both urine and blood based RDT that had a negative microscopy result. However, there was no significant difference in both urine -based RDT positive cases and Blood-based RDT positive cases that had one plus and two pluses of malaria. The Urine-based RDT recorded a low sensitivity (10.8%) when compared to Blood-based RDT (54.0%). Malaria is still a problem in the study area. The RDT used in the diagnosis is parasite density dependence and may show low or poor performance in low infected population.

## 966

### CONTRIBUTION OF RAPID DIAGNOSIS TEST IN MALARIA CASE MANAGEMENT STRATEGY IN SENEGAL

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According to WHO recommendations issued in 2004 for RDT use to improve quality and accuracy of the diagnosis, Senegal had introduced RDT (based on the detection of the antigen Histidin Rich Protein-2/HRP-2 specific for *Plasmodium falciparum*) in October, 2007. This diagnosis tool was supposed to: allow rational use of CTA, reduce the risk of CTA resistance development in the parasite, increase accuracy and reliability of morbidity data, avoid useless care-costs. In 2015, Senegal recorded 929,010 suspected cases (tested negative), all these cases should have been treated with an antimalarial if RDT was not conducted. Thus, by considering the cost of a CTA treatment at one US dollar, NMCP has realized almost 929,010 USD savings. RDT introduction into malaria case management was a realistic alternative to no functionality of the laboratories in the peripheral structures and to operational issues in the realization of the microscopic diagnosis. Before RDT introduction, the confirmation cases rate by microscopy was just around 10%. In 2007, at the beginning, RDT realization was around 15%, it jumps to 99, 31% in 2015. This was possible as RDT contributed to increase access to biological diagnostic to end users within the community by the mean of community health workers, due to easy use and rapidity in result delivery. Moreover, RDT was accepted by caregivers at any level as it allowed rational use of time, credibility for the health system and increased confidence of the populations. RDT had largely contributed to the decrease of morbidity and mortality due to malaria, since it made possible detecting earlier and accurately malaria cases, treating its with rapidity and by reducing the risks of transmission and evolution of cases to severe ones.

## 967

### A NEW HIGH SENSITIVITY RAPID DIAGNOSTIC TEST FOR *PLASMODIUM FALCIPARUM* DETECTION IN AN ELIMINATION SETTING: INDIVIDUAL DIAGNOSIS OF INFECTION AND MEASUREMENT OF PREVALENCE

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The role of asymptomatic carriage of parasites in the persistence of falciparum malaria is increasingly described in low transmission settings where ultrasensitive molecular methods allowed to fully measure the extent of parasite reservoir. However, no point of care test is currently available to detect the parasite densities commonly found in asymptomatic carriers, thus limiting the options to identify and target all parasites as required for active elimination strategies. This is necessary in the Greater Mekong Subregion where rapid elimination of *P. falciparum* has been undertaken to prevent the spread of artemisinin-resistant parasites and the emergence of multi-drug resistant parasites. We tested the Malaria Ag Pf high sensitive RDT (hsRDT) developed by Standard Diagnostics/Alere aiming at a 10-fold increase in sensitivity of PfHRP2 detection compared to standard RDT. From June 2016 to January 2017, prevalence surveys were conducted in 39 villages from Eastern Myanmar by the Malaria Elimination Task Force. In each village, a RDT and hsRDT were performed on site to all participants who provided informed consent. A 2-mL venous sample was collected from a random sub-sample of adults to allow laboratory tests to compare *P. falciparum* detection by RDT, hsRDT, microscopy, ultrasensitive qPCR assay (uPCR) and highly sensitive quantitative HRP2 assay (Quansys). Overall 1730 samples were analyzed with all methods in the laboratory. Preliminary results indicated that laboratory-performed hsRDT could detect 62% (151/243) of all uPCR PF-positive samples, and over 56% (116/208) when restricting the analysis to asymptomatic individuals. In contrast, RDT detected 40% (97/243) of all uPCR PF-positive, and 31% (67/208) of asymptomatics. The specificity was 99.3% compared to uPCR, awaiting confirmatory HRP2 results. The correlation between hsRDT- and uPCR-measured prevalence was 0.955 (compared to 0.822 for RDT). Comparison with results of field hsRDT and RDT is ongoing. The hsRDT significantly increased the sensitivity of the individual diagnostic of asymptomatic infection and provided reliable estimates of community-level prevalence.

## 968

### MAXIMIZED DIAGNOSTIC SENSITIVITY REVEALS UNEXPECTED RESERVOIR OF MALARIA INFECTIONS

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Sub-microscopic malaria infections are prevalent particularly in low-endemic settings and are considered an important reservoir for mosquito infection. Ultra-low density infections below the detection limit even of standard PCR present a challenge for malaria diagnosis, which is limited mainly by the volume of blood analyzed, and thus their relevance for malaria transmission and control remains unclear. In Papua New Guinea (PNG), where intensified control efforts during 2004 to 2014 had reduced malaria prevalence drastically, venous blood samples were collected from 302 participants from Madang province during the wet season of 2016/17 and aliquoted to clarify the proportion of infections missed by standard

molecular diagnosis. Malaria prevalence in the study area by standard molecular diagnosis procedures, i.e. 18S rRNA qPCR on finger-prick blood volumes (200 µL), has tripled between 2014 and 2017 for *P. falciparum* (2014, 11%; 2017, 30%) but remained constant for *P. vivax* (2014, 21%; 2017, 20%). Preliminary data suggests that in 2017 one third of *P. falciparum* and *P. vivax* infections are missed when using small volumes (200 µL) and standard 18S rRNA qPCR compared to high volumes (2 mL) combined with ultra-sensitive qPCR. However, finger-pricking is more feasible for routine surveillance studies. 40% of those samples positive in high blood volumes only can also be detected by ultra-sensitive qPCR done in small-volume blood samples. The proportion of ultra-low density *P. falciparum* and *P. vivax* infections is comparable between villages, age groups, men and women, with the exception of children <10 years. In this young age group *P. falciparum* densities, but not *P. vivax* densities, are well detected by standard 18S rRNA qPCR. The high proportion of ultra-low density infections and rapid resurgence of *P. falciparum* between 2014 and 2017 in PNG raises the possibility that sub-microscopic malaria infections even of ultra-low parasite density may constitute an important reservoir for malaria transmission. RNA work for investigation of gametocyte prevalence in these ultra-low density infections was on-going at time of abstract submission.

## 969

### ESTABLISHING NATIONAL MALARIA SLIDE BANK: IN ETHIOPIA

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Despite the provision of microscopy trainings for health workers and implementation of proficiency testing (PT) external quality assurance (EQA) programs implemented in most countries, few countries have the capacity to produce and use validated blood film slides to ensure the sustained reliability of the trainings and EQA programs. Public Health Institute (EPHI), with support from the U.S. President's Malaria Initiative (PMI), has partnered with the ICAP and Malaria Care for mass production of validated malaria blood film slides with the aim of establishing the nation's first malaria slide bank. *Plasmodium falciparum* and *Plasmodium vivax* positive slides and dry blood spots were prepared at Adama Malaria Control Center from blood specimens collected from consented adult patients. Negative slides were prepared from blood collected from volunteer visitors from non-malaria endemic countries with no history of malaria and travel to malarious areas. Between 200-400 blood film slides were prepared from each donor. The blood film slides were examined by WHO-certified expert microscopists for species identification, for MSP typing and characterized and validated by Polymerase Chain Reaction (PCR). The slides were archived using a custom-made slide bank database and storage cabinets with capacity for 10,000 slides. A total of 10,742 (7,042 *Plasmodium falciparum* and 3,700 *Plasmodium vivax* positive; 1,697 negative) validated blood film slides were collected from 35 donors. Validated malaria slides sets containing blood films slides of negative, mixed from 35 samples for MSP typing 28 were 3D7 and 3 were Dd2 the rest 4 were unknown. Establishment of the slide bank enabled the national malaria program to use standardized and validated slides for quality in-service and pre-service malaria microscopy trainings, competency assessment of microscopists, laboratory mentorship programs, and regional malaria microscopy proficiency testing EQA programs.

## 970

### ANTIBIOTIC PRESCRIPTION PRACTICE FOLLOWING INTRODUCTION OF THE MALARIA 'TEST AND TREAT' POLICY IN UGANDA

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The WHO recommends parasitological confirmation of all suspected malaria cases before antimalarial treatment. Where malaria testing facilities are lacking, health workers treat suspected malaria cases presumptively with an antimalarial, antibiotic, or both. The malaria 'Test and Treat' policy adopted by Uganda in 2011 contributed to rational antimalarial prescription, however its impact on antibiotic use among those with negative malaria tests is not known. Misuse of antibiotics may contribute to the development of Antimicrobial Resistance since bacterial infections are not routinely confirmed. We assessed antibiotic prescription practices among patients attending Malaria Reference Health Centers (MRCs) in Uganda following the introduction of the malaria 'Test and Treat' policy. We analyzed antibiotic prescription data from the Outpatient Department (OPD) Registers (HMIS Form 031) at 21 MRCs covering 5 years (2012 - 2017). Of 2,417,772 patients who attended OPD, 1,042,263 (43%) had a malaria test done of which 525,154 (50%) tested positive. Antibiotics were prescribed to 163,100/525,154 (31%) patients with a positive test result for malaria and to 338,776/517,107 (66%) with a negative test result. Patients with positive malaria test results were less likely to receive an antibiotic prescription compared to those with a negative test result (OR = 0.211, 95% CI = 0.209 - 0.213). Among patients with a negative test result, those who were not prescribed antimalarials were more likely to receive an antibiotic compared to those who were prescribed an antimalarial (OR = 3.93, 95% CI = 3.84-4.03). Achieving high testing rates for malaria to reduce unnecessary use of antimalarials could significantly increase empirical use of antibiotics. More studies are needed to examine antibiotic prescription practices among patients with acute febrile non malaria illness and the potential drivers of such practices.

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### ULTRASENSITIVE DETECTION OF HISTIDINE-RICH PROTEIN 2 (HRP2) AS A ROBUST METRIC IN ESTIMATING ACTIVE OR RECENT *PLASMODIUM FALCIPARUM* INFECTION IN HAITI

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Estimating residual malaria transmission becomes increasingly difficult as parasite prevalence is decreased. Increases in proportion of infections with subpatent characteristics and low parasite densities cause field diagnostics of microscopy and rapid diagnostic tests (RDT), as well as lab-based nucleic acid amplification assays, to become more unreliable. Histidine-rich protein 2 (HRP2) is produced exclusively by only one of the human malarial, *Plasmodium falciparum*, and has been an effective diagnostic marker utilized for decades due to its high expression levels and species specificity. Recently, development of a novel laboratory assay for detecting HRP2 allows picogram detection of the antigen in human biospecimens. We employed this assay to a school-based survey in a concentrated sampling area in the low-endemic nation of Haiti (n=1,230 students in 52 schools), where all participants were given a HRP2-based RDT test, and provided a dried blood spot (DBS) for later testing by PET-PCR, ultrasensitive HRP2 assay, and serology assay for IgG against *P. falciparum* antigens. Of all students sampled, 7 (0.57%) tested positive by RDT, 9 (0.73%) positive by PET-PCR, and 26 (2.11%) positive by the HRP2 assay. Discordance was noted among these three assays, with just 4 children testing positive for all, 3 RDT only positives, 3 PET-PCR only positives, and 20 HRP2 only

positives. Children only positive for the HRP2 lab assay consistently had lower concentrations of the antigen, indicating a very low-density infection (below PET-PCR limit of detection), or lingering antigen in the blood from a previously-resolved infection. Regardless of the assay used, similar geospatial clustering was observed to estimate regions of active or recent malaria exposure. Serological data for IgG prevalence to *P. falciparum* antigens corroborated these findings, showing higher percentage of children with malaria antibodies in clustered regions. Direct comparisons of estimates provided by each of these assays provides evidence of HRP2 detection and quantification as a potentially useful metric for population-based malaria surveillance purposes.

## 972

### IMPROVING QUALITY OF BLOOD SMEAR MALARIA MICROSCOPY THROUGH A DISTRICT-BASED EXTERNAL QUALITY ASSURANCE SCHEME IN UGANDA, 2014-2016

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World Health Organization (WHO) recommends Microscopic examination of blood smears as the gold standard method for malaria diagnosis. However, its quality in most malaria endemic and resource limited settings remains modest. To improve the quality of malaria microscopy, we conducted refresher training before a WHO recommended External Quality Assurance (EQA) system was implemented in Uganda. We report on findings of a district-based slide cross-checking program that aimed at improving the quality of malaria microscopy in 21 districts of Uganda. From 2014 to 2016, 54 health facilities located across 21 districts were selected to participate in an EQA program. Each health facility randomly selected and stored two blood slides (positive and negative) from routinely examined slides per day. On a monthly basis, a district laboratory coordinator randomly selected and transported 10 positive and 10 negative slides to the district hospital for cross-checking in an independent and blinded manner. Ability to correctly classify blood smears as positive or negative was compared for parasite detection with those at the district hospital. Discordant slides were tie broken by WHO certified expert slide readers whose decision was final. Blinding of readers was done at all levels. The Kappa statistic was used to measure accuracy and level of agreement between the slide readers. Between January 2014 and November 2016, a total of 18,000 blood slides were collected, of these, 5200 (28%) were discordant after cross-checking at the district level. Overall, the proportion of laboratories with accurate parasite detection increased from 49% (26) in 2014 [Kappa=0.66] to 78% (42) in 2016 [Kappa=0.88]. Improvement in accuracy varied across districts with the highest and lowest being 67% in 2014 to 82% in 2016, [p<0.02] and 67% in 2014 to 77% in 2016 [p<0.04] respectively. Implementation and maintenance of an external Quality assurance system can potentially improve quality of malaria microscopy in support of malaria case management and surveillance systems.

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### CLINICAL PERFORMANCE OF A HIGH-SENSITIVITY HISTIDINE-RICH PROTEIN 2 (HRP2)-BASED ELISA FOR DETECTION OF *PLASMODIUM FALCIPARUM* MALARIA

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Detection of individuals harboring low-density *Plasmodium falciparum* (*Pf*) infections is critical for assessing malaria intervention efforts, as well as *Pf* vaccine efficacy and drug sensitivity studies. The gold standard, microscopy, lacks reproducibility and has high false-positive rates and low sensitivity at submicroscopic parasitemias (200 parasites/ $\mu$ L). As a result, biomarkers such as HRP2 that are abundant throughout the parasite asexual stage are increasingly being used as a surrogate for *Pf* detection. Current commercial enzyme-linked immunosorbent assays (ELISAs) are used because they have higher sensitivity than current diagnostics, but they have only a 400 pg/mL HRP2 limit of detection (LOD) and are therefore unable to detect and quantify HRP2 at low parasitemias. Recently, Alere™ developed the high-sensitivity Malaria Ag P.f ELISA (HS ELISA) with an LOD of 25 pg/mL HRP2. As part of the PATH Diagnostics for Malaria Elimination Toward Eradication (DIAMETER) project, a performance evaluation of the Alere HS ELISA was conducted using field-collected whole blood specimens from malaria-endemic areas. The HS ELISA study used 300 whole blood specimens, of which 100 were from Nagongera, Tororo District, Uganda, and 200 were from Karen Village, Myanmar. The Uganda specimens were collected from May through September 2015 in a high-transmission area, and the Myanmar specimens were collected from April through May 2015 in a low-transmission area. All 100 specimens from Uganda were *Pf* positive by both microscopy and qRT-PCR, and the 200 specimens from Myanmar were *Pf* negative by both microscopy and qRT-PCR. The results showed an HS ELISA sensitivity of 100% (96.4-100) and a specificity of 97.9% (94.8-99.4) compared to concordant microscopy and qRT-PCR results. The findings suggest that the Alere™ HS ELISA has excellent sensitivity and specificity against clinical specimens when compared to gold standard assays, making it a promising tool for successful malaria programs.

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### A RETROSPECTIVE CLINICAL RECORD REVIEW OF MALARIA DIAGNOSES IN HAITI: 2008-2016

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The national policy for malaria diagnosis in Haiti changed in 2012, switching from microscopy to rapid diagnostic tests (RDTs). This study aims to examine the temporal trends of malaria diagnosis among five clinics in the Ouest Department that captures the impact of this change in policy on reporting. Monthly clinic records from 2008 to 2016 were acquired from the African Methodist Episcopal Church Service and Development Agency, Inc. (AME-SADA) for five different clinics within the Ouest Department of Haiti. Data was dichotomized between "microscopy only" for the years 2008 to 2011 and "RDT" for the years 2012 to 2016. Descriptive statistics and logistic regression were used to analyze data. From 2008 to 2016, the percentage of malaria positive samples out of the total number tested was approximately 9% overall. The microscopy group was found to have 533 malaria positives out of 1678 suspected cases (31.8%), while the RDT group had 92 malaria positives out of 5272 suspected cases (1.7%). After adjusting for the number of people tested, patients examined by microscopy were approximately 1.8 times more likely to have a malaria positive diagnosis than patients examined by RDTs (OR = 1.79 95% CI:

1.44-2.24). The significant difference between percentages of confirmed cases before and after the implementation of RDTs in Haiti may be evidence of improved diagnostics with the switch to RDTs and possible previous over diagnosis with microscopy. However, a plausible reason for this could be differences in thresholds of detection or that microscopy was identifying asymptomatic gametocytemia while RDTs identified the acute clinical phase. Regardless, 98% of suspected malaria cases tested negative, warranting further investigations into the underlying cause of undifferentiated febrile illness in Haiti.

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### EXPLORING THE ANTIMALARIAL POTENTIAL OF WHOLE PLANT *CYMOPOGON CITRATUS*

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Malaria is a health problem concentrated in the poorest Sub-Saharan African countries. Because of the development of resistance and adverse reactions to common antimalarials as well as their limited affordability and accessibility, it is important to investigate other options for malaria therapy. Plants have been used as therapeutics for centuries and as source of pharmaceutical antimalarial drugs. In this study, the antimalarial activity of the dried whole plant (WP) *Cymbopogon citratus* (Lemon Grass) was evaluated. This plant has been used as herbal infusion against fever and malaria. The antimalarial activity of the whole plant was assessed in two rodent malaria models, *Plasmodium chabaudi* AS, which generates a self-cured infection, and *P. berghei* ANKA, which is lethal. Results showed that the WP *C. citratus* treatment produced prolonged antimalarial activity against both parasites. In addition, two whole plant doses of 1600mg/kg and 3200mg/kg were respectively tested. The low dose elicited higher antimalarial activity than the high dose, indicating a potential saturation of receptors with the high dose. As a prophylactic treatment, the whole plant displayed higher antimalarial activity than chloroquine as well as the plant's infusion. These findings provide evidence of the antimalarial effectiveness of the dried whole plant *C. citratus* and supports continued efforts towards developing whole plant therapies for the management of malaria and other infectious diseases in resource poor settings.

## 976

### PYRIDO[1,2-A]BENZIMIDAZOLES: A NOVEL NON-QUINOLINE B-HAEMATIN INHIBITING ANTIMALARIAL CHEMOTYPE

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The emergence of *Plasmodium falciparum* strains tolerant to artemisinin combination therapies underscores the urgent need to expand the antimalarial drug arsenal. One approach to this involves exploring novel compound classes, preferably with multistage activity, good safety profiles and targeting critical pathways within the parasite. Pyrido[1,2-a]benzimidazoles (PBIs), which possess a chloroquine (CQ)-like planar heterocyclic moiety, are likely to interact through  $\pi$ - $\pi$  stacking with ferriprotoporphyrin IX - a toxic byproduct of haemoglobin degradation by *P. falciparum*, leading to inhibition of haemozoin (Hz) formation and consequent parasite death. The aim was to profile a series of PBIs using *in vitro* analyses to determine their activity against various stages of *P. falciparum* lifecycle and, as mechanistic evaluation, test their ability to inhibit *in vitro*  $\beta$ -haematin and with-in cell Hz formation as well as *in vivo* efficacy in a mouse model. Characterization of the antiplasmodial potency, cytotoxicity,  $\beta$ -haematin/Hz inhibition activity and metabolic profiles of each compound was performed using established *in vitro* and *in vivo* assays. All test compounds had nanomolar activity against CQ-sensitive and -resistant *P. falciparum* strains and displayed favourable toxicity indices. Representative analogues in the series were effective against liver and gametocytic stages, and showed *in vivo* oral efficacy in the *P. berghei* mouse model. Most of the compounds tested inhibited  $\beta$ -haematin formation in the same range as the standard inhibitor, amodiaquine and

further showed dose-dependent Hz inhibition signatures similar to CQ in trophozoites. These PBIs are therefore potential candidates for pre-clinical development and inhibition of Hz formation is a likely mechanism of action contributing to their activity in *P. falciparum*.

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### BOOSTING IVERMECTIN FOR VECTOR CONTROL: CYTOCHROME-P-450/ABC-TRANSPORTER INHIBITION SYNERGIZES IVERMECTIN AND INCREASES THE MORTALITY OF *ANOPHELES GAMBIAE*

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Insecticide resistance and mosquito behavioural adaptations that allow avoidance of home-centred vector control measures are two of the major challenges faced by the malaria community today. In this context, the mass use of drugs that can kill mosquitoes feeding on treated subjects has potential to become a new paradigm for vector control. Ivermectin is one of the leading candidates given its excellent safety profile. In the phase of the challenge posed by insecticide resistance, a thorough understanding of the mosquito metabolic pathways and potential defence mechanisms from ivermectin is of paramount importance for vigilance purposes and early assessment if this novel strategy is to be used in the field. Simple synergistic bioassays have been previously used to identify metabolic resistance in *Anopheles* adults and larvae. We have conducted a synergistic bioassay to evaluate the potential involvement of ABC transporters and cytochrome P450 in the metabolism of ivermectin in *Anopheles gambiae*. Mosquitoes were membrane fed with matched pairs of blood samples containing the same ivermectin concentration with or without Ketoconazole (a dual cytochrome-P450/ABC-transporter inhibitor). Mosquitoes in the ivermectin plus ketoconazole group had a significantly reduced mean survival and time to median mortality as assessed by Kaplan-Meier analysis. Our results show that pharmacological inhibition of P450 and ABC transporters could further improve the efficacy of ivermectin as a vector control tool and extend the spectrum towards less susceptible vectors.

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### ANTIMALARIAL THIOTRIAZOLES: DISCOVERY OF A NOVEL PRECLINICAL CANDIDATE WITH CLINICALLY VALIDATED MODE OF ACTION

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Malaria continues to be a major global disease still causing impermissible number of deaths. The effectiveness of current antimalarial therapy is under continuous threat through the spread of resistance. Consequently, there is an urgent need to replace those drugs compromised by resistance, as well as identifying potential novel therapies that offer significant advantages over the current standard of care. Within the TCAM set of phenotypic hits identified at GSK, a thiothiazole chemical series was selected because its chemical novelty offering exciting properties as antimalarial. Thiothiazole molecules are dual acting antimalarials with demonstrated activity against both asexual and sexual intra-erythrocytic *Plasmodium* stages, with nanomolar *in vitro* potency in sensitive and MDR strains (including clinical isolates). Potential to block disease transmission has been validated using a standard membrane feeding assay (SMFA)

with *Anopheles* mosquitoes. These properties are accompanied by an oral efficacy characterized by a rapid parasite clearance in the *P. falciparum* mouse model. Thiotriazole series displays a novel mechanism of action and its parasitological profile and genetic studies point at compounds targeting the PfATP4 pathway. Lead optimization efforts within the series led to the identification of GSK3212030 molecule as a new preclinical candidate that will offer opportunities linked to its rapid antimalarial mode of action expected to maximize efficacy, achieving fast clinical resolution, and minimizing the in-patient window of opportunity for resistant parasite selection and dissemination. The overall properties of GSK3212030 constitute a promising profile supporting further development to provide novel antimalarial therapeutic opportunities.

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### DISCOVERY OF NOVEL ANTI-MALARIALS BY HIGH THROUGHPUT SCREENING AND COMBINATORIAL CHEMISTRY

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Malaria has been a major global health concern for many years. Despite some reduction in mortality, malaria still have a hideous impact on people's health, especially on children. Swift spread of drug resistant malaria parasites and limited available drugs - accelerate the necessity of novel anti-malarial compound development. In our previous study (doi:10.1128/AAC.01607-16), core 9600 compounds assigned with various structural diversity of >200,000 compounds from the University of Tokyo chemical library were screened. 224 compounds among 9600, were identified as potential hemozoin inhibitors after an initial *in vitro* anti-hemozoin high throughput screening (HTS). In our current study, an *in vitro* erythrocytic anti-malarial assay was performed at 10  $\mu$ M to screen positive compounds exhibiting anti-malarial activity using Chloroquine/Mefloquine sensitive *Plasmodium falciparum* (Pf) strain, 3D7. Subsequently, dose-response assay was done at ten different dilutions ranged between 0.5 nM and 10  $\mu$ M to measure the 50% inhibitory concentration (IC<sub>50</sub>). Each experiment was conducted twice. Eventually, an *in vitro* anti-malarial assay followed by dose-response assay were performed using Chloroquine/Mefloquine resistant *P. falciparum* strain, Dd2, at similar condition as mentioned above. SYBR Green-I was used for the staining and detection of parasite. Cytotoxicity was measured using human liver carcinoma (HepG2) and adult mouse brain (AMB) cells at 20  $\mu$ M concentration. A total of 22 compounds were identified to exhibit anti-malarial activity against 3D7 strain with IC<sub>50</sub>  $\leq$  10  $\mu$ M. Out of 22, only six compounds possessed IC<sub>50</sub> < 7  $\mu$ M for both *Plasmodium falciparum* (Pf) strains, 3D7 and Dd2. However, five compounds demonstrated anti-malarial activity at IC<sub>50</sub> < 7  $\mu$ M for both Pf strains and CC<sub>50</sub> > 20  $\mu$ M. Among them, 3 compounds showed IC<sub>50</sub> < 0.8  $\mu$ M. In conclusion, 5 compounds have selected as potential "Hit compounds". Based on the chemical similarity scores using "Tanimoto similarity measure", all the available analogues (>1500) of the five hit compounds were purchased from University of Tokyo for further analysis.

### OPTIMIZATION OF POLYMERIC BLENDS-ARTESUNATE-AMODIAQUINE HCL MICROPARTICLES USING DESIGN EXPERT SOFTWARE

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A 23 Factorial design of cissus-gelatin B polymer blends was developed to formulate amodiaquine HCl-artesunate microparticles by varying the polymer blend concentration (2 %w/v, 5 %w/v), crosslinking time (0.5 h, 1 h) and glutaraldehyde volume (0.5 ml, 1 ml). The formulations were evaluated using drug entrapment efficiency (EE), particle size, polydispersity index, thermal behaviour with differential scanning calorimetry, crystallinity with powder X-ray diffraction, morphology with scanning electron microscope and *in vitro* release using combination of simulated gastric fluid (SGF, pH= 7.4, 95 %) and methanol (5 %). The expected responses, EE and *in vitro* release were fitted into Design Expert®. The polymer blends exhibited pseudoplastic behavior and there was no marked change in rheology behaviour of 2 % w/v dispersion at 55 oC. The amodiaquine HCl-artesunate formulated microparticles were dark brownish discrete mass, physically stabilized, irregular shape, polydisperse, and semi-amorphous system. An optimal formulation comprising polymer blend (5%w/v), glutaraldehyde (1 ml) and cross-linking time (0.5 h) was identified to provide desired values for EE, amodiaquine HCl (47.41%), artesunate (36.42%) and *in vitro* release. This study proposes the best opportunity for selection of factors required for optimum microparticles formulation using the polymer blends and the drugs.

**Keywords:** microparticles, crystallinity, entrapment efficiency, polymer blend, *in vitro* release.

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### EVALUATION OF THE ANTIOXIDANT POWER AND THE EFFECT OF POTENTIATION AND STUDY OF TOXICITY PARAMETERS OF 2 EXTRACTS OF PLANTS WITH STRONG ANTIMALARIAL ACTIVITY

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In the aim to seek an alternative face to the *Plasmodium falciparum* resistance to known antimalarials, especially artemisinin derivatives, and as part of the promotion of African traditional pharmacopoeia by the development of ITM, we have in this study evaluated *ex vivo* and *in vitro* antiparasmodial activity of several extracts from two plants traditionally used as antimalarials in Ivory Coast: *Erigeron floribundus* and *Terminalia glaucescens*. Then we explored the chemical composition of active and promising extracts, assessed their antioxidant activity and studied their toxicological profile. After this work, we noted that the methanol extracts of the leaves and roots bark of *T. glaucescens* showed the best activities with respective IC<sub>50</sub>s of 2.20  $\mu$ g/mL and 1.40  $\mu$ g/mL on chloroquine-sensitive isolates and 3.65  $\mu$ g/mL and 1.38  $\mu$ g/mL on chloroquine-resistant isolates. These extracts have been more active on chloroquine-sensitive and chloroquine-resistant reference strains and also showed a high potentiating power over chloroquine. The evaluation of the antioxidant activity of these extracts showed their wealth in polyphenols, tannins and flavonoids. In addition, we determined that methanol extracts of *T. glaucescens* leaves and roots bark have nearly three times the reducing power of vitamin C on the DPPH radical. On the toxicological aspect, we noted that these extracts showed no immediate toxicity

by oral administration. From the experience of repeated doses, rather we noted several beneficial effects of these extracts on body weight, hemoglobin, blood levels of cholesterol and triglycerides, and a remarkable hepatoprotective action. The signs of toxicity appeared only at very high doses. Thus the traditional therapeutic dose of 13.90 mg/kg body weight of methanol extracts of *T. glaucescens* leaves and root bark is not toxic and may be admixable in human. NOAEL have been determined below or close to 1390 mg / kg body weight.

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### MECHANISTIC AND SAR EVALUATION OF HEMOZOIN-INHIBITING COMPOUNDS IDENTIFIED VIA HTS ACTIVE AGAINST PLASMODIUM FALCIPARUM

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Quinoline antimalarials target hemozoin (Hz) formation causing a cytotoxic accumulation of ferriprotoporphyrin IX (Fe(III)PPIX). Well-developed SAR models exist for  $\beta$ -hematin ( $\beta$ H); synthetic Hz) inhibition, parasite activity and cellular mechanisms for this compound class, but no comparably detailed investigations exist for other Hz-inhibiting chemotypes. In a previous study, target based screening was carried out for inhibitors of  $\beta$ H formation and series of benzimidazoles and triarylimidazoles were identified as active against *Plasmodium falciparum*. Here we report the subsequent synthesis and testing of derivatives with varying substituents for these series. The results indicated that only benzamide derivatives containing an electron deficient aromatic ring and capable of adopting flat conformations, optimal for  $\pi$ - $\pi$  interactions with Fe(III)PPIX, can inhibit  $\beta$ H formation. In contrast to quinolines, introduction of amine side chains for pH trapping did not lead to benzamide accumulation in the parasite. Furthermore, a 2-hydroxy-1,3-dimethoxy substitution pattern on the 2-phenyl ring of the triarylimidazoles is required for submicromolar parasite activity. The two most potent analogues showed nanomolar IC50s, with little CQ cross-resistance, low cytotoxicity and high *in vitro* microsomal stability. Selected  $\beta$ H inhibiting analogues were shown to dose-dependently inhibit Hz formation and parasite survival in parasitized red blood cells causing unprecedented levels of intracellular exchangeable (free) heme at their relative IC50. In contrast, active compounds which do not inhibit  $\beta$ H formation, did not show this effect *in vitro*, suggesting an alternative mechanism of action. These data reveal complex relationships between heme binding, free heme levels, cellular accumulation and *in vitro* activity of potential novel antimalarials.

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### IDENTIFICATION OF APPROVED DRUGS THAT HAVE ACTIVITY AGAINST PLASMODIUM FALCIPARUM USING IN SILICO AND IN VITRO APPROACHES

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Recent reports of artemisinin resistance in South-East Asia warrant urgent discovery of novel drugs for treatment of malaria. Unfortunately, the success rate of bringing new drugs to the market is low. This is mainly because most bioactive compounds don't get approved due to safety concerns and the prohibitive cost and time involved in drug discovery and development. Therefore, repositioning of already approved drugs

can be sustainable since these have passed safety tests in the treatment of some diseases. This study screened approved drugs for antiparasmodial activity using *in silico* chemogenomics approach and *in vitro* assays. All *Plasmodium falciparum* proteins available at NCBI RefSeq database were retrieved in FASTA format. Sequence similarity search was performed against these proteins and putative target proteins of approved drugs in Therapeutic Target Database, DrugBank and STITCH databases. Functional residues of the drug targets were determined using ConSurf server which were used to fine tune the similarity search. Druggability of the *P. falciparum* proteins were also obtained from TDR targets database. Of the drugs predicted to have antiparasmodial activity, those that have been previously tested against malaria were identified by doing a published literature search. Finally, drug susceptibility assays using SYBR Green I method were done to validate their antimalarial activity. Preliminary results showed 133 approved drugs could target specific *Plasmodium* proteins. Though, published literature showed that only 34 out of 133 drugs not to have been tested. Interestingly, most of those tested did show significant antimalarial activity. We did *in vitro* tests on 12 out of the 34 drugs, 10 had IC50 below 100  $\mu$ M while 3 had less than 10  $\mu$ M. This study validates the use of target-similarity in identifying approved drugs that have activity against the *Plasmodium* parasite and hence reposition them for antimalarial treatment. This approach would circumvent many challenges involved in preliminary stages of drug discovery and development hence could save on cost and time spent in introducing new drugs into the market.

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### HIGH THROUGHPUT DISCOVERY OF NEW DRUGS TARGETING MALARIA PARASITE TRANSMISSION - TOWARDS THE ALTRUISTIC ANTIMALARIAL

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Growing evidence of resistance to artemisinin threatens the shelf-life of the current frontline antimalarial combination therapies and underscores the need for the discovery of novel drug targets with new modes of action. Historically, antimalarial chemotherapy has been an arms race between drug-development and resistance acquisition of asexual parasites. Multi-stage targeted approaches are therefore a necessity if future gains are to be made and held. Since only 0.2-1 % of asexual parasites differentiate into gametocytes, targeting this natural bottleneck provides a tangible route to interrupt transmission whilst avoiding resistance selection. In recent years, development of high-throughput antimalarial assays has permitted access to previously unexplored chemical space. Towards discovery of transmission blocking antimalarials and novel targets, a Dual Gamete Formation Assay (DGFA) has been developed as a high-throughput alternative to the Standard Membrane Feeding Assay (SMFA). The DGFA determines viability of male and female gametocytes independently, using different readouts of the same well, following drug exposure. Here we present the results from a large-scale high-throughput screen of the Global Health Chemical Diversity Library, with nearly 70,000 different chemical scaffolds, identifying several classes of novel drug hits. The screening campaign has uncovered compounds with different activity profiles (male-specific activity, dual male-female activity or dual-asexual-sexual activity), providing new options to target parasite transmission. We present results from ongoing work aimed at validating *in vivo* activity of each compound along with efforts to determine their precise mode of action. The discovery of novel antimalarials targeting transmission sets the groundwork for future therapeutics that would prevent transmission of parasites through the population, altruistically blocking the spread of malaria in communities. Combined with schizonticides, we believe dual-stage combination therapies envisaged this way would become key assets for future global eradication campaigns.

## TRANSLATIONAL PRECLINICAL PLATFORM FOR EVALUATION OF ANTIMALARIAL COMBINATION THERAPY

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Malaria is a global health problem that causes significant mortality and morbidity in many developing countries, where young children and pregnant women are the groups most affected. Most antimalarial drugs face decreased efficacy due to the spread of resistance. The past decade has seen a transformation of the portfolio of malaria medicines, with new chemical entities, that act through novel mechanisms of action, entering clinical development. The challenge in drug discovery and development is to show that a medicine is effective in the clinical setting. Drug combinations are essential for optimal control of malaria, as they should delay the selection of anti-malarial drug resistance and could offer additional advantages if the separate agents are active against different parasite stages. A translational preclinical platform aimed at identifying novel and optimal combinations to optimize efficacy and avoid selection of resistance has been established. This platform is supported by the Bill and Melinda Gates Foundation and encompasses a complete profiling which involves several phases: a/ To map the compatibility of a range of current and new antimalarials based on its different Mode of Action (MoA) and establishment of quantitative *in vitro* and *in vivo* assays to analyze combination interactions. b/ To conduct *in vitro/in vivo* efficacy analysis of selected pairs of antimalarials c/ To provide a detailed PK/PD analysis of selected drug combinations for Human Dose Prediction. d/To analyse the effect of antimalarial combinations on resistance selection. The combinations that are to be thoroughly investigated will be selected by a joint Steering Committee of both MMV and GSK members. Novel combinations will be studied extensively with the main aim of identifying key parameters that will assist the design of future clinical trials. "The human biological samples were sourced ethically and their research use was in accord with the terms of the informed consents" "All animal studies were ethically reviewed and carried out in accordance with European Directive 86/609/EEC and the GSK Policy on the Care, Welfare and Treatment of Animals."

## MEPICIDES: POTENT INHIBITORS OF ISOPRENOID METABOLISM IN *PLASMODIUM FALCIPARUM*

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The emergence of *Plasmodium falciparum* strains resistant to current antimalarial therapies has prompted efforts to identify and validate agents with novel mechanisms of action. The enzymes of the methylerythritol phosphate (MEP) pathway of isoprenoid biosynthesis are promising drug targets, as pathway enzymes are essential, conserved, and lack mammalian homologues. We specifically targeted the first dedicated enzyme of the MEP pathway, deoxyxylulose phosphate reductoisomerase (Dxr), and have both genetically and chemically validated the enzyme as an antimalarial target. The ultrastructural changes that occur when Dxr is disrupted were characterized by transmission electron microscopy. Our data indicate that Dxr function is essential for maintaining normal

cellular morphology. Further, we identified a new series of Dxr inhibitors, termed MEPicides, which have low nM potency against asexual blood stage parasites. Targeted metabolite analysis demonstrated that MEP pathway metabolites are substantially reduced in *P. falciparum* following treatment with the inhibitors. In addition, isoprenoid precursors rescued growth of MEPicide-treated parasites, and *P. falciparum* mutants that produce higher levels of the Dxr substrate were resistant to MEPicide treatment. Importantly, multidrug-resistant parasites were sensitive to the inhibitors, and there was no observable toxicity to human cell lines. While the MEPicides lacked activity against mature gametocytes, the compounds effectively killed parasites committed to gametocytogenesis, suggesting that the Dxr inhibitors may reduce transmission. Finally, our data indicate that this novel class of compounds is both safe and efficacious in malaria-infected mice. Collectively, our findings demonstrate that the MEPicides are promising antimalarial agents, and likewise, are candidates for further drug development.

## SCREENING THE PATHOGEN BOX FOR HITS AGAINST ARTEMISININ-RESISTANT *PLASMODIUM FALCIPARUM* MALARIA

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The Pathogen Box contains 400 diverse, drug-like molecules active against neglected diseases of interest and is available free of charge from Medicines for Malaria Venture. A dramatic increase in artemisinin and partner drug resistance in SE Asia has forced the need for new drugs against *P. falciparum* malaria. In an effort to identify new anti-malarials with such activity, we screened n=80 compounds from The Pathogen Box against artemisinin-resistant *Plasmodium falciparum* malaria strains MRA-1236 (*Plasmodium falciparum* IPC3445 Pailin Cambodia 2010) and MRA-1240 (*Plasmodium falciparum* IPC 5202 Battambang Cambodia 2011) using the standard erythrocyte stage assay. Both strains harbour mutations in the kelch 13 gene which confers resistance to artemisinin. *P. falciparum* was cultured under standard conditions, synchronized, and tested using the histidine-rich protein ELISA assay. Drugs were screened at 10uM in order to identify hits. Our results confirm that 4 out of 80 compounds have significant activity (>65% inhibition versus control, triplicate experiments performed twice) against both strains MRA-1236 and MRA-1240 in our screen. Of the 4 molecules, 3 were known to have activity against malaria previously, whereas 1 had previously only shown activity against tuberculosis. While these data are preliminary, we will present the final screening results on all 400 compounds together with their 50% inhibitory concentration, known DMPK data, and chemical structures. We anticipate uncovering further novel agents against artemisinin-resistant *P. falciparum* malaria in this collection which may be repurposed as leads in drug development.

## A PHASE IB STUDY TO INVESTIGATE THE ANTIMALARIAL ACTIVITY OF (+)-SJ000557733 (SJ733) IN INDUCED BLOOD STAGE *PLASMODIUM FALCIPARUM* MALARIA

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SJ733 is a new oral antimalarial that targets the ATPase 4 ion transporter of plasmodia. A two cohort single-dose study was undertaken to define its antimalarial activity in the induced blood-stage malaria (IBSM) system. A dose of 150 mg was selected for the first cohort by combining the

minimal parasitocidal concentration (MPC) predicted from preclinical data (0.1-0.2 µg/mL) with the emerging human PK, aiming to observe parasite clearance kinetics, including recrudescence. Testing of 150 mg in 6 healthy volunteers resulted in transient antimalarial activity over 24 hrs, with a parasite clearance half-life of 6.5 hrs (95%CI: 5.9 - 7.2 hrs). However, rapid recrudescence occurred with all subjects, requiring artemether-lumefantrine rescue 2 days post SJ733 dosing. In the second cohort where 8 subjects were administered 600 mg, clearance of parasitemia was quicker, with a parasite clearance half-life of 3.6 hrs (95%CI: 3.3 - 3.9 hrs); a longer duration of action (~30hrs), as well as a longer interval to recrudescence (days 2 [2], 3, 5, 6 [2], 7, and 11). SJ733 pharmacokinetic parameters in this IBMS study were similar to the ones previously observed in the First-In-Man study. Although no SJ733-related adverse events were reported, transient inoculum-related moderate clinical and laboratory adverse effects were observed. Laboratory changes included significant elevations in ALT in 3 subjects (>5xULN) only in the first cohort where early and rapid recrudescence occurred. Clinical and laboratory adverse events were less common in cohort 2, with only 1 subject experiencing ALT >3xULN. A population PK/PD model led to an estimate of the human MPC of 0.3 µg/mL. These data suggest that SJ733 shows promise as a component of a new combination 3 day treatment for malaria.

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### TARGETED-REACTIVE CASE DETECTION AT SLEEPING SITES TO INTERRUPT MALARIA TRANSMISSION IN VIETNAM II: REPORTED AND OBSERVED MALARIA PREVENTION, TREATMENT AND RISK BEHAVIORS

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Reactive case detection (RACD) has limited impact and high costs in low endemicity areas where malaria cases sleep outside of their homes. In Vietnam, over 60% of malaria cases sleep in forests or on farms. We applied a targeted-RACD approach to collect information from participants at their sleeping sites and identify risk behaviors associated with malaria cases in a low malaria transmission setting. A cross-sectional study was conducted in three mountainous communes in Phu Yen province, 2016. An index case was defined as someone who routinely slept in the forest or farm and tested positive for malaria using rapid diagnostic test or microscopy at a community health facility, between 2014 and 2016. A list of 110 index cases was obtained from three commune health centers. Index cases came from 71 huts (referred to as an "index hut"). We found 109 neighbor huts, located within 500m of the index hut. Participants were interviewed face-to-face using a standardized questionnaire and a tool for direct observation. Logistic regression models were used to calculate prevalence odds ratios (PORs) and 95% confidence interval (CI) for risk factors, after adjusting for socio-demographic characteristics. The respondents lived in 180 huts: 21 huts had two or more malaria cases per hut, 50 had one case, and 109 had no cases. Neighbor huts were significantly closer to their official homes than the index huts—they were 3.10 times more likely to be within a 30 minute motorbike ride (95% CI 1.87-5.13). Significantly more index huts than neighbour huts had more than three occupants (POR=4.63; 95% CI 2.74-7.81), and were surrounded by more than 3 huts (POR=2.48; 95% CI 1.28-2.79). Access to a cell phone network was significantly higher among the neighbour huts (POR = 6.33; 95% CI 3.11-12.90). Direct observation showed low ownership of treated nets in both groups (13.8% vs 5.6% among the

index huts). Targeted education and malaria prevention strategies can be developed to address the specific risk factors identified for those primarily sleeping in farms and forests.

## 990

### IMPACT OF BIOLARVICIDES ON THE MALARIA MORBIDITY IN SCHOOLCHILDREN LIVING IN OUAGADOUGOU, BURKINA FASO

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Larval source management is considered as one of the major strategies to reduce malaria transmission in endemic countries. A pilot study was conducted in the urban area of Ouagadougou, Burkina Faso to evaluate to what extent using biolarvicide (Griselesf® + Bactivec®) to treat mosquitoes breeding sites will reduce malaria vectors population density, and hence the transmission. To complement the entomological evaluations, a parallel study was conducted to measure potential reduction of malaria morbidity indicators. The intervention consisted of weekly applications of the biolarvicides in the main Anopheles larval habitats during the year. Two cross sectional surveys were conducted in parallel to measure malaria morbidity indicators before (first survey) and after (second survey) the intervention in randomized schools and children. Clinical and biological data were collected. A total of 4190 children aged 5 to 14 years were randomly included during the two surveys. Prevalence of *Plasmodium falciparum* infection was 16.5% (ic95% [14.8%-18.0%]) and 15.2% (ic95% [13.7%-16.8%]) respectively during the first and second surveys. The risk to present febrile episode was 1.26 (1.1<OR<1.7) during the second survey compare to the first survey. Clinical malaria prevalence was 18.8% (ic95% [13.9%-24.6%]) at the first survey and 27.6% (ic95% [22.4%-33.4%]) at the end of season. Parasite density was 281.80 ic95% [204.08-330.78] vs. 946.12 ic95% [776.92-1152.17]. Mean haemoglobin level was 11.7±2.2 g/dL (first survey) vs. 11.6±1.2 g/dL (second survey) (p<< 0.0001). This study has not shown any efficacy of the intervention in reducing malaria burden in this age category.

## 991

### CHALLENGES IN LLIN INTERVENTION AMONG MOBILE MIGRANT POPULATIONS ALONG THE SURINAME-FRENCH GUIANA BORDER

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In the past decade, Suriname has rapidly decreased its malaria burden. The Malaria Elimination program in Suriname currently focuses on the last remaining foci of transmission in small-scale gold mining (SSM) sites along the Suriname-French Guiana border. The border also is a crossing point for migrant mobile populations working in French Guiana SSM areas, where they have no access to malaria diagnosis and treatment. For the past decade, the distribution of Long Lasting Insecticide-treated Nets (LLIN) has been a key component of the National Strategic Plan. In 2016, LLINs were mass-distributed among inhabitants of high malaria risk areas. To assess the effectiveness of this strategy, interviews were conducted with gold miners and mining service providers in two border-crossing points, who had suffered from (suspected) malaria in the 18 months preceding the interview, both prior to (N=141) and after (N=152) distribution of LLINs. Results suggest that both knowledge of LLIN as an effective way to protect oneself against malaria (from 69.5% to 82.2%) and LLIN possession (10.6% to 36.8%) significantly increased in the project period (X<sup>2</sup>, p<0.001; p<0.001). Nevertheless, 3 months after LLIN distribution, only 36.8% of interviewees reported possession of an LLIN. Moreover,

only 11.8% of respondents slept under an LLIN in the night prior to the interview. The low LLIN coverage is partly due to the high mobility of the target population, which results in high population turn-over and loss or abandonment of LLIN. Another factor is the French repressive policy towards clandestine SSM, which includes the burning of mining camps -including bed nets. Reported factors discouraging LLIN use included absence of mosquitoes, low malaria prevalence, discomfort, and the nets hinder quick fleeing in case of French military raids. We conclude that the distribution of LLINs to at-risk gold mining populations in the border region has not achieved the desired results (80% LLIN usage). Reaching and engaging the members of this highly mobile, superfluous SSM community remains challenging, and will require regional cooperation.

## 992

### QUALITATIVE FINDINGS FROM A CROSS-SECTIONAL MALARIA RISK FACTOR AND PARASITEMIA SURVEY, NORTHERN LAO PDR

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Northern Laos has experienced large reductions in reported malaria cases in recent years despite limited changes in interventional coverage. In Sept-Oct 2016, a mixed-methods study was conducted in four northern districts to measure parasite prevalence, intervention coverage, and risk factors, and to improve understanding of the observed changes in malaria epidemiology. Semi-structured qualitative interviews with local health staff and high-risk populations were conducted to triangulate quantitative survey data. Four focus group discussions and six key informant interviews were conducted in each of the four study districts. Focus groups were separately comprised of health center staff, village malaria workers, forest-goers, and recently diagnosed malaria patients, while key informants included provincial and district malaria staff and other government officials. Interview topics included malaria risk factors, temporal changes in malaria burden, care-seeking behaviors, access to treatment, travel patterns, and any recent changes in employment patterns. A total of 145 respondents were interviewed across 16 focus groups and 24 in-depth interviews. Preliminary analyses suggest that overnight stays in forests or rice fields, work-related travel to higher-burden areas, ethnic minority status, failure to use bednets, and poor food hygiene were all perceived as malaria risk factors. Changes in labor and land use patterns varied by district, with some citing increased mobility among adult men for work and shifts from rice to other crops (including tea, and *Jatropha curcas* for biodiesel). Commonly cited challenges among malaria staff included RDT and ACT stock-outs and limited capacity for supervision due to resource constraints and poor road access. While the malaria burden in northern Lao PDR has declined in recent years, further studies are needed to validate these changes to inform elimination policies nationally and mitigate the potential for resurgence. Increasing population mobility and weak supply chain and supervision structures should be addressed to strengthen systems for sustainable malaria elimination.

### MALARIA ELIMINATION PLANNING TOOL: AN OPERATIONAL CAPACITY ASSESSMENT FOR MALARIA ELIMINATION EFFORTS IN ETHIOPIA

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The Ethiopian National Malaria Control Program (NMCP) has set a goal to eliminate malaria by 2030. Malaria elimination readiness is often assessed using epidemiological criteria. However, a country's operational capacity to implement malaria elimination efforts is a factor that also needs consideration. To assist the Ethiopia NMCP to achieve its goal, a Malaria Elimination Planning Tool (EPT) was developed to assess the operational capacity of woredas (districts) to implement critical surveillance, vector control, and case management activities required to achieve and sustain malaria elimination. The approach used to develop the tool included consulting WHO guidance, the NMCP's malaria elimination strategy guideline, and technical experts from the NMCP and other malaria partners. The EPT contains 9 capacity assessment categories: (1) human resource, (2) governance and political support, (3) financial resource, (4) technical resource, (5) malaria case management, (6) surveillance, monitoring and evaluation, (7) vector control, (8) social and behavior change communication, and (9) malaria commodities. The tool is designed to assess various health facility levels including the Woreda Health Office (WoHO), Health Centers (HCs) and Health Posts (HPs). Interviews using the tool will be conducted with WoHO heads, malaria focal persons (WoHO), HMIS focal persons (WoHO; HC), PHEM focal persons (WoHO; HC), pharmacy personnel (WoHO; HC), laboratory staff (HC), clinical staff (HC) and Health Extension Workers (HP). With Ethiopia's NMCP officially declaring the start of malaria elimination activities in March 2017, the EPT is a key component to assess operational capacity in the 239 woredas targeted for elimination. The EPT is easily adaptable to be used by other countries aspiring to eliminate malaria.

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### DATA-DRIVEN APPROACHES FOR FINER TEMPORAL AND SPATIAL RESOLUTION COVERAGE AND PROGRESS ESTIMATES FOR INDOOR RESIDUAL SPRAYING (IRS) CAMPAIGNS IN CHOBE DISTRICT, BOTSWANA

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As Botswana approaches its 2018 malaria elimination target, evidence-based operational planning is important to target malaria interventions. Indoor Residual Spraying (IRS) is a cornerstone of the National Malaria Programme's (NMP) vector control program, with a goal to achieve 80% target coverage nationally. Historically, coverage has been evaluated by the total number of rooms sprayed compared to rooms seen, aggregated by village at the end of the season from paper spray forms. In 2016, the NMP collaborated with the Clinton Health Access Initiative (CHAI) to implement a novel, data-driven approach in Chobe district that would allow better estimations at finer spatial and temporal resolutions. A remote enumeration of structures (13,466 buildings in Chobe) was conducted to build a household footprint database for what appears to be a building on freely available satellite imagery. An electronic data collection solution,

including collection of GPS points of each household, was implemented for real-time data collection during IRS implementation. The electronic data was automatically fed into a web-based dashboard, which was used to visualize key decision indicators for progress of field teams. Post-season data analysis was conducted to compare completeness and quality between the paper and electronic data, and assess coverage of the intervention. Approximately 9,514 (71%) buildings were targeted for IRS, given some areas will receive bed nets. Assuming 2 buildings and an average of 4 rooms per household, a total of 38,056 rooms were targeted for IRS. Based on electronically collected data, 7188 (19%) of the targeted rooms were visited for IRS and 4947 (13%) were sprayed. However, using the paper aggregated data, 7,871 (21%) were visited and 5,744 (15%) were sprayed. Comparing the electronic and paper-based system, 91% of rooms reported by the paper-based spray card system (N=7871) were collected in the electronic system (N=7188). The introduction of electronic data collection provided a detailed understanding of IRS coverage and progress in real-time via web, informing improvements required for future IRS seasons.

## 995

### GENOMIC METHODS OF SURVEILLANCE REVEAL MARKED PATTERNS IN *PLASMODIUM FALCIPARUM* PARASITE POPULATIONS ACROSS THE TRANSMISSION GRADIENT IN SENEGAL

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Senegal is characterized by three broad transmission zones from low transmission in the northern, moderate transmission in the central, and relatively high transmission in the southern regions of the country. To better assess patterns of transmission, we deployed a molecular barcode to genotype 24 independent single nucleotide polymorphisms (SNPs) utilizing discarded rapid diagnostic tests at multiple clinics across these transmission zones. Based on the hypothesis that the general patterns related to the proportion of monogenomic infections and the frequency of clonal parasites within these monogenomic infections may reveal distinct patterns of transmission and migration, we analyzed samples from multiple clinics across these transmission zones in Senegal, including extensive collections from the northern Richard Toll district, which is moving toward elimination. This study assessed nearly 750 cases positive for *Plasmodium falciparum* infection in Richard Toll and more than 1000 samples collected from surveillance sites nationwide from 2012 to 2015. These data reveal patterns of changing indicators related to the proportion of monogenomic infections and clonality among these monogenomic infections, as well indicate several parasite genetic signatures that were shared across transmission zones, suggesting that human migration may be an important factor with regard to sustaining transmission in low transmission settings such as Richard Toll. Delineation of these parasite signatures inform sites of ongoing transmission and those dominated by imported parasites which may inform actionable responses to malaria infections and stratify regions according to the optimal intervention based on their genomic characteristics. Evaluation of the genetic relatedness and population characteristics using simple tools may offer new insight for policy decision-makers for malaria control and elimination.

## 996

### THE LONG-TERM DURABILITY OF MASS DRUG ADMINISTRATION USING DIHYDROARTEMISININ-PIPERAQUINE AS PART OF A COMPREHENSIVE MALARIA ELIMINATION STRATEGY IN SOUTHERN PROVINCE ZAMBIA

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A community randomized controlled trial in a population of ~300,000 people in 60 health facility catchment areas (HFCA) in Southern Province Zambia was conducted to assess the impact of 4 rounds of community-wide (MDA) and household-level mass treatment (focal MDA) with dihydroartemisinin-piperazine from 2014-2016. The entire study area received a standard of care of sustained high vector control coverage and improved access to facility and community case management. Across all treatment groups, child malaria parasite prevalence declined from 31% at baseline to 4% after 4 MDA rounds. MDA reduced child infection prevalence in low transmission areas by a further 72% [adjusted odds ratio 0.28 (95% CI 0.09 - 0.87)] compared to the control of no MDA measured 3-months after the 4th MDA round. Following the trial, the Zambia national program implemented an additional 2 rounds of MDA in 2016-17 in 'high transmission' HFCA that included 35 from the previous trial areas with the highest malaria cases. As a result there were 12 HFCA that received 6 rounds of MDA, 8 HFCA that received the initial 4 MDA rounds only, and 6 control HFCA that never received MDA. In the HFCA that received no additional MDA after the trial, the sustained impact of the initial 4 MDA rounds (14 months since the last round) will be compared to control HFCA that never received any MDA, using a parasite survey in April-May 2017. Facility-based confirmed malaria case incidence will serve as a secondary endpoint. Among the facilities that received no additional MDA, confirmed malaria incidence was 0.56 and 2.11 per 1,000 in the previous MDA and control HFCA, respectively, during the peak malaria season (January-March) at the end of the trial in 2016. Preliminary results show confirmed malaria incidence was 0.82 and 3.48 per 1,000 in MDA and control HFCA, respectively, in January-March 2017, about a year after the last MDA round. However, confirmed incidence is still well below 2014 and 2015 levels during this period. These results will be critical in understanding the long-term durability of MDA when implemented as part of a comprehensive elimination strategy in this context of Southern Africa.

## 997

### ASSESSING BIAS AND IMPACT ON DECISION-MAKING FOR MALARIA ELIMINATION WHEN RELYING ON RISK MAPS DERIVED USING CONVENIENCE BASED SAMPLING STRATEGIES IN HAITI

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One of the challenges with controlling and eliminating malaria transmission is being able to estimate where transmission is actually occurring. The gold standard for determining transmission intensity is to use household surveys. However, these are time and cost intensive and are not a viable option as a tool for routine data collection. The use of easy access groups (EAG) such as health facilities, schools, or churches provide a convenient option to quickly collect data on malaria. However, due to issues with selection bias such as access and differing age profiles than the target population present in the EAGs, the resulting estimates of malaria may be biased. Generating malaria risk maps using data collected as part of convenience sampling can be done but how the predicted risk surface compares with those generated using data generated as part of a gold-standard household survey is not known. Data from 6300 participants in 10 health facilities, 25 schools, and 8 churches from the Artibonite valley in Haiti will be integrated into risk maps using Bayesian geostatistical models and compared to risk maps generated using a contemporaneous household survey. Malaria prevalence surfaces according to the convenience and household data are then compared to determine the concordance between the estimates. There is considerable heterogeneity in malaria infection in this area with RDT positivity in health facilities ranging from very low to over 10%. Preliminary evidence suggests that maps generated using an intensive household survey provided a more granular picture compared to the EAG surveys. However, risk surfaces based on all three venue types led to a similar stratification of burden. The ability to quantify these biases and validate the use of EAG surveys to generate malaria risk maps has important implications for malaria control and elimination decision-making.

## 998

### THE IMPACT OF PRIMAQUINE (PQ) DEPLOYMENT AND INSECTICIDE TREATED UNIFORMS ON *PLASMODIUM VIVAX* INCIDENCE IN A PILOT MALARIA ELIMINATION STUDY IN CAMBODIA

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Elimination strategies targeting *Plasmodium vivax* (Pv) and its hidden hypnozoite reservoir remain elusive. We conducted a two-arm, cluster-randomized, open-label malaria elimination study, to determine the effectiveness of monthly malaria prophylaxis (MMP) with dihydroartemisinin-piperazine (DP) and weekly primaquine (PQ), against PCR-based monthly screening and treatment (FSAT), over a 6-month period. In the MMP arm (n=534), all volunteers over 12 years old were treated with weekly PQ of 22.5 mg for 12 weeks. In the FSAT arm (n=516), volunteers received radical cure with PQ only if Pv positive. Half of volunteers had permethrin treatment of uniforms (ITU), while the other half had sham treatment (sITU). Overall Pv failure risk during 6 months of follow up was 19%, 8%, 8%, and 12% in FSAT+sITU, FSAT+ITU, MMP+sITU and MMP+ITU, respectively. The MMP group had a PCR-corrected Pv prevalence of 9% on D0 which was highest in the MMP+ITU, high transmission area (12%). At the 6 month follow up, Pv prevalence decreased to 5%, further stratified to 3%, 3%, 10%, and 4% for MMP+ITU (low endemic), MMP+sITU (low endemic), MMP+ITU

(high endemic), and MMP+sITU (high endemic) interventions, respectively. Volunteers in MMP group who were less than 13 years old and not treated with PQ (n=57) had Pv prevalence of 7% on D0 and 4% on month 6. In the MMP arm, Pv failure risk was 30% (95% CI: 17-43%) and 7.1% (95% CI: 4.9-10%) in volunteers with and without Pv on enrollment, respectively. In FSAT arm, risk of Pv infection in children under 18 years old (n=55) was low during 6 months of follow up, with only 3 cases reported. Interventions in FSAT+ITU, MMP+sITU, and MMP+ITU all showed benefit over FSAT+sITU with ARR of 11% (95% CI 5-18%), 11% (95% CI 5-18%), and 8% (95% CI 1-15%), respectively. However, effectiveness of presumptive antirelapse therapy was lower than expected despite the 3 months of weekly PQ in MMP arm. There was no benefit of ITU over drug therapy in MMP arm. In the FSAT arm, the additional benefit of insecticide-treated uniforms was only observed in high endemic area. Multidisciplinary approach is needed for targeting vivax malaria elimination.

## 999

### MOSQUITO DIRECT MEMBRANE FEEDING ASSAY: OVERCOME THE FIELD CONSTRAINTS AND ADAPT THE METHOD FOR THE EVALUATION OF MALARIA TRANSMISSION-BLOCKING INTERVENTIONS

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The Direct Membrane Feeding Assay (DMFA) is one of the main methods for measuring human to mosquito malaria transmission. This method is routinely used to evaluate the transmission blocking interventions (TBIs) for malaria control. However, DMFA can be limited by some field and lab constraints. It is usually considered that gametocyte containing blood must be provided to mosquitoes as fast as possible after withdrawal to insure infectivity. This imposes that the volunteers come to the lab which can be distant from their living location. This constitutes a logistic challenge or even an obstacle in some cases. Here we aimed at determining the timeframe between withdrawal of gametocyte containing blood and mosquito blood meal that allows relevant mosquito infection for subsequent experiments. We also tested the effect of blood transportation on *Plasmodium* infectivity in mosquitoes. For each replicate, blood was collected from a gametocyte carrier in heparinized tubes and assigned to one of 5 treatments: immediately used for mosquito feeding (0H) or; kept at 37°C in the lab and provided to mosquitoes 2H, 4H, 6H or 8H afterwards. Fully fed mosquitoes were dissected 7 days-post feeding and oocysts were counted to compare infection levels in the mosquito's groups. Moreover, the effect of blood transportation was tested by comparing infectivity of gametocyte-infected blood either kept at 37°C in the lab or placed in a moving car for 1 to 3 hours before mosquito blood feeding. We found a significant time effect on both the proportion of successfully infected mosquitoes (infection prevalence: X24=73.5, P<0.001) and on the number of oocyst (infection density: X24=42, P<0.001). The relationship of these two traits with time followed a bell-shaped curve with maximal infectivity at 2 to 4h post blood drawing. Also, we detected no blood transportation effect on the gametocyte infectivity (X22=3, P= 0.2176). These findings suggest that delaying and transporting blood for few hours before mosquito blood meal may not prevent the infectivity. Further studies are required to confirm this pattern and explore the proximate mechanisms.

## 1000

**QUANTITATIVE POINT OF CARE G6PD TESTS FOR RADICAL CURE OF VIVAX MALARIA**

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Currently, the only drugs that can completely cure a patient of *Plasmodium (P.) vivax* parasites (radical cure), thus reducing the risk of relapse, are those in the 8-aminoquinoline family, such as the registered drug primaquine. This class of drugs presents a safety risk to subjects with glucose-6-phosphate dehydrogenase (G6PD) deficiency. G6PD deficiency is an X-linked disorder that affects more than 400 million people worldwide. Moderate to severe life-threatening hemolytic anemia episodes can develop if G6PD-deficient individuals are treated with 8-aminoquinoline drugs. This risk represents a major barrier to wide-scale adoption of radical cure. Therefore, determination of a malaria patient's G6PD activity level is critically important before 8-aminoquinoline drug therapy. We have evaluated novel G6PD-Hb integrated quantitative test prototypes that simultaneously measure red blood cell G6PD and hemoglobin (Hb) in whole blood in a point-of-care setting with results available in 2 to 5 minutes; we have also evaluated qualitative tests. Analytical performance of the tests has been evaluated following the criteria of the target product profile. Analytical sensitivity and accuracy have been measured against the reference assay over the critical G6PD activity dynamic range.

## 1001

**TARGETED-REACTIVE CASE DETECTION AT SLEEPING SITES TO INTERRUPT MALARIA TRANSMISSION IN VIETNAM I. RISK BEHAVIORS ASSOCIATED WITH MALARIA CASES SLEEPING IN A FARM OR FOREST**

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Reactive case detection (RACD) has limited impact and high costs to identify malaria cases in low endemicity areas where cases sleep outside their home. In Vietnam, over 60% of malaria cases sleep either in forests or on farms. We piloted a targeted-RACD approach to collect information from participants at their sleeping sites and identify risk behaviors associated with malaria cases in a low malaria transmission setting. A cross-sectional study was conducted in three mountainous communes in Phu Yen province, 2016. An index case was defined as someone who routinely slept in forest or farm and positive for malaria using rapid diagnostic test or microscopy. A list of index cases was obtained from three commune health centers. All index cases and neighbors from huts within 500m were interviewed face-to-face at their sleeping sites. Logistic regression models were used to calculate prevalence odds ratios (PORs) and 95% confidence interval (CI) for risk factors after adjusting for socio-demographic characteristics. Of 110 index cases, 82% were males with a mean age of 36.6 years; illiteracy proportion was 23%. Among 93 participants who slept in the forest, index cases were less likely to use treated bed nets (adjusted-POR=0.10; 95% CI 0.02–0.58), and more likely not to use any net when sleeping (POR=2.95; 95% CI 1.26–6.92). Index cases were also more likely than neighbors to sleep in huts without walls or outdoors (POR=44.0; 95% CI 13.0–148), and to work after dark

(adjusted POR=6.33; 95% CI 1.92–20.9). A significantly higher proportion of forest-based index cases worked in natural resource occupations (hunting, trapping) (POR=11.7; 95% CI 4.37–31.2), than did neighbors. Among 204 respondents who slept on a farm, the proportion using treated nets and no nets were not significantly different between index cases and neighbors. A significantly higher proportion of index cases were involved in planting or logging on farms (POR=2.74; 95% CI 1.27–5.91), than were neighbors. Targeted education and malaria prevention strategies can be developed to address the specific risk factors identified for forest and farm workers, particularly for illiterate group.

## 1002

**MALARIA PARASITEMIA AND SEROLOGICAL PREVALENCE IN NEAR-ZERO TRANSMISSION SETTINGS IN SENEGAL**

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Senegal has made enormous progress in the fight against malaria and is close to elimination in certain regions. Passive incidence of malaria cases is the only metric available in near-zero transmission settings, and other methods to assess potential interruption of transmission at the sub-national level are needed. In Richard Toll, Northern Senegal, four health post catchment areas with zero or very low malaria incidence ( $\leq 3$  confirmed, locally-acquired malaria cases in the last two years) were selected. A community-based cross-sectional study was conducted in 1,200 randomly selected individuals (400 aged 1-4 years old, 400 aged 5-9 years old, and 400  $\geq 10$  years old) to estimate prevalence of *Plasmodium falciparum* infection by rapid diagnostic test (RDT), microscopy and PCR, sero-prevalence of anti-AMA-1 and MSP-1 antibodies, and serological conversion rate. To assess whether an operationally easier convenience survey could be used instead, a survey in a convenience sample of 400 children 1-4 years old (recruited at health facilities and during community health outreach activities in the same areas) was also conducted, and prevalence of infection and sero-prevalence results were compared. Prevalence of *P. falciparum* by RDT and microscopy was zero in both the community and the convenience surveys. PCR analysis is ongoing and results will be available by mid-2017. Preliminary data from the community survey show that respective MSP-1 and AMA-1 sero-prevalence increased with age, being 0.8% and 0.5% in 1-4 years of age, 3.1% and 3.3% in 5-9 years, 3.5% and 7.9% in 10-19 years old, and 32.2% and 40.8% in  $\geq 20$  years old. In the convenience survey the sero-prevalence in 1-4 years old was 2.1% both for MSP-1 and AMA-1. Serological conversion rates will also be estimated. Preliminary results suggest that there is almost zero transmission in the study areas—mirroring the passively-detected malaria incidence—and that transmission intensity decreased around 20 years ago, and has remained low since that time.

## 1003

### ZAMBIAN MALARIA RAPID REPORTING SYSTEM: VARIATIONS IN DATA QUALITY ACROSS HEALTH FACILITIES IN SOUTHERN PROVINCE

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As part of increased investment in surveillance, Zambia's National Malaria Elimination Centre has implemented strategies designed to improve the timeliness with which malaria incidence is communicated to the national level. The Malaria Rapid Reporting (MRR) system uses inexpensive mobile phones to transmit malaria indicator data from health facilities on a weekly basis. To understand how well information transmitted through this system reflects what is captured in health facility registers, audits were conducted reviewing 24-week periods in both 2014 and 2015 among 172 and 165 health facilities, respectively, in Southern Province. Program review of audits described data quality across 4 dimensions—completeness, timeliness, reporting, and accuracy—aggregated at the district level. Further insight can be gained from this information by understanding how data quality indicators vary at a more granular scale—i.e., at the health facility level—and what factors are associated with reporting data quality. To this end, this analysis aims to describe the variation in MRR data accuracy, completeness, and reporting rates across health facilities, assess the degree of spatial clustering of poorly performing facilities, and determine associations of other non-spatial characteristics with data quality. Accuracy of reports varied from 100% to 0% (med: 87.5%, IQR: 65.3% - 94.6%) across facilities. Facility reporting ranged from 100% to 0% (med: 97.9%, IQR: 91.7% - 100%). Analysis of variance demonstrated significant differences in average facility reporting accuracy between districts ( $F=1.77$ ,  $p<0.05$ ), and initial review of the spatial distribution of reporting accuracy suggests clear spatial clustering. Further analysis will describe spatial clustering of MRR data quality and assess characteristics of health facilities—such as distance from the district health office, facility type, facility case load, and total malaria incidence—associated with individual indicator and overall accuracy, reporting rates, and completeness of data reported through the MRR system, and thereby help direct efforts to improve malaria surveillance.

## 1004

### MALARIA CONTROL IN MIGRANT LABORERS WORKING IN AGRICULTURAL FARMS IN METEMA REGION, ETHIOPIA: CURRENT PRACTICES, FEASIBILITY AND ACCEPTABILITY OF NEW MALARIA INTERVENTIONS

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Every year, an estimated 400,000 seasonal migrant workers move to large-scale agricultural farms in western Amhara—where malaria transmission is moderate—and then return home, mostly to lower-transmission areas. They represent a high-risk group for malaria infection during their stay at the farms, and risk bringing malaria back to their home villages (20% were

infected upon return in a pilot study). To design malaria control strategies, we conducted a mixed methods evaluation to describe the current malaria control interventions at farms, and assess the feasibility and acceptability of implementing other strategies at the farms and upon workers' return. All 285 agricultural farms in Metema district were mapped and a survey was conducted in a subset of 102 farms randomly selected to collect information on farm operations, malaria risk-related behaviors, and current malaria control. Focus group discussions with laborers and key informant interviews with farm managers and health stakeholders were also conducted. Migrants begin arriving in the region in July-August and most stay through November-December. The living conditions are poor and the majority sleep outdoors or in thatched shelters. Only 25% of the farms use malaria control strategies (18% in smaller farms and 67% in larger farms), of which 80% provide bednets, 8% spray sleeping quarters with insecticide, 36% conduct source reduction activities, and 4% offer traditional repellents. Formal onsite or offsite healthcare is available at 30% of the farms (15-33% in smaller farms and 67% in larger farms), but most ill laborers seek presumptive antimalarial treatment from the farm managers instead. Most stakeholders indicated the need to provide healthcare at the farms either through mobile or stationary clinics. Most of the migrants and farm managers would be willing to use other control tools (such as bednets) if they were distributed for free, or would participate in drug-based interventions (such as mass test and treat) at the farms or upon arrival at their home village. Thus, with appropriate funding, malaria control could greatly improve the laborers' and their families' health.

## 1005

### DATA USE FOR DECISION-MAKING THROUGH DATA MONITORING POSTERS IN KRIBI CAMEROON

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Malaria is the leading cause of morbidity and mortality in Cameroon, where an estimated 500,000 cases occur every year and led to 55% of hospitalizations and 241 deaths among pregnant women in 2010. In order to measure the long-term impact of malaria prevention and treatment interventions at the facility level through outcome indicators, Jhpiego developed a data analysis process using an *affiche de monitoring* or data monitoring poster, which includes indicators on case management, suspected cases tested, intermittent preventive treatment for pregnant women (IPTp) and use of long-lasting insecticide-treated net (LLINs). Jhpiego and the National Malaria Control Program (NMCP) identified inadequate and irregular data collection and data use as a systemic problem throughout Kribi district. In response, Jhpiego developed and implemented training sessions on the data posters that focused on: the context and rationale for this type of data visualization; techniques for data collection, analysis, and interpretation for decision-making; and practical sessions enabling health providers to practice mapping data onto the posters. In September 2015, Jhpiego introduced the posters in 26 health facilities in Kribi that were already trained in malaria prevention and case management interventions. Jhpiego then provided blank copies of the data posters and supported sites via biannual supervision visits during which they reviewed data posters for accuracy against facility registers. Furthermore, supervisors assessed facility's progress on their objectives, identified gaps and their causes, and discussed corrective actions. As a result of Jhpiego's efforts by June 2017, 61% of the trained facilities use the data poster for decision-making. With the introduction of the posters, the percentage of health facilities that did not experience stock shortages increased by 17 percentage points, from 21% in October 2016 to 38% in March 2017. Additionally, the Ministry of Health requested Jhpiego to lead a training of 181 health administrators and providers aimed at scaling-up the use of the data posters across all 9 districts of the South Region of Cameroon.

## 1006

### PREVALENCE OF MALARIA AND ANEMIA AMONG PATIENTS ATTENDING REFERENCE HEALTH CENTER IN NIORO DU SAHEL, MALI, WEST AFRICA

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Despite several studies on malaria in the south of Mali where its transmission is seasonal from June to October, there is scarcity of data on malaria prevalence in the Sahelian part of the country, where its transmission is short and seasonal. We carried out this study during three annual transmission seasons (specify months and years) in order to assess both malaria and anemia in NiORO-du-Sahel located in the Sahelian area in the north-west of Mali. We conducted a cross-sectional study at the Reference Health Centre of NiORO-du-Sahel, where 1090 all-cause, patients selected randomly of any age were seen during medical visit and enrolled after obtaining consent. Capillary blood was collected for determination of malaria parasitemia and hemoglobin levels. Malaria infection and anemia proportion (Hb<11g/dL) among all patients examined was 45.3% and 38.6%, respectively. Fever was the main reason for medical visit, accounting for 61.8%. Self-reported ITN use was 83.3%. Participants with parasitemia  $\geq 10,000$  Parasites/ $\mu$ L represented 16.8%. Severe anemia (Hb < 7g/dL with parasitemia >0) was observed in 12.3% of study participants. Both malaria (61.2%) and anemia (55.6%) frequencies were higher in patients <18 years old compared to those  $\geq 18$  years old (35% and 25%, respectively,  $p < 0.05$ ). The lowest malaria infection prevalence was found among adults >37 years (32%;  $P < 0.05$ ). Anemia was significantly higher among children less than 5 years compared to adults aged 28 – 37 years ( $P < 0.05$ ). Malaria prevalence among ITN users was 43.8% compared to 56.4% observed in non-ITN users ( $P = 0.003$ ). Malaria infection peak was observed in October (56.1%;  $p < 0.001$ ) while the peak of anemia was in November (58.8%;  $P < 0.001$ ). Malaria infection was significantly associated with anemia among patients presenting in NiORO du Sahel ( $r_2 = 0.83$ ;  $P = 0.043$ ). In conclusion, despite high ITN coverage, malaria infection remains common among patients presenting to the main health centre in NiORO-du-Sahel. Anemia was also prevalent, particularly in children below 5 years, and was significantly associated with malaria infection.

## 1007

### HAS SEASONAL MALARIA CHEMOPREVENTION DECREASED THE MALARIA BURDEN AMONG CHILDREN UNDER FIVE YEARS IN SENEGAL?

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Senegal, a West African country, has made great strides in malaria control during the last decade, but its four southeastern regions continue to suffer a high malaria burden. In 2014, Senegal introduced seasonal malaria chemoprevention (SMC) - treatment with sulfadoxine-pyrimethamine and amodiaquine monthly during rainy season, for children aged 3 -120 months in the four high transmission regions, reaching  $\geq 95\%$  of the approximately 625,000 eligible children annually. The Ministry of Health also increased access to care, particularly for children under five years, through a variety of initiatives, and increased access to malaria diagnostic testing beginning in 2014. We analyzed routine malaria information system data, provided by all publicly supported health facilities, for the three regions that received SMC and for which completeness of

reporting was at least 95%, for the period from 2013 (the year before introduction) to 2016. Data were analyzed for children under 5 years and for non-pregnant patients 5 years and older. From 2013 to 2016, the reported annual incidence of malaria among children < 5 years increased 6%; however, total curative outpatient consultations for children < 5 years increased 63%, the number tested for malaria increased 117%, the proportion of all outpatient curative consultations associated with confirmed malaria (proportional morbidity) decreased from 15% to 10%, and the test positivity rate decreased from 66% to 32%. Among non-pregnant patients 5 years and older, reported annual incidence increased 48% from 2013 to 2016; however, total curative outpatient consultations increased 40%, the number tested for malaria increased 90%, the proportional morbidity did not change (18% to 19%), and the test positivity rate decreased from 72% to 56%. While assessment of SMC results based on reported annual incidence is complicated by increasing care seeking and diagnostic testing, particularly among children under 5 years, proportional morbidity and test positivity rate have declined substantially among children under 5 years compared to older patients, suggesting decreased malaria burden among this age group.

## 1008

### PREVALENCE OF PARASITEMIA DURING TWO SEASONS IN AN AREA RECEIVING SEASONAL MALARIA CHEMOPREVENTION (SMC) IN NIGER

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Seasonal malaria chemoprevention (SMC) is recommended in the Sahel: monthly courses of sulfadoxine-pyrimethamine and amodiaquine (SPAQ) are given to children aged 3-59 months during the high transmission season. In some areas, SMC has been expanded to include children up to 10 years old. We performed two malaria prevalence surveys to evaluate the burden of parasitemia in the expanded SMC target age group in Niger, aiming to estimate the prevalence of parasitemia during two different transmission seasons in among those 3-59m, 5-9y, and  $\geq 10y$ . We performed household-based surveys in October and December 2016 in Magaria District, Niger. (October is traditionally the end of the high season, and late December is traditionally the beginning of the low season.) The target sample size was 396 households in October and 266 households in December, because of the expected lower prevalence of parasitemia in December. Households were randomly selected with probability proportional to population size of the village. One household member in each age group was selected randomly for participation. Thick and thin smears were prepared, and slides were read by two microscopists. Overall prevalence of parasitemia was 43% [95%CI: 39-48] in October and 40% [37-44] in December. In both surveys, prevalence was higher among 5-9y than among 3-59m: 66% [58-74] vs. 52% [45-59],  $p = 0.005$ , in October; 64% [56-71] vs. 48% [41-55],  $p = 0.002$ , in December. Among  $\geq 10y$ , prevalence was 24% [19-29] and 21% [17-26], respectively. Gametocytemia was present in 19% [15-24] of children <10 in October and 13% [9-17] in December, with no difference between age groups ( $p = 0.81$  in October,  $p = 0.83$  in December). Parasite burden was higher in 3-59m than in 5-9y (geometric mean 4663 [3123-6962] vs 1404 [989-1995] ( $p = 0.001$ ) in October; and 1545 [1061-2250] vs 614 [453-832] ( $p = 0.001$ ) in December). In conclusion, prevalence of parasitemia among 3-59m was elevated despite SMC. Children aged 5-9 years in this area have a high prevalence of parasitemia and gametocytemia, though their overall parasite burden is lower than in younger children. They would potentially benefit from receiving SMC.

### GEOGRAPHICAL, TEMPORAL AND SEASONAL TRENDS IN *PLASMODIUM OVALE* AND *P. MALARIAE* INFECTIONS IMPORTED TO THE UK BETWEEN 1987 AND 2015

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*Plasmodium ovale* and *P. malariae* are less common than *P. falciparum* and *P. vivax* and cause milder clinical infection. Nevertheless, they are both important causes of febrile illness in endemic regions and in returning travellers. Using national surveillance data collected between 1st January 1987 and 31st December 2015 by Public Health England's Malaria Reference Laboratory (MRL), we describe the epidemiology of *P. ovale* and *P. malariae* infections imported to the UK. By collating this with data from the International Passenger Survey and climatic data from East and West Africa, we describe geographical, temporal and seasonal trends over this period. 52,242 cases of malaria were notified to the MRL during this period of which 6.04% (3157) were caused by *P. ovale* and 1.61% (841) by *P. malariae*. Most affected travellers had acquired infection in West or East Africa; those infected in West Africa had principally visited friends and family whilst those infected in East Africa had travelled for holidaying purposes. Mortality was rare at just 0.03% (1) and 0.12% (1) in patients with *P. ovale* and *P. malariae* respectively compared to 0.62% in those with *P. falciparum* infection. The time between arrival in the UK and onset of symptoms (latency) was 76 days for *P. ovale* and 18 days for *P. malariae*, reflecting the existence of hypnozoites in the former. Interestingly, there was seasonal variation in latency of *P. ovale* infections imported from West Africa with shorter latent periods in those arriving in the UK during the West African monsoon season (July to November, 49 days) than in those arriving at other times of the year (December to June, 92 days,  $P=0.0001$ ). This trend was not seen in *P. ovale* imported from East Africa or in patients with *P. malariae* infection. Between 1987 and 2015, infection rate fell five fold, from 0.62 to 0.13/1000 travellers to West Africa and 0.19 to 0.038/1000 travellers to East Africa. These data suggest that West African *P. ovale* is adapted to relapse during the West African monsoon. This is of evolutionary benefit to the parasite, offering the greatest chance of onward transmission to a new host in the period of maximal mosquito density.

### 1010

### A LONGITUDINAL STUDY OVER THREE YEARS LEADS TO THE IDENTIFICATION OF *PLASMODIUM VIVAX* INFECTIONS IN DUFFY BLOOD GROUP NEGATIVE CHILDREN IN BANDIAGARA, MALI

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*Plasmodium vivax* was thought to only infect the red blood cells of Duffy blood group positive people particularly in Asia and South America. In the last decade, *P. vivax* has appeared in Africa in areas such as Madagascar and Ethiopia, where Duffy positive and negative people live side-by-side. We sought evidence of *P. vivax* infections in a cohort of 300 children in

Bandiagara, Mali, West Africa (a Sahelian area of Mali where people are primarily Duffy negative) from the beginning of one transmission season through a second season and into the beginning of a third season.

We used quantitative PCR of blood samples dried onto filter paper to determine the prevalence of *P. vivax* and *P. falciparum*. We observed the occurrence of *P. vivax* in 25 children in Bandiagara, Mali. Duffy negativity was confirmed in all 25 children by Sanger sequencing the single point mutation (T to C) in the GATA1 binding region 5' prime of the Duffy blood group antigen open reading frame. The prevalence of *P. vivax* infection was 2.0-2.5% at every time point (June 2009 to June 2010). While experiencing *P. vivax* infections, none of the children had a history of fever, chills, headache or muscle ache, which are symptoms that may be associated with malaria. The parasite densities were extremely low between 8 and 157 parasites per microliter. The present data indicate that at some time in the past, *P. vivax* has adapted to infect Duffy-negative people in Africa which could become a burden to sub-Saharan Africa in future. Hence, the evidence of *P. vivax* existence needs to be taken into consideration in designing malaria control and elimination strategies in Africa.

### 1011

### GROWTH TRAJECTORIES OF CHILDREN IN SEASONAL AND PERENNIAL MALARIA TRANSMISSION SETTINGS

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Pediatric growth curves and velocities can provide an overall clinical impression at the individual level, but little is known about variation in individual z scores related to malaria infection. To determine whether there was an impact of malaria infection on the z score we used the anthropometric measurements in the Immuno-Epidemiology cohort in Oulessebouyou, Mali. We contrast these data with measurements from the previously described Mother-Offspring Malaria Study (MOMS) cohort data from Muheza, Tanzania, to compare growth trajectories in both seasonal and perennial transmission settings. In the Immuno-Epidemiology cohort 1,764 children were followed from birth to 5 years from 2011-2016, with an average of 26 measurements per child over the study period. The sex distribution of the cohort was 49.6% males and 50.3% females. Anthropometric measures of weight, length, height, head and arm circumference for both cohorts were compared with the WHO Child Growth Standards using the WHO Anthro macro version 3.2.2 for SAS, published January 2011, to define growth velocity and z scores by age and sex for each individual in the cohort. Head circumference was directly comparable between the cohorts. In the seasonal malaria transmission setting of Oulessebouyou head circumference for age mean z scores was 0.03 (SD 1.19) for 0-5 month olds, -0.12 (SD 1.10) for 6-11 month olds, -0.23 (SD 1.09) for 12-23 month olds, -0.31 (SD 1.05) for 24-35 month olds, -0.31 (SD 1.04) for 36-47 month olds, and -0.24 (SD 0.99) for 48-60 month olds. In the MOMS cohort head circumference for age mean z scores were 0.47 (SD 1.20) for 0-5 month olds, 0.76 (SD 1.02) for 6-11 month olds, 0.63 (SD 0.97) for 12-23 month olds, 0.61 (SD 0.94) for 24-35 months, and 0.83 (SD 0.90) for 36-74 month olds. The findings of this study highlight the differences in anthropometric measures between the two cohorts and describe differences in growth trajectory related to malaria infection.

## 1012

**DETERMINANTS OF MALARIA PARASITEMIA AMONG CHILDREN UNDER 5 IN NIGERIA: AN ANALYSIS OF THE DRIVING FORCES THAT INFLUENCE PARASITEMIA**

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Results from 2015 Nigeria malaria indicator survey (MIS) showed a drop in prevalence of malaria in reference to the 2010 survey. The survey showed regions with high levels of Parasitaemia also have high levels of ownership and use of Insecticide Treated Nets (ITN). This brought about the question, what could be the cause of the high levels of Parasitaemia in the face of high utilisation of preventive methods, especially the ITN? Could there be other factors? A secondary data analysis of the 2015 Nigeria MIS was carried out among children aged between 6 to 59 months (n=5,766) tested for Parasitaemia using microscopy. Multivariate logistic regression was used to compare odds of having malaria among children who slept under ITN the night before the survey while controlling for other determinants like children's age in months, residence, mother's educational status, wealth quintile. Sleeping under an ITN has weak evidence of association on the odds of having malaria (OR=1.2, CI: 0.99-1.44). However, children living in the rural residence were faced with over 4 times the odds of having malaria (OR=4.3, CI: 3.18-5.83). Similarly, mother's educational level and household's wealth quintile showed decrease odds of having malaria with increasing educational level (OR=0.53, CI: 0.47-0.60) and wealth quintile (OR=0.56, CI: 0.51-0.61). After adjusting for Age in months, ITN Use among children Under 5, Mother's education in years and Wealth quintile, there was still strong evidence of association among children living in the rural residence and the risk of having malaria (OR=1.62, CI: 1.2-2.2). It can therefore be concluded that the use of ITN is not the main driver of malaria Parasitaemia in Children under 5. Low mother's educational level, low household's wealth quintile and living in rural areas have presented to be the main drivers of malaria Parasitaemia in Nigeria. These factors can be seen to be indicators of development, stressing the position that malaria is a disease of poverty and lack of general human development. The regions in Nigeria with high ITN ownership and use but still have high Parasitaemia qualify as the areas with these factors as described.

## 1013

**DISTRIBUTION OF MALARIA BURDEN BY TRANSMISSION STRATUM SENEGAL, 2016**

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Senegal, a West African country, scaled up malaria control interventions aggressively over the past decade, aiming for universal coverage with long lasting insecticidal nets and case management with rapid diagnostic tests and artemisinin-based combination therapy in health facilities and at the community level. Parasite prevalence in children under 5 years of age fell from 6% in 2008 to 1.2% in 2014. Malaria transmission varies greatly, increasing in intensity from north to south, and starting in 2014, Senegal introduced additional interventions targeted according to transmission intensity, implementing seasonal malaria chemoprevention and proactive community case management in high transmission zones, and reactive case detection in low transmission zones. We analyzed data reported in 2016 from every publicly supported health facility in all 76 health districts, including 35 hospitals, categorizing them by reported annual malaria

incidence, to assess the malaria burden in each transmission zone and to improve intervention targeting. Completeness of reporting was 99%; nationwide, the incidence was 23.6 cases / 1,000 residents (349,540 cases). Thirty three districts with malaria incidence less than 5 / 1,000 comprise 47% of the population and accounted for 6% of cases (20,390). Nineteen districts with incidence of 5 to 15 / 1,000 residents comprise 23% of the population and accounted for 9% of cases (30,488). Twenty-four districts with incidence more than 15 / 1000 residents comprise 30% of the population but accounted 85% of cases (298,682); among these are ten districts in which incidence was more than 100 / 1,000 residents, comprising only 8% of the population but 55% of cases (191,342). Almost half the nation lives in districts at pre-elimination status in 2016, highlighting the need for expansion of active case detection activities in all these districts. However, incidence remains high among a small population that accounts for the majority of cases despite high coverage of a package of interventions, requiring a better understanding of the local epidemiological context and more aggressive approaches to reduce the malaria burden.

## 1014

**STUDY OF PREGNANCY OUTCOMES IN ASSOCIATION WITH MALARIA AND CO-INFECTION WITHIN MYANMAR'S PUBLIC HEALTH SYSTEM**

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In Myanmar, no specific intervention for malaria prevention is recommended for pregnant women, primarily due to a lack of sufficient data on how malaria impacts maternal or newborn health in areas of low or unstable malaria transmission. To measure the incidence of adverse pregnancy outcomes and their association with malaria and intestinal parasitic infections, a prospective, multi-center, longitudinal study was embedded into the existing structure of public healthcare services for pregnant women in 12 villages of two rural townships in Myanmar. From these historically malaria endemic areas, eligible and consenting pregnant women were enrolled and followed throughout the perinatal period. Demographic and clinical data, including reproductive health history, were collected. Blood was obtained for hemoglobin measurement and malaria evaluation. Stool was tested for intestinal parasites. Pregnancy outcome data were analyzed for the prevalence of adverse events. 752 pregnant women were enrolled, among whom were detected 137 (18.2%) intestinal parasitic infections and 43 (5.7%) asymptomatic malaria infections; these contributed to 10 (1.3%) co-infections. 670 (89.1%) birth events were recorded; 161 (24%) mothers were noted to be anemic (hemoglobin concentration <10 g/dL) during pregnancy. There were 657 (98.1%) live births, 7 (1%) stillbirths, 72 (10.7%) preterm births, 68 (10.1%) operative deliveries, 15 (2.2%) low birth weight infants, and 10 (1.5%) small size for gestational age infants. There were no statistically significant associations between infections and adverse pregnancy outcomes, likely owing to insufficient statistical power to detect associations between low frequency events. We conclude that treatable infections and potentially correctable anemia are common during pregnancy in rural Myanmar, and that more expansive studies would be needed to characterize the contribution of malaria and co-existing parasitic infections to poor maternal or newborn health. Our experience also demonstrated that long-term research integrated into Myanmar's maternal-newborn public healthcare system is feasible.

## 1015

**THE HIGHLY VARIABLE EPIDEMIOLOGY OF BLACKWATER FEVER****G. Dennis Shanks***Australian Army Malaria Institute, Enoggera, Australia*

Blackwater fever is a massive hemolytic event usually occurring in the context of repeated falciparum malaria infections and intermittent quinine use. Its aetiology is poorly understood and it is rarely seen today. Historical epidemiological observations from the 20th century demonstrated a very wide range of blackwater fever in prisoners in Andaman Islands, refugees in Macedonia, canal workers in Panama, expatriates in Rhodesia and Second World War soldiers in New Guinea and West Africa. Rates of blackwater fever (BWF) per 1000 malaria cases varied over two orders of magnitude. Islands such as the Andaman Islands (0.5 BWF cases / 1000 malaria cases) and New Guinea (0.2 BWF cases / 1000 malaria cases) had lower blackwater fever rates than continental areas such as Macedonia (36.8 BWF cases / 1000 malaria cases) and Rhodesia (74.6 BWF cases / 1000 malaria cases). Panama Canal workers (6.4 BWF cases / 1000 malaria cases) and British soldiers in West Africa (8.3 BWF cases / 1000 malaria cases) had intermediate rates. During the Second World War, blackwater fever rates in British soldiers in West Africa and Australian soldiers in New Guinea differed by a factor of forty despite similar treatment regimens and falciparum transmission risks. Fifteen of the 25 blackwater fever cases reported by the US Army from the Southwest Pacific during the Second World War were in black soldiers suggesting a role for G6PD deficiency. Blackwater fever is a complex interaction between host erythrocyte, falciparum malaria and antimalarial drugs which only occasionally combines to cause massive intravascular hemolysis for reasons that remain obscure.

## 1016

**APPROPRIATENESS OF MALARIA DIAGNOSIS AND TREATMENT OF FEVER EPISODE ACCORDING TO PATIENT HISTORY AND ANTIMALARIAL BLOOD MEASUREMENT****Joanna Gallay<sup>1</sup>**, Emilie Pothin<sup>1</sup>, Dominic Mosha<sup>2</sup>, Martin Zuakulu<sup>2</sup>, Erick Lutahakana<sup>2</sup>, Laurent Decosterd<sup>3</sup>, Blaise Genton<sup>4</sup>

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Monitoring the impact of case management strategies in terms of use and compliance to RDT result as well as drug use is essential to evaluate the public health benefit they confer. Methodologies that rely on objective and standardized endpoints such as drug levels in the blood should be used. We evaluated diagnosis and treatment appropriateness in case of fever according to patient history and antimalarials blood concentration. The survey took place in 2015 in three regions of Tanzania with different levels of malaria endemicity. Community surveys were conducted to collect information on demographics, health seeking behavior and case management in case of fever. Blood samples were collected as dried blood spots for further antimalarials measurements by LC-MS/MS. Health facility surveys collected information on diagnosis and treatment practices. Appropriate testing was defined as a patient with history of fever being tested for malaria and appropriate treatment as having antimalarial in the blood if the test result was positive or if the person was not tested. In total, 6485 individuals were surveyed and 1344 (21%) had antimalarials detected in the blood. 1021 reported a febrile episode in the previous 2 weeks but only 664 (65%) of them sought care. 69% (172/248) of the individuals who sought care in health facilities were tested and 52% (130/248) appropriately treated. When other providers were sought, 6% (23/382) of persons were appropriately tested and 44% (169/382) appropriately treated. Results obtained through exit interviews in health facilities were close with 67% (151/226) of individuals tested and 66%

(149/226) appropriately treated. Overall, the proportion of individuals treated was larger than that being tested (45% (301/664) treated, 30% (196/664) tested). Only about half of individuals with detectable levels of antimalarials in the blood were those having a test result positive or not being tested. These results show that treatments are often not targeted to individuals that are sick with malaria. Efforts should be made to better follow WHO recommendations of systematic diagnostic testing and treatment upon result.

## 1017

**LONGITUDINAL SEROLOGICAL EVALUATION OF MALARIA TRANSMISSION PATTERNS IN BIKO ISLAND, EQUATORIAL GUINEA****Jackie Cook<sup>1</sup>**, Joseph Biggs<sup>1</sup>, Catherine Mair<sup>1</sup>, Dianna Hergott<sup>2</sup>, Aveika Akum<sup>3</sup>, Lou Herman<sup>1</sup>, Julie Niemczura de Carvalho<sup>2</sup>, Guillermo Garcia<sup>2</sup>, Christopher Schwabe<sup>2</sup>, Immo Kleinschmidt<sup>1</sup>, Chris J. Drakeley<sup>1</sup><sup>1</sup>London School of Hygiene & Tropical Medicine, London, United Kingdom,<sup>2</sup>Medical Care Development International, Silver Spring, MD, United States,<sup>3</sup>Medical Care Development International, Malabo, Equatorial Guinea

Monitoring and evaluation of malaria control programmes is a key element of refining intervention strategies. Alongside parasitological measures, serological markers of malaria transmission have been utilised to investigate impact, as well as to identify heterogeneities in effect. Three cross-sectional serological surveys have taken in place in 18 sentinel sites in Bioko Island, Equatorial Guinea, in 2008, 2012 and 2015, encompassing a period when the island was experiencing a decrease in transmission, due to extensive roll-out of indoor residual spraying (IRS) and insecticide treated nets (ITN). There was a sharp reduction in seropositivity to AMA-1 between 2008 and 2012 (from 52% to 35%) which remained similar between 2012 and 2015 (33%). Estimated seroconversion rates correlates strongly with parasitological data in all three years and the method was able to highlight differential impact of interventions between regions on the island, with several sites appearing close to elimination in 2015. Modelling of serological data can help to reconstruct historical transmission patterns and these three repeated surveys highlight the integrity of the method in an area undergoing changes in transmission patterns.

## 1018

**TRENDS AND SEASONALITY OF SEVERE MALARIA DEATHS IN RWANDA, 2007-2016****Jean Pierre Habimana<sup>1</sup>**, Alphonse Rukundo<sup>1</sup>, Nsengiyumva Divine<sup>2</sup><sup>1</sup>Private Firm working with Rwanda BioMedical Center, Kigali, Rwanda,<sup>2</sup>Kabgayi District Hospital, Muhanga/South Province, Rwanda

Malaria remains one of the most important parasitic disease causes of death, accounting for nearly three million annual globally. In Rwanda, 5% of deaths are due to severe malaria. We did this study to determine the trend in malaria deaths and to describe the weather seasonal patterns of severe malaria deaths during a ten-year period. Rwanda Health Information System (RHMS) contains monthly reports from District and referral hospitals. Severe malaria death was defined as death occurred in hospital with diagnostic of confirmed severe malaria. We used STATA for data analysis. Descriptive analysis was used to assess the trend in malaria deaths and a negative log-binomial regression was done to assess the relationship between weather seasons and malaria deaths. Relative Risks (RR) and 95% confidence intervals (CI) were calculated. A total of 5,880 patients died with a diagnosis of severe malaria from 2007 to 2016. Of the severe malaria deaths, 29% (1,696) were children less than five years. The total number of malaria deaths was 2,593 during 2007-2011 and 2,287 during 2012-2016, representing a decline of 11.8%. Comparing malaria deaths in children under-five for the years 2007-2011 and 2012-2016, malaria deaths declined by 37% (1042 to 654). Our study has shown also a relationship between seasonality in weather patterns. The short dry

season indicated the relative risk of all malaria deaths (RR=1.16, 95%CI: 1.04-1.29). In addition, the short dry season indicated the highest relative risk of malaria deaths in fewer than five years (RR=1.35, 95%CI: 1.17-1.60). In conclusion, Slight increase in malaria deaths was observed in five and above patients during the period of our study. A decline of mortality among children fewer than five years was observed during the same period of study. Results showed a correlation between malaria deaths and weather season. We recommend targeted early malaria case management interventions.

## 1019

### ASSESSMENT OF THE DYNAMICS OF *PLASMODIUM FALCIPARUM* PARASITEMIA REGARDING THREE ARTEMINININ COMBINATION REGIMENS FOR ACUTE UNCOMPLICATED MALARIA TREATMENT, BANFORA, BURKINA FASO

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The two recommended Artemisinin Combination based Therapy (ACTs: ) for the uncomplicated malaria treatment in Burkina Faso are suffering from the mass used from the Seasonal Malaria Chemoprevention strategy (Amodiaquine) and from the high frequency of the new infections cases with the Artemether-Lumefantrine Combination. Therefore the assessment of alternative effective combination treatment is highly recommended. In that context, a comparative, randomized, open label longitudinal clinical trial involving children and adults with uncomplicated *Plasmodium* sp. malaria was carried out. The study was a two years follow up involving 2 new ACT, Artesunate-pyronaridine (PYR) and Dihydroartemisinin-piperazine (DHA-PQ) compared to Artesunate-Amodiaquine (ASAQ) in the treatment of the uncomplicated acute *Plasmodium falciparum* malaria cases in Banfora, located at the southern part of Burkina Faso. Each of participant enrolled received during their subsequent episodes the same drug and went through the same trial procedures as for the initial episode. Sexual and asexual parasite prevalence were evaluated during 42-days follow-up to assess the dynamics of *Plasmodium falciparum* regarding each of the 3 ACTs. All treatment doses were supervised and administered according to patients' weight. A total of 763 persons aged from 6 month and above (mean  $\pm$  SD age = 7.3  $\pm$  5.9 years, median age = 5.5 years) were included in the study; 50.3% were female. The mean  $\pm$  SD number of malarial episodes occurring in a person's during the two years follow up was 2.9  $\pm$  2.1 episodes. The therapeutic efficacy rates (PCR-non corrected) were 93% for ASAQ, 97.3% for DHA-PQ, and 93.3% for PYR. Sexual and asexual parasitemia dynamics of each treatment regimen showed no statistically significant differences between the three Artemisinin Combination based Therapy (by log-rank test).

## 1020

### THE ECONOMIC BURDEN OF MALARIA CASES IMPORTED FROM HISPANIOLA TO OTHER NON-ENDEMIC COUNTRIES IN THE WESTERN HEMISPHERE (2007- 2013)

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Hispaniola is the only island endemic for malaria in the Caribbean region. Widening income disparity and natural disasters have hindered malaria control. Routine travel between Hispaniola and other non- endemic countries in the Western Hemisphere could pose a risk for the re-introduction of malaria in non- endemic countries. Given the paucity of

information on the cost of imported cases to non-endemic countries in the Americas, this study sought to estimate the cost and evaluate the economic burden of malaria cases imported from Hispaniola to non-endemic countries in Americas. Epidemiologic data on imported malaria cases came from reports from the US Centers for Disease Control and Prevention, Public Health Agency of Canada, the Canadian Malaria Network, the World Health Organization and the Pan American Health Organization. Calculation of costs per disability adjusted life-year (DALY), were based upon the WHO burden of disease estimates of DALY loss due to malaria in non- endemic countries in Americas, and the costs of diagnosis and treatment of malaria. During 2007-2013, the estimated number of malaria cases, imported from Hispaniola, to non-endemic countries in North America ranged between 30 and 192. Disease management costs varied between \$100,018 and \$1,229,320; the Cost/DALY range was \$121,377 to \$1,079,557. Between 2011 and 2013, 24 cases from Haiti reported in non-endemic Caribbean Islands; they cost \$53,076, with no loss of DALY reported. Understanding the economic and human impact of malaria on endemic countries as well as on their non-endemic neighbors helps strengthen the case for worldwide malaria elimination.

## 1021

### DIVIDE AND CONQUER: PARTITIONING MOSQUITO BITING HETEROGENEITY AND IDENTIFYING MALARIA HOTSPOTS FOR INTERVENTION

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Heterogeneity in malaria transmission varies with the intensity of transmission. A large portion of heterogeneity in transmission over time and space can be attributed to seasonality, individual household attractiveness, and environmental noise. With the ability to identify households which act as hotspots for sustaining a major portion of malaria transmission we can increase the efficiency of control interventions. This study focuses on mosquito count data from entomological surveillance conducted between October 2011 and September 2016 at three study sites in Uganda. A total of 330 households were involved in the surveillance. The intensity of malaria transmission varies considerably across the three sites. Using a Bayesian zero-inflated negative binomial model and a prior distribution for seasonal signals, we partitioned the heterogeneity in mosquito abundance into household biting propensities, seasonality, and environmental noise. For each study site, we contrasted household biting propensities in different scenarios - dry vs. rainy seasons; before the enrolment of indoor residual spraying vs. after; during the first half period of the surveillance vs. the second half period of the surveillance. We also conducted hotspot analysis using the Getis-Ord (Gi\*) statistic for all three sites to identify hotspots of malaria risk. This work provides an understanding of heterogeneity in malaria exposure and offers a critical appraisal of the possibility of targeting interventions at households with the most mosquitoes, which differ depending on various environments and various levels of endemicity. Once transmission in an area has decreased but is maintained in hotspots of malaria transmission, such targeted interventions are likely to become increasingly important tools in malaria elimination efforts. Focusing malaria interventions on households which contribute disproportionately to malaria transmission could achieve community protection by eliminating transmission in a relatively small fraction of human hosts.

### TRACKING MALARIA SLIDE POSITIVITY RATES IN 30 SENTINEL SITES ACROSS GHANA

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In Ghana, effective case management remains one of the main interventions for the achievement of the primary goal of malaria control, which is to reduce malaria-related morbidity and mortality. Key to the provision of effective malaria case management is adequate diagnosis, which is vital for assessing the impact of preventive strategies such as deployment and use of long lasting insecticide-treated Nets (LLINs), indoor residual spraying (IRS), intermittent preventive treatment in pregnancy (IPTp), and seasonal malaria chemoprevention (SMC). Over the years malaria diagnosis has largely been presumptive leading to poor case management and poor data on malaria parasite positivity rates, which is necessary for the assessment of the country's progress along the control-elimination continuum. It is in this light that the National Malaria Control Programme (NMCP), in collaboration with the Noguchi Memorial Institute for Medical Research (NMIMR), established sentinel sites across the country to generate data for monitoring malaria slide positivity rates (mSPRs). The surveillance activity aims at tracking mSPRs in 30 sentinel health care facilities across Ghana with 2014 as baseline. Thick and thin blood smears for microscopy are prepared for all 3rd suspected malaria cases attending the health care facility. Blood smears are stained with 10% Giemsa stain, dried and stored in plastic slide boxes for reading by experienced microscopists from NMIMR and the Ghana Health Service (GHS). Data generated over the past 3 years show a significant decline in the overall mSPR from 23.7% (95% CI: 23.3-24.1) in 2014 to 18.8% (95% CI: 18.5-19.1) in 2016 with regional variations. *Plasmodium falciparum* mono-infection remains the most prevalent infection-type in the country: 97.5% (95% CI: 97.2-97.8). The majority of slide-positive patients were under 5 years old: 37.5% (95% CI: 36.7-38.4). We conclude that malaria interventions over the years have had a significant positive impact on mSPR in Ghana.

### 1023

#### PREVALENCE OF ASYMPTOMATIC MALARIA INFECTION AND GLUCOSE-6-PHOSPHATE (G6PD) DEFICIENCY IN A *PLASMODIUM VIVAX*-ENDEMIC SETTING, LAO PDR: IMPLICATIONS FOR SUB-NATIONAL ELIMINATION GOALS

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Confirmed malaria case incidence reported through the health system in Northern Laos has declined rapidly over the past 5 years, and *Plasmodium vivax* now accounts for the majority of cases. As a result, the national malaria program is aiming for sub-national elimination of *P. falciparum* by 2020, and *P. vivax* by 2025. A cross-sectional survey was conducted in four districts of Northern Laos in Sept-Oct 2016 to determine the remaining prevalence of malaria parasite infection and the prevalence of genotypic G6PD deficiency. 1,500 households across 100 villages were selected using two-stage cluster sampling, and household heads interviewed using a demographic and risk factor survey. Rapid diagnostic tests (RDTs) and dried

blood spots were collected for all consenting individuals > 18 months. All blood samples were tested via PCR for parasitemia and a random subset of males genotyped for G6PDd. Out of a total sample of 5,082 individuals, zero infections were detected by RDTs; 39 infections were detected by PCR (weighted prevalence 0.77%; 95% CI: 0.40-1.47%), with 3 *P. falciparum*, 28 *P. vivax*, 2 *P. malariae*, 5 *P. falciparum/P. vivax*, and 1 *P. vivax/P. malariae* infections. Infection prevalence was greatest in adolescents and young adults, and similar by gender, with substantial clustering within households and villages. In preliminary analyses, lack of household bednet ownership [odds ratio= 31.9; (95% CI: 4.3 - 234.1),  $p = 0.001$ ] and any household members sleeping overnight in forest or rice field areas [OR= 3.4; (95 %CI: 1.1 - 10.2),  $p = 0.03$ ] were associated with PCR-positivity; G6PD genotyping is ongoing. The very low parasite prevalence suggests subnational elimination goals in Northern Laos are feasible, and case-based surveillance should be rapidly scaled-up to target interventions to remaining transmission foci. The higher prevalence of *P. vivax* infections highlights the critical need for G6PDd data for safe and efficacious deployment of primaquine towards elimination goals.

### 1024

#### GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6PD) DEFICIENCY GENETIC VARIANTS IN MALARIA PATIENTS IN SOUTHWESTERN ETHIOPIA

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Malaria remains a major public health concern in Ethiopia, affecting over five million individuals each year. *Plasmodium vivax* infections underlie a third of malaria cases and are often treated with primaquine. One disadvantage to primaquine use is the risk for hemolytic anemia in patients with the X-linked enzymatic disorder, glucose-6-phosphate dehydrogenase (G6PD) deficiency. This risk is increased when treating *P. vivax* infections, as these pathogens require a high primaquine dose. As primaquine becomes more widely used in Ethiopia, it is important to identify G6PD deficiency mutations in the population. In the present study, mutations in the *G6PD* gene are identified in a sample of patients with malaria in Jimma town in southwest Ethiopia. Polymerase chain reaction (PCR) and gel electrophoresis were used to detect *Plasmodium* species of infection from blood spot samples collected from malaria patients. For G6PD deficiency, PCR and Sanger sequencing were performed to observe a portion of the *G6PD* gene where the common G6PD mutations, A376G and G202A, are found. The majority of the samples were identified as single *P. vivax* infections (83.7%). For G6PD genotyping, A376G (rs1050829, A>G) was frequent in this study sample set (23.26%) while G202A (rs1050828, G>A) was absent. Three mutations were also identified at *G6PD* locations that have not yet been characterized for their association with G6PD enzyme activity. One mutation occurs in the coding region (rs782669677, G>A,) and two mutations occur in non-coding regions (rs370658483, G>T and a new mutation at chrX:154535443, C>T). Analysis of the functional properties of the three additional mutations are on-going. The data presented here add to the growing literature on the diversity of the *G6PD* gene in Ethiopia needed to inform policy on appropriate primaquine use in malaria control programs.

## 1025

**HIGH PREVALENCE OF CLINICAL MALARIA IN A POPULATION OF PREGNANT WOMEN LIVING IN LIBREVILLE, GABON**

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Pregnant women in malaria endemic areas are at high risk of *Plasmodium falciparum* infection and its complications. This study investigated for the first time in Libreville, the prevalence and risk factors for *P. falciparum* infection among pregnant women. We conducted a cross-sectional study from May and November 2015 at gynecological services of l'Hôpital d'Instruction des Armées Omar Bongo Ondimba (HIABO) of Libreville. For each pregnant women who was hospitalized for fever and non febrile ones seen during their antenatal care visit, socio-demographic, obstetrical and clinical data, history of fever and antimalarial treatment, use of IPTp-SP, and delivery outcome were recorded. Peripheral blood was collected for *falciparum* malaria detection by thick and thin blood smears and for hemoglobin concentration measurements. The association between the studied variables and malaria were analyzed by logistic regression. A total of 179 pregnant women were included in the study, 94 were febrile and hospitalized, 85 non-febrile and non-hospitalized. Nearly half, 47.5% were seen before the third antenatal care visit, 56.4% received IPTp-SP and 38.5% used bed net. The preliminary analysis shows that the prevalence of confirmed clinical malaria was 60.6% among the hospitalized pregnant women. The factors associated with malaria infection were a maternal age below 25 years old (71% vs. 52.9%), a high education level, a low number of ANC visits (93%), being at the second trimester of pregnancy (78%) ( $p < 0.01$ ). The median number of IPTp-SP doses was significantly low ( $p = 0.02$ ) and the frequency of women who did not used IPTp-SP ( $p = 0.02$ ) or bednet ( $p = 0.04$ ) were significantly high among infected women compared to those without malaria. In conclusion, the frequency of clinical malaria is high in febrile hospitalized pregnant women. It is associated with an infrequent use of preventive measures.

## 1026

**PROXIMITY OF HUMAN RESIDENCE TO IRRIGATION DETERMINES MALARIA RISK AT AN IRRIGATED AGRO-ECOSYSTEM IN MALAWI**

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Although malaria control interventions have led to a decline in transmission intensity, the disease continues to be a major public health problem in Malawi. Proximity of vector breeding sites to human residences affects malaria risk. Irrigation plays an increasingly important role in Malawi to improve food security and the rural economy. However, land transformation for irrigated agriculture could increase malaria vulnerability for those residing in proximity to irrigation schemes, negatively impacting the anticipated improvements in rural life. Therefore, we aimed to determine the effect of irrigated agriculture on variation in malaria risk in villages around Bwanje Valley rice irrigation Scheme. Two cross-sectional surveys were conducted at the end of the rainy seasons of April 2016 and 2017. Demographic and health information was collected for household members. Blood samples were obtained for malaria rapid test and microscopic identification of *Plasmodium falciparum*. Malaria risk was assessed by village clusters, defined by distance of village from irrigation scheme; within 3km or 3-6 km radius. Among 2,709 individuals aged greater than six months, prevalence of infection by microscopy was 33.2 % and was significantly in residents of villages within a 3 km

radius of the scheme (40.1%) compared to 3-6 km from the scheme (28.4%). Prevalence estimates by rapid diagnostic test were higher. Study participants living within 3 km radius from the boundary of the scheme were at significantly higher risk of malaria infection than were those living further away (aOR=1.7; 95% CI, 1.4-2.2). Most (90%) infected people were asymptomatic. Compared with the younger and older age groups, children aged 6-15 years had higher risk of malaria infection (aOR=3.2; 95% CI, 2.4-4.2), higher parasite densities, higher gametocytemia, and reported less frequent use of bed nets. We conclude that proximity of human residence to irrigation in Malawi increases malaria risk. Additionally, among the community members, school-age children share the greater burden to malaria infection.

## 1027

**EVALUATING THREE YEARS OF A TARGETED INDOOR RESIDUAL SPRAYING (IRS) CAMPAIGN IN A HIGH TRANSMISSION AREA OF NORTHERN ZAMBIA**

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Evidence-based approaches are needed to meet malaria control and elimination goals in endemic countries. The World Health Organization has identified vector control as a key strategy to reduce transmission, however limited resources and surveillance capacity remain a challenge in regions with the greatest need. An increase in malaria cases has been reported in Luapula Province, northern Zambia since 2007 despite consistent reductions nationwide. In response, a large-scale indoor residual spraying (IRS) campaign was conducted using novel methodology to target high-transmission areas through household satellite mapping and health center malaria reports. Annual targeted spraying was done prior to the rainy season in 11 districts in Luapula Province starting in 2014 using the organophosphate insecticide Actellic. A multi-year evaluation of the IRS campaign was conducted in Nchelenge District, Luapula Province using active malaria surveillance data collected by the Southern Africa ICEMR project. From April 2012-March 2017, 3,345 participants from 1,030 households participated in cross-sectional surveys in sprayed and unsprayed areas, with an overall parasite prevalence by RDT of 50%. Fifty five percent of households within IRS-targeted areas reported being sprayed with Actellic. In multivariate regression analyses controlling for demographics, weather, and geographic factors, there was a 25% reduction in rainy season malaria prevalence by RDT within the IRS-targeted area compared with the same geographical area pre-intervention ( $P < 0.001$ ) but no decrease in dry season malaria prevalence. However, malaria prevalence in adjacent unsprayed areas increased each year throughout the period of surveillance, and a difference-in-differences analysis to compare pre- vs. post-intervention malaria prevalence between sprayed and unsprayed areas revealed a significant effect of IRS for both rainy and dry seasons ( $P = 0.02$  for each). Targeted IRS using Actellic had a moderate but measurable impact on parasite prevalence in Nchelenge District, but more intensive interventions are needed to achieve Zambia's malaria elimination goals.

### A SITUATIONAL ASSESSMENT OF THE DRIVERS OF MALARIA IN COMMUNITIES ALONG THE ZIMBABWE-MOZAMBIQUE BORDER OF MANICALAND PROVINCE

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In Zimbabwe (ZIM), the 3 provinces bordering Mozambique (MOZ) reported 86% of malaria cases and 60% of malaria deaths. The President's Malaria Initiative supported the Government of Zimbabwe's National Malaria Control Program (NMCP) to assess the potential drivers of malaria transmission in Manicaland on the ZIM-MOZ border. Methods included a household (HH) survey (n=522), interviews with individuals (n=850), key informants (n=55), cross-border travelers and traders (n=106), MOZ patients seeking care in ZIM (n=138) and facility and community medical records review (n=2,331). Findings indicate that neighboring Manica (MOZ) and Manicaland (ZIM) Provinces are not equally prioritized to receive malaria interventions. Interviews suggest that cross-border collaboration efforts initiated by the leadership from the two provinces over the years have not progressed to concrete implementation steps. Just over 50% of the 850 individuals interviewed reported hearing messages on malaria prevention during the preceding 6 months. About 64% of HHs surveyed had long-lasting insecticide nets (LLIN), although only 38% reported using LLINs the previous night, and the number of nets per HH was insufficient for sleeping spaces. About 65% of HHs surveyed reported indoor residual spraying in the previous year. 37% of the border population reported regular cross-border travel. Less than 20% of travelers reported using personal protection when crossing the border. Outpatient registers do not collect travel history, so the extent of imported malaria cases wasn't determined. ZIM cross-border travelers with malaria often seek care at ZIM health facilities; as do MOZ nationals (4% of malaria cases reviewed). The assessment illuminated intervention coverage gaps on the ZIM side. Communities on both sides of the border are dynamic and interactive, thus the ZIM NMCP should continue coordinate and collaborate with MOZ counterparts. Enhanced social and behavior change communication would benefit border communities on both sides. Cross border traders and travelers form a significant group at risk and should be deliberately targeted for malaria interventions.

### 1029

#### THE EFFECT OF DROUGHT ASSOCIATED INDICATORS ON MALARIA IN SOUTHERN ZAMBIA

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Malaria is an infectious disease endemic to Zambia transmitted by anopheline mosquitoes. Due to their key role in transmission, malaria incidence is strongly influenced by environmental variables (e.g. vegetation and elevation) and climatic variables (e.g. rainfall and temperature). Drought strongly affects most of these variables. While previous studies have examined associations between climatic factors and malaria, few have considered drought measures such as soil moisture or drought index as predictors of malaria. Furthermore, when considering the lag effects of weather-related variables on malaria incidence, most studies assumed lagged weather at one time. However, weather occurring over an interval of time within a lag might better correlate with the biology and ecology of vector mosquitoes and hence have a stronger association with malaria

incidence. Diagnosed weekly malaria incident cases from 14 rural health centers (RHCs) in Southern Province, Zambia were recorded between 2012 and 2016. All predictor parameters were remotely sensed except relative humidity and daily rainfall, which were recorded using a weather station. Weekly data from all 14 RHCs were aggregated for analysis. Optimal point temporal lags and interval lags were determined using cross correlation maps. Univariate and multivariate negative binomial regressions were performed to determine the best predictors of malaria counts. To account for nonlinear relationships between malaria counts and predictor variables, models using splines were also considered. Drought index and soil moisture were significantly associated with malaria counts; however, the best predictors were rainfall, night temperature, and normalized difference vegetation index. These three variables could be used in future studies of the impact of drought on malaria transmission. Point lag and lag interval models resulted in similar prediction quality. Accounting for the nonlinear association of night temperature and malaria counts improved prediction quality but an increase in malaria counts in 2016 remained underestimated, due to suspected differences in rainfall effects.

### 1030

#### OUTBREAK OF MALARIA IN UBON RATCHATHANI, THAILAND (2012-2015)

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Thailand has made significant progress reducing its malaria cases by 85% between 2000 and 2015, but the burden of disease remains a challenge in areas along the country's borders. Moreover, evidence of anti-malarial resistance has been observed on the Thai-Cambodian border, making prevention and control efforts for malaria less effective. As Thailand aims to eliminate malaria by 2024, it is necessary to have a detailed and up to date understanding of local disease patterns and possible drivers of transmission. In 2014, there was a large outbreak of malaria in Ubon Ratchathani Province in northeast Thailand. This outbreak has not been studied in detail but could have important implications for other malaria eliminating countries. A detailed analysis of routine incidence data over space and time was conducted to examine the spatial and temporal epidemiology of malaria in this province. Malaria surveillance data were obtained from the Thai Ministry of Public Health from 2012 to 2015. Subdistrict-level analyses of numbers of confirmed malaria cases by species and annual parasite index were produced. Data were explored for any relationships between numbers of cases and other factors such as temperature and rainfall. There were 15,459 malaria cases of all species reported between 2012 and 2015. The majority were men (92%), and of Thai nationality (96%). In 2014, a 6.8-fold increase in both *Plasmodium falciparum* and *Plasmodium vivax* cases was observed compared to the previous year from 2.6% of the national total in 2013 to 22.9% in 2014. Peak incidence was in June and May 2014, with most of the increase being in three neighboring districts of Buntharik, Na Chaluai and Nam Yuen, all geographically sharing borders with southern Lao PDR and northern Cambodia. This large outbreak was very focal, and although it was not possible to determine a definite cause from available data, cross-border importation and travel into the forest may have played a role. Field studies are underway to examine the current situation in more detail.

## 1031

### DEMOGRAPHIC AND REGIONAL RISK FACTORS FOR MALARIA-ASSOCIATED HOSPITALIZATIONS IN WESTERN AND COASTAL KENYA

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Malaria is a major cause of morbidity and mortality in Kenya. Children can present with diverse clinical manifestations ranging from asymptomatic parasitemia to severe malaria and death. It is unclear why some individuals progress to severe disease. Risk factors for severe malaria could be linked to the host, parasite, socio-economic status and/or environmental factors. We enrolled children aged 1 to 17 years who presented with fever (Jan 2014- present) at each of four Kenyan study sites: Chulaimbo (rural) and Kisumu (urban) in Western Kenya, and Msambweni (rural) and Ukunda (urban) on the coast. All patients were tested for malaria by light microscopic examination of peripheral blood smears and detailed questionnaire data were collected on demographics, education level, socioeconomic status, and household environment, along with full clinical history and physical examination. "Severe" malaria cases, defined as those requiring hospitalization due to malaria, were compared to "mild" malaria cases, defined as those sent home after the clinic visit. Incidence of malaria in the cohort by blood smear was 42.1% (1,393/3,310). The majority of cases (98.0%) were due to *P. falciparum*, and the remaining were due to *P. ovale* (0.6%) and *P. malariae* (1.3%). Of the 1,393 cases, 94 (6.75%) were classified as having severe malaria. Severe cases were significantly more common in western Kenya (59.6% vs. 40.4%  $p < 0.001$ ), were younger (median age=4 vs. 5 years;  $p < 0.001$ ) and were more likely to have a history of prior malaria (95.7% vs. 71.0%;  $p < 0.001$ ) than "mild" malaria cases. No association was found between risk of severe malaria and family wealth, maternal education level, hygiene, or mosquito prevention indices. This study highlights the remaining large burden of malaria in young children, especially in western Kenya, and the critical need for more vector-borne disease control and prevention resources targeting this vulnerable population for severe malaria.

## 1032

### SPATIOTEMPORAL EPIDEMIOLOGY OF MALARIA IN THAILAND 2012-2015

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Thailand has a plan to eliminate malaria by 2024 and has made significant progress reducing the number of cases from around 150,000 in 2000 to 25,000 in 2015. Over the past few years, most malaria cases were found along the borders with Myanmar, Lao PDR and Cambodia with many cases thought to be imported. In addition, resistance to first-line antimalarial drugs has been identified in different parts of Thailand and there is concern that this could derail efforts to reaching elimination by 2024. To support these elimination efforts, it is essential to have a highly effective surveillance system to provide a detailed understanding of the disease patterns over space and time, as well as information on risk factors and groups at higher risk of malaria. Working in partnership with the Ministry of Public Health (MOPH), detailed routine malaria surveillance data from the whole of Thailand from 2012 to 2015 were analysed and mapped at subdistrict level including calculation of annual parasite index (API) using data on population, identification of areas of highest risk and

the stability of these risk areas over time. In addition, detailed climate data from the Thai government to assess the relationship between climate and incidence. The primary goal was to generate results to support elimination efforts by the MOPH. Malaria incidence was found to be highest in four provinces (59% of total cases during 2012-2015) along international borders: Tak, Kanchanaburi, Yala (Thai-Burmese border) and Ubon Ratchathani (Thai-Cambodia-Lao PDR border). *Plasmodium vivax* was the predominant species in three of these provinces, with falciparum being commoner in Yala. The majority were male (42%), aged 5 to 19 years and of Thai (66%) or Burmese nationality (25%). The incidence and risk maps produced will be combined with population movement data from travel surveys and analysis of anonymized aggregated mobile phone call detail records to determine the effect of population movement on the distribution of malaria in the country and internationally.

## 1033

### PROACTIVE COMMUNITY TREATMENT OF CHILDREN UNDER-5: RESULTS OF A PILOT PROJECT IN NORTHERN BENIN

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The ProActive Community Treatment (ProACT) model uses weekly household visits by community health workers (CHWs) to detect and treat illnesses like malaria in children. ProACT had not been tested in a year-round high malaria transmission country like Benin. The National Malaria Control Program worked with Peace Corps volunteers and the US President's Malaria Initiative to implement a ProACT pilot in four villages in Northern Benin. The villages were economically, demographically, and culturally similar; all were located  $\geq 5$  km from a health center. CHWs identified children with malaria or diarrhea during "sweeps" conducted weekly (two villages) or intermittently (at 0, 8, and 16 weeks; two villages). Children were evaluated and treated or referred for treatment per existing CHW protocols. During the pilot, CHWs diagnostically confirmed and treated 95 malaria cases among the 440 children under 5 years of age living in the four villages. Sixty-five cases were identified during sweeps (43 in villages with weekly sweeps, 22 in villages with intermittent sweeps). Illness identified during sweeps accounted for 64% of the total care provided. Weekly active detection identified substantial numbers of children with malaria and may have led to earlier treatment and/or treatment of cases that would have otherwise gone untreated. More rigorous evaluation is needed to determine ProACT's ability to reduce morbidity during high malaria transmission periods in Benin.

## 1034

### A TLR1 POLYMORPHISM INCREASES THE RISK OF VIVAX MALARIA IN SOUTHERN INDIA

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Malaria is considered the single most important selective force in the history of the human genome but the extent to which this notion applies to *Plasmodium vivax* is not well established. In Mangalore, Karnataka, southern India, we conducted an unmatched case-control study among malaria patients attending the largest governmental hospital and representatively selected community controls, and genotyped Toll-like

receptor 1 (TLR1) I602S and the MBL -221 X/Y promoter polymorphisms as exemplary members of the innate immune response. 960 patients with PCR-confirmed malaria (*P. vivax*, 69.4%; *P. falciparum*, 9.0%; mixed species, 21.4%) as well as 1017 community controls were included in analysis. The *TLR1* 602S variant was present in 13.3% of patients and 11.1% of controls. Adjusting for significant differences in age, sex, and migration status between cases and controls, the odds of malaria *per se* tended to be increased in *TLR1* 602S carriers (aOR, 1.32; 95%CI, 0.96-1.81), and it was significantly increased with respect to *P. vivax* mono-infection (aOR, 1.49; 95%CI, 1.05-2.09). MBL -221 X/Y had no such impact. We show that TLR1 is involved in the risk of vivax malaria in India. Ongoing work will show whether this applies to further members of the TLR and the innate immune system. Geographical variation of innate immunity polymorphisms may contribute to differing risks and manifestation patterns of malaria in India.

## 1035

### VARIATION AT THE VAR2CSA LOCUS: RESULTS FROM A CROSS-SECTIONAL STUDY IN DEMOCRATIC REPUBLIC OF CONGO

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*P.falciparum* affects over 1 million pregnancies in Democratic Republic of Congo (DRC) each year, placing both pregnant women and newborns at risk. The adverse effects of pregnancy associated malaria (PAM) are mediated by the binding of infected erythrocytes to the placental microvasculature via the VAR2CSA surface antigen. Using next generation deep sequencing, we categorized the diversity of the *var2csa* gene in 120 survey cluster sites from the 2013-4 Demographic and Health Survey in DRC. We found that *var2csa* is highly variable. We identified 583 different haplotypes, only about 1/3 of which were found in 2 or more clusters. Our results indicate that there is extensive connectivity and gene flow across large parts of DRC, combined with balancing selection maintaining diversity at this locus. These results impact on how we perceive and manage parasite populations within DRC, and ultimately our ability to develop an effective vaccine against PAM.

## 1036

### WITHIN-VECTOR PARASITE DIVERSITY: INSIGHTS FROM PLASMODIUM FALCIPARUM DEEP WHOLE-GENOME SEQUENCING FROM FIELD-CAUGHT MOSQUITOES IN NORTHERN ZAMBIA

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In regions of moderate and high malaria transmission where infectious mosquito bites occur frequently, human infections with *Plasmodium falciparum* typically comprise multiple clones. Because parasites undergo

sexual recombination within the mosquito, cross-fertilization between genetically distinct parasite gametes may occur more frequently than inbreeding, possibly contributing to the observed genetic diversity of the parasite population. Most parasite genomics studies have focused on monoclonal infections in human samples to characterize transmission intensity and model the impact of interventions, and few empirical studies characterizing parasite diversity and relatedness within mosquitoes exist. Better understanding of the extent of within-vector parasite diversity, genetic relatedness, and which parasite clone(s) are transmitted to humans, may aid reconstruction of transmission chains and inform vector control strategies targeted to disrupt malaria transmission. To gain insights into parasite diversity within mosquitoes, we used a multiplexed hybrid capture technique to selectively enrich and deep sequence 20 whole genomes (WGS) of *P. falciparum* directly from field-caught mosquitoes from a high transmission setting in Nchelenge District in northern Zambia. Using WGS and genome-wide SNPs, we will infer parasite diversity and genetic relatedness resulting from recombination and different bottleneck and expansion events across these *P. falciparum* samples from mosquitoes. We anticipate that population genomic studies of mosquito-derived *P. falciparum* samples, coupled with spatially and temporally paired human samples, could greatly aid understanding of transmission dynamics in malaria endemic regions by identifying hotspots of parasite diversity and clarifying the role of mosquitoes in modulating parasite diversity at the population level.

## 1037

### MATCHED PLACENTAL AND PERIPHERAL BLOOD PARASITES ARE GENETICALLY HOMOLOGOUS AT THE VAR2CSA ID1-DBL2X LOCUS BY DEEP SEQUENCING

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In pregnancy-associated malaria (PAM), infected erythrocytes (IEs) accumulate in the placenta. It is unclear if in polyclonal infections, which are common in natural infections, placental sequestration of IEs results in distinct peripheral and placental parasite populations. We used long amplicon deep sequencing of *Plasmodium falciparum* *var2csa*-ID1-DBL2X, the 1.6kb region encoding the minimal binding epitope to human CSA, in 15 matched peripheral and placental samples at delivery (i.e. a total of 30 samples from 15 women) in a high transmission area of Benin. We aimed to determine the within-patient genetic homology between sample pairs. Despite substantial sequence diversity (580 variable sites of 1738 total nucleotide sites, including INDELS) and within-host allele frequencies as low as 1%, the majority of infections (28/30, 93.3%) were monoclonal or had a predominant haplotype. The mean multiplicity of infection (MOI) in peripheral and placental samples was 1.4 (range = 1-2) and 1.6 (range = 1-5), respectively. Pairs mostly contained the same genetic variants, with 11/15 pairs sharing 100% of their haplotypes, while other pairs showed some heterogeneity. These findings suggest that at the time of delivery, placental parasites are not a selected subset of those in the periphery, and the parasites in the two compartments appear to intermix. Whether this intermixing reflects that cytoadherent variants are at a selective advantage over non-cytoadherent genotypes will be discussed. In conclusion, these data show that the placental population can be inferred through peripheral blood sampling.

## 1038

**EVALUATING CROSS-BORDER MALARIA TRANSMISSION BETWEEN ZAMBIA AND THE DEMOCRATIC REPUBLIC OF CONGO: A PARASITE GENETICS APPROACH**

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In Zambia, the national goal to eliminate malaria by 2020 is threatened by potential carriage of parasites across borders in human or mosquito hosts. Cross-border malaria transmission was evaluated using parasite genetics between Nchelenge District, Luapula Province (malaria RDT+ prevalence 30-50%) in northwest Zambia and two villages, Kilwa and Kashobwe, across the border from Nchelenge District in the Democratic Republic of Congo (DRC) (malaria RDT+ prevalence 47%). Randomly selected households in Nchelenge, Kilwa, and Kashobwe were visited in June and July 2016 and consenting individuals provided a dried blood spot (DBS) for detection of *Plasmodium falciparum* by PCR and genetic analysis. Among the DBS which tested positive for *P. falciparum* by PCR, 41 samples from Nchelenge, 39 from DRC, and 15 positive controls (spiked with known concentrations and varying ratios of laboratory *P. falciparum* strains NF54, 3d7, and 7g8) were extracted and prepared for amplicon deep sequencing at the CSP, var2csa, and AMA1 loci. By analyzing haplotype diversity and genetic relatedness between human-derived samples from Nchelenge District and the DRC, the extent of gene flow between parasite populations will be estimated to indicate the extent of cross-border malaria transmission. This research will inform regional control programs on the need to coordinate malaria elimination efforts, particularly along border regions.

## 1039

**EUPATHDB: POWERFUL DATA-MINING TOOLS FOR EXPLORING THE BIOLOGY OF HOST-PATHOGEN INTERACTIONS**

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The Eukaryotic Pathogen Database (EuPathDB, <http://EuPathDB.org>) is a free, online data mining resource facilitating the discovery of meaningful biological and clinical relationships from large volumes of genomic and epidemiological data. This platform places the power of bioinformatic analysis in the hands of the scientific community, supporting hypothesis-driven discovery research by providing guided queries and powerful tools for data mapping and analysis in a graphical web-interface. EuPathDB supports 170+ species including pathogenic protists (*Amoebae*, *Cryptosporidium*, *Giardia*, *Leishmania*, *Plasmodium*, *Trypanosoma*, etc), fungi (*Aspergillus*, *Cryptococcus*, *Candida*, etc), and related non-pathogenic species. EuPathDB integrates a wide range of omics-scale data types, applies standard bioinformatics workflows, and creates orthology profiles and domain predictions. Data exploration and interrogation proceed via direct examination of record pages representing genes, pathways, study subjects, etc; dynamic visualization of sequence-based data aligned to the genome; analyses such as functional enrichment or Galaxy workflows; and a sophisticated Search Strategy system with

>100 pre-configured searches that can associate diverse evidence with specific hypotheses. EuPathDB makes it easy to interrogate biological questions relating to stage-specific expression, gene model integrity or alternative splice variants, etc, and to compile lists of genes that share multiple biological characteristics (e.g. kinases secreted at a particular time, where they may affect host responses). This platform has recently expanded to incorporate data from Systems Biology programs (including the Malaria Host-Pathogen Interactions project) and clinical studies (e.g. the International Centers of Excellence in Malaria Research, and the GEMS and MAL-ED projects supported by the Gates Fdn Enteric and Diarrheal Diseases program). Active user support includes an email help desk (Help@EuPathDB.org), social media, YouTube tutorials, and a global program of workshops. Please visit our poster or exhibitor hall booth for a demonstration.

## 1040

**A LARGE-SCALE GENETIC SCREEN OF PLASMODIUM FALCIPARUM IDENTIFIES GENOTYPY-PHENOTYPE MUTATIONS AFFECTING TOLERANCE TO FEBRILE TEMPERATURES**

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Intermittent fever is a characteristic clinical feature of malaria that reflects the synchronized cyclical development of blood-stage parasites. Importantly, malarial fever is lethal to blood-stage *Plasmodium falciparum*, except for early intraerythrocytic ring stages that can tolerate febrile temperatures. Because parasite survival of febrile temperatures represents an essential adaptive mechanism, we seek to understand the pathways and processes associated with parasite tolerance of febrile temperatures through a forward-genetic approach using ~1000 unique *piggYbac* (*pB*) mutants of *P. falciparum* NF54. Our experimental and computational approaches rely on Quantitative Insertion-site Sequencing (QISeq) to quantify differences in growth of *pB* mutants before and after phenotype selection. QISeq is a next-generation sequencing method that counts each *pB* insertion within a mutant pool and in this study enabled tracking of individual *P. falciparum* mutations affecting sensitivity to heat-shock stress. The *in vitro* phenotypic selection was a heat-shock stress assay used to simulate malarial fever by exposing mutant parasites to 41C for 8 hours. First, we optimized the heat-shock screen SOP with a pilot clone library of 128 extensively characterized unique *pB* mutant clones and then scaled up to a library containing ~ 1000 unique *pB* mutants. We identified ~100 *pB* mutants that have strong positive phenotypes adversely affected by heat-shock stress. Analysis of genes linked to the heat-shock mutants and RNAseq of febrile-sensitive mutants indicated that genes associated with protein folding, unfolded protein binding, and protein trafficking were the most dysregulated in response to febrile temperatures. Additionally, these data suggest that terpenoid biosynthesis in the apicoplast coupled with thiamine synthesis might be a uniquely required process of primate malaria parasites to cope with febrile stress. Large-scale application of the approach used in this study offers the potential for broader functional annotation of the *P. falciparum* genome for the genetic factors associated with other important phenotypes.

## G6PD DEFICIENCY IN CHILDREN IN AN AREA ENDEMIC FOR MALARIA IN BENGO PROVINCE, ANGOLA

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Glucose 6-phosphate dehydrogenase (G6PD) is the rate-limiting enzyme of the pentose phosphate pathway, important in the protection of cells against oxidative stress. The G6PD deficiency is the most common enzymopathy X linked worldwide. The majority of the G6PD deficient do not manifest any symptoms, however, acute hemolytic anemia may be triggered by several agents, such as primaquine. Current WHO guidelines state that in elimination areas a single 0.25 mg base/kg primaquine dose should be given, as a gametocytocide, to all patients with parasitological-confirmed *P. falciparum* malaria on the first day of treatment in addition to an ACT. In face of this recommendation, endemic malaria countries should be informed of the prevalence G6PD deficiency, in order to make safe and appropriate decisions regarding the use of potentially unsafe drugs for G6PD deficient individuals. The aim of this study is to determine the prevalence of G6PD deficiency in an holoendemic region in Africa for *P. falciparum*, evaluating the genotype and the phenotype of the enzyme. This is a longitudinal prospective cohort study, involving 1692 children. The G202A, A376G genotypes were determined through Real Time PCR methods. For the enzyme activity NeolISA kit was used for Neonatal screening of G6PD deficiency. The prevalence of the G6PD A- allele was 19.4%, with 19% of hemizygous males and 4.5 % of homozygous females. Moreover 22.2% and 8,2% of heterozygous B/A- and A/A- heterozygous females respectively were observed. The enzyme activity was low in G6PD deficient in both sexes and with statistical significance from A and B alleles respectively for boys (1, 66U/gHb [0.55 -2.76] p < 0.001) and for girls (0, 97U/gHb [0.32 -1.62] p < 0.003). There seems to be a protection against malaria in heterozygous girls, since the lowest number of confirmed cases (7.7%) was observed in the class B/A- in a 18 months period. The prevalence of G6PD deficiency among children in Bengo is considerable and is similar to that found in other parts of Africa. This data, can be used by MoH in order to make safe and appropriate decisions regarding the use of potentially unsafe drugs for G6PD deficient individuals.

## EFFECT OF PLASMODIUM FALCIPARUM CRT SINGLE NUCLEOTIDE POLYMORPHISM AND PLASMEPSIN 2-3 COPY NUMBER INCREASE ON EX VIVO PIPERAQUINE RESISTANCE IN P. FALCIPARUM ISOLATES FROM NORTHWESTERN CAMBODIA, 2012-2015

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*Plasmodium falciparum* resistance to piperazine (PPQ) emerged rapidly across western Cambodia over the last 5 years, leading to high rates of dihydroartemisinin-piperazine (DHA-PPQ) treatment failure. Molecular correlates of PPQ resistance would allow timely surveillance, important for monitoring the efficacy of DHA-PPQ outside Southeast Asia and helping to determine if and when DHA-PPQ could be reintroduced in the region. We are investigating the relationship of *ex vivo* PPQ susceptibility amongst 162 Cambodian isolates collected in Anlong Veng, northwestern Cambodia, to copy number variation of the *plasmepsin 2-3* gene and single nucleotide polymorphisms in the *Pfcrtr* gene, including a *Pfcrtr* F145I mutation recently implicated in DHA-PPQ treatment failure. Isolates were collected pre-treatment from two cohorts, the first enrolled in Dec 2012-Feb 2014, the second between Dec 2014-Sep 2015. Median PPQ IC90, measured using an HRP2-based assay on fresh isolates, rose from 104 nM (IQR: 65 – 368) in the earlier cohort to 837 nM (IQR: 113 - 12050) in the later cohort. Thus far, real-time PCR and whole genome sequencing of 78 isolates from the earlier cohort show that 38 (49%) have multiple copies of *plasmepsin 2-3*, 11 (14%) contain the F145I mutation, and 8 (10%) have both the mutation and increased *plasmepsin* copy number. When PPQ IC90 is dichotomized at a cut point of 200uM, a previously determined pharmacologically relevant dose, increased *plasmepsin 2-3* copy number is associated with an odds of increased IC90 more than six times that of the odds in those with only one copy (OR = 6.6, 95% CI = 2.2 - 22.8, p = 0.001). Preliminary SNP analysis suggests the presence of the F145I mutation is also associated with increased mean IC90 (6513 nM vs. 258 nM). This study confirms the previously described association between increased *plasmepsin 2-3* copy number and increased IC90, and supports an additional role for the *crt* F145I mutation in PPQ resistance. Future analyses will define the independent and joint effects of these molecular correlates on PPQ resistance.

## QUANTIFYING VAR GENE EXPRESSION IN UNCOMPLICATED MALARIA INFECTIONS USING WHOLE GENOME SEQUENCE DATA

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*Plasmodium falciparum* erythrocyte membrane protein-1s (PfEMP1s), expressed on the surface of infected erythrocytes, play a critical role in immune evasion by mediating cytoadhesion and sequestration in host capillaries. PfEMP1s are encoded by *var* genes, with each parasite genome containing ~40-60 copies. Sequencing and assembling these genes has been a challenge due to extreme sequence diversity and motif repetition. Full *var* repertoires are known for only a few reference genomes and clinical isolates, and this lack of knowledge has posed a challenge for studying *var* gene expression, particularly in clinical samples. We previously sequenced *var* gene repertoires from symptomatic uncomplicated malaria infections in 12 Malian children. To measure *var* expression in these samples, we first evaluated the ability of published degenerate primer sets targeting the hypervariable DBL $\alpha$  region to optimally capture these repertoires. We complemented this primer set with additional degenerate primers predicted *in silico* to maximize the number of *vars* amplified from the total pool of ~1092 available *var* sequences. To identify predominantly

expressed *vars* in these uncomplicated malaria infections, we extracted RNA from all 12 samples, generated cDNA via reverse transcription, and amplified *vars* from each sample using our comprehensive primer set, barcoded by sample. The resulting amplicons were pooled in equimolar amounts and deep-sequenced via Illumina to measure the abundance of *var* transcripts identified by this degenerate primer set. We report the identity of *vars* and relative proportions in each sample. In addition, classes of *var* genes expressed in these uncomplicated infections were identified and compared to results from previous *var* expression profiles in different clinical presentations in this population. This approach provides an in-depth analysis of *var* expression in clinical infections using a fully sequenced *var* repertoire and may provide insights into further use of next generation sequencing to better understand parasite variant surface antigen expression.

## 1044

### GENETIC DIVERSITY OF *PLASMODIUM FALCIPARUM* BASED ON *MSP-1* BLOCK2 GENEPOLYMORPHISM ANALYSIS IN ISOLATES FROM TSARATANANA COMMUNE, IFANADINA DISTRICT, SOUTHEAST OF MADAGASCAR

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Malaria drug efficacy tests are indispensable for malaria control in endemic countries. In Madagascar, ASAQ is the antimalarial therapeutic recommended by the National Malaria Control Program (NMCP). In contrast to Asia and Africa, no resistance to Artesunate + Amodiaquine (ACT) so far has been detected proving for ACT to be an efficient treatment. In this study, we were interested in the *Plasmodium falciparum* merozoite surface protein 1 (*mSP1*) genetic diversity among symptomatic patients enrolled during a therapeutic efficacy test in the southeast of Madagascar in April 2016. The genetic diversity was analysed in 65 isolates from Tsaratanana commune in the Ifanadina district. Children between six months to fifteen year-old and only *P. falciparum* infected were included. After DNA extraction from blood spot, allele frequency and diversity of *mSP1* block2 genes within this population were investigated by nested polymerase chain reaction. Over the 65 samples, 89.23% were identified with *mSP1* genes including 49.23% and 40% of single and multiple infections respectively. The multiplicity of infection (MOI) was 1.5. A same MOI value of 1.4 was found for patients between 6 months and 5 year-old and from 6 to 15 year-old. Among the single infections, 33.84%, 9.23% and 6.15% corresponded respectively to K1, MAD20 and RO33 allelic types. As observed worldwide, the K1 allele is always present in single infections but also in the mixed infections among the 65 Malagasy isolates. Within the mixed infections, the K1+MAD20 allelic types were preponderant with 15.38% followed by the K1+RO33 genotype with 12.30%, MAD20+RO33 genotype with 7.69% and K1+Mad20+RO33 genotype with 4.61%. This genetic diversity study showed the presence of seven allelic *mSP1* block2 types in total in this malaria endemic area with the K1 dominant genotype. K1 genotype has been found to be the major one in Africa. Based on antigenic marker, this study illustrates the presence of 7 parasitic strains circulating in this endemic area.

## 1045

### GENOME-WIDE SCAN OF GENE LOCI UNDER POSITIVE SELECTION IN IMPORTED *PLASMODIUM VIVAX* FROM CHINA-MYANMAR BORDER AREA

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High importation risk from Myanmar and wide distribution of malaria vectors in the China-Myanmar border region sustain risk for secondary infections among local populations. High recombination rates of *Plasmodium vivax* populations associated with varied transmission intensity might cause distinct local selective pressures. The information on the genetic variability of *P. vivax* in this area is scant. Our study assessed the genetic diversity of *P. vivax* genome sequence in CMB area aim to provide information on the positive selection of new gene locus. Blood samples were collected from 6 clinical malaria cases that are microscopically positive for *P. vivax* and PCR confirmed for single infection. Our sequencing generated an average of 120M paired-end reads of 125 bp and 188,757 SNPs after filtering. We found that considerable genetic diversity and mean pair-wise divergence among the sequenced *P. vivax* isolates are higher in some important gene families. Using the standardized integrated haplotype score (IiHS) for all SNPs in chromosomal regions with SNPs above the top 1% distribution, it was observed that the top score locus involved 356 genes and most of them are associated with red blood cell invasion and immune evasion. The XP-EHH test was also applied and some important genes associated with anti-malarial drug resistance were observed in high positive scores list. This result suggest that *P. vivax* in CMB area is facing high pressure and this has led to the strong positive selection of genes that are associated with host-parasite interactions. Our study suggests that greater genetic diversity in *P. vivax* from CMB area and the positive selection signals in invasion and drug resistance genes are consistent with the history of drug use during malaria elimination programme. Furthermore, our work also demonstrates that haplotype-based detecting selection can assist the genome-wide methods to identify the determinants of *P. vivax* diversity.

## 1046

### COMPLEX GENOMIC EVOLUTION OF INSECTICIDE RESISTANCE IN THE MAJOR AFRICAN MALARIA VECTOR *ANOPHELES FUNESTUS*

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Insecticide resistance in malaria vectors such as *Anopheles funestus* is hindering malaria prevention throughout Africa. To help design suitable insecticide resistance management (IRM) strategies, we elucidated the dynamics and genetic basis of resistance across Africa revealing a complex genomic evolution of resistance Africa-wide. Susceptibility surveys showed that resistance including against pyrethroids, the only class recommended for bed nets, is spreading with increasing cases of multiple resistance and higher resistance intensity. Lab and field studies revealed that increased resistance is reducing the efficacy of control tools with PBO-based nets providing better efficacy. Significant contrast in the gene RNAseq expression profiles of resistant mosquitoes is observed between African regions suggesting differences in the molecular basis of resistance. For example, the P450s CYP6P9a/b are dominant in southern Africa but not in other regions where other genes operate. This variation correlates with high genetic differentiation between regions and the distribution patterns of resistance markers suggesting that barriers to gene flow impact the spread of resistance. Whole genome sequencing detected signatures of selective sweeps but these varied by regions as for gene expression. Fine-scale analysis of key resistance genomic regions revealed a complex evolution of resistance mechanisms with evidences of various copy number

variations suggesting independent selective events across the continent. Analysis of pre and post-intervention samples reveals that selective sweep is likely caused by the scale up of control tools which are selecting alleles the most metabolically efficient in conferring resistance. The near fixation of such alleles in populations would limit options for IRM.

## 1047

### STUDY ON MOLECULAR MARKERS ASSOCIATED WITH DIHYDROARTEMISIN-PIPERAQUINE AND OTHER DRUG RESISTANCE IN *PLASMODIUM FALCIPARUM* POPULATION IN BINH PHUOC PROVINCE, VIETNAM 2015-2016

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Artemisinin-based combination therapies (ACT) have been recommended by WHO as first-line treatment for uncomplicated *Plasmodium falciparum* infections in all malaria endemic areas. Recently, there were the worrying sign that the efficacy of ACT have been declined in Mekong Delta regions especially in Binh Phuoc, Vietnam. Artemisinin-resistance in *P. falciparum* has been proved that associated with mutations in K13-propeller gene. There were new molecular markers related to piperazine resistance: (a) plasmepsin 2 gene copy number variation (CNV) and (b) mutation E415G in exonuclease encoding gene (Exo-E415G). (c) the chloroquine (CQ), sulphadoxine and pyrimethamine (SP) resistance associated with multidrug resistance gene 1 (pfmdr1), dihydrofolate reductase gene (pfdhfr) and dihydropteroate synthetase gene (pfdhps). An understanding of those genetic factors that determine how resistant parasite emerges and spreads is necessary. From 2015 to 2016, 84 blood samples were collected from uncomplicated *P. falciparum* patients in Binh Phuoc province. Mutations of K13 propeller domain gene, exonuclease gene are detected by sequencing method and plasmepsin 2 gene copy number is determined by qPCR. Among the samples, C580Y mutation in the K13 propeller domain showed the highest frequency with 71.4% (60/84). Multicopy of plasmepsin 2 gene was detected in 25% (21/84) of the samples, while single copy was found in 36.9% (31/84) of them. The Exo-E415G mutant was found 67.8% (57/84). The others were the frequency of the single mutant Y184F in pfmdr1 gene was 65.78% (25/38) and there are no mutations detected at N86Y and D1246Y. The prevalence of IRNL quadruple-mutant haplotype accounted for 74% (27/38) and was more popular than IRNI triple-mutant, 26% (10/38) in pfdhfr. The triple-mutant GNGA haplotype was much more common (61%) than the double mutant GEAA (39%) in pfdhps gene. Identification of those marker genes and its frequency in population of *P. falciparum* in Vietnam will support surveillance efforts to contain, prevent the artemisinin resistance and facilitate the development of effective strategy to combat drug resistance.

## 1048

### MALARIA IN HAITI: A GENOMIC APPROACH TO ITS EPIDEMIOLOGY AND BIOLOGY

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The malaria parasite (*Plasmodium falciparum*) was introduced into Hispaniola through the slave trade when, during a 300 year period ending in the early 1800s, ~10 million Africans were brought to the Caribbean. It is likely that a substantial percentage of these individuals carried the parasite, since malaria is holoendemic in West Africa. Hispaniola is now the only island in the Caribbean where malaria is still actively transmitted. Haiti occupies the western half of the island and has the bulk of malaria cases with epidemics occurring during rainy seasons. We hypothesize

that in establishing transmission through its new vector, *Anopheles albimanus*, the parasite population underwent several bottlenecks driven by powerful selection pressures. Timing the founder events of the parasite, and understanding its underlying population dynamic in Hispaniola, represents a unique opportunity to examine the evolution of *P. falciparum*. We have undertaken a detailed study of the genetics of the parasite population utilizing whole genome sequencing. Principal component and phylogenetic analyses based upon single nucleotide polymorphisms (SNPs) indicate that the Haitian parasites have, as expected, an ancestral relationship with parasites from Africa, but they clearly belong to a phylogenetic lineage distinct from the ones found in South America. Loci under selection, representing mutations allowing *P. falciparum* to adapt to its novel vector and environmental conditions on Hispaniola, were mapped using maximum likelihood and Bayesian algorithms that take into account evolutionary relationships. Mutations in drug resistance loci and vaccine antigens, as well as those suited to tracking the movement of the parasite between countries, are under study and may be useful in support of elimination plans.

## 1049

### POPULATION STRUCTURE OF *PLASMODIUM FALCIPARUM* IS DETECTABLE AT SMALL SPATIAL SCALES IN KIHIFI, UGANDA

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Clustering of malaria incidence is apparent over various spatial scales. Evaluation of genetic relatedness of malaria parasites may be a useful tool for understanding transmission patterns, but may not be easily detectable in areas with moderate-high transmission. To evaluate the potential to detect genetic relatedness within a community with moderate-high malaria transmission, we measured relatedness of isolates from cohort participants recruited from 100 randomly selected households in the Kihifi sub-county of Uganda (annual EIR=27 infectious bites per person; area ~12x24 km). All infections detected via microscopy or *Plasmodium* specific loop mediated isothermal amplification from passive and active case detection during the first 6 months of the study were genotyped at 26 microsatellite loci, providing data for 301 samples from 267 participants living in 89 households. As expected, genetic diversity was high (mean He=0.73), and 85% of samples were polyclonal. Despite the high diversity, spatiotemporal structure in genotypes was detectable. Identical haplotypes were observed in 3 pairs of monoclonal infections; 1 pair was from a single household on the same day, and the other 2 were pairs living ~7km apart and detected within 2 weeks of each other. Pairwise genetic distance, identity by state (IBS), was calculated using the number of non-shared alleles at each locus weighted by the total number of alleles detected. Pairs of infections which were closely related genetically (IBS 0-0.2) were significantly more likely to have been collected from nearby locations than those with moderately (IBS 0.2-0.4) and more distantly related (mean distances of 4.6 km, 6.4 km, and 8.4 km, respectively, p=0.04 and <0.001) infections. Household members were more likely to have closely or moderately related infections (with IBS <0.4) vs. comparisons to other community members (5% vs. 0.15%, p<0.001). These data suggest malaria transmission within a household or small spacial scale, which could be targeted by interventions such as reactive case detection with treatment or IRS. Additional genotyping and analyses on samples from this cohort are ongoing.

## 1050

### PATTERNS OF INFLAMMATORY RESPONSES AND PARASITE TOLERANCE VARY WITH MALARIA TRANSMISSION INTENSITY

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In individuals living in malaria-endemic regions, parasitaemia thresholds for the onset of clinical symptoms vary with transmission intensity. The mechanisms that mediate this relationship are however, unclear. Since inflammatory responses to parasite infection contribute to the clinical manifestation of malaria, this study investigated inflammatory cytokine responses in children with malaria from areas of different transmission intensities (ranging from low to high). Blood samples were obtained from children presenting with clinical signs of malaria (at clinic) from sites of distinct transmission intensities. Cytokine levels were assessed using the Luminex® based magnetic-bead array system, and levels were compared across sites using appropriate statistical tests. A multivariate analysis was used to determine the influence of age, gender, parasitaemia and transmission intensity on cytokine levels. Parasite density increased with increasing transmission intensity in children presenting to hospital with symptomatic malaria, indicating that the parasitaemia threshold for clinical malaria increases with increasing transmission intensity. Furthermore, levels of pro-inflammatory cytokines, including tumour necrosis factor (TNF)- $\alpha$ , interferon (IFN)- $\gamma$ , interleukin (IL)-1b, IL-2, IL-6, IL-8, and IL-12, decreased with increasing transmission intensity, and correlated significantly with parasitaemia levels in the low transmission area but not in high transmission areas. Similarly, levels of anti-inflammatory cytokines, including IL-4, IL-7, IL-10 and IL-13, decreased with increasing transmission intensity, with IL-10 showing strong correlation with parasitaemia levels in the low transmission area. Multiple linear regression analyses revealed that transmission intensity was a stronger predictor of cytokine levels than age, gender and parasitaemia. Taken together, the data demonstrate a strong relationship between the prevailing transmission intensity, parasitaemia levels and the magnitude of inflammatory responses induced during clinical malaria.

## 1051

### CHARACTERIZATION OF B CELL SUBSETS OVER THE COURSE OF *PLASMODIUM YOELII* INFECTION AND ROLE OF CD73+ B CELLS IN PROTECTION

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Despite the critical importance of humoral and B cell mediated immunity in protecting against malaria, the biological nature of anti-malaria memory B cells (MBC) has not been sufficiently delineated. In this study, we used the *Plasmodium yoelii* 17XNL (PyNL) mouse model of malaria to confirm the importance of anti-malarial B-cell immunity and identified subsets of B cells as critical components of protective immunity against malaria. MBC subsets were identified using flow cytometry by quantification of PD-L2, CD80, and CD73 markers on splenic B cells. Evaluations were done at days 0, 6, 13 and 22 over the course of a primary PyNL self-resolving infection and from mice which had recovered from the primary infection and were protected against a second infection. When compared to naïve mice, we found that the frequencies of PD-L2<sup>lo</sup>CD80<sup>lo</sup>CD73<sup>hi</sup> cells during a primary infection significantly increased from 2.1 $\pm$ 0.1% at day 6 to 39.1 $\pm$ 1.2% at day 22 post-infection. Interestingly, significantly increased relative frequencies of PD-L2<sup>hi</sup>CD80<sup>hi</sup>CD73<sup>lo</sup> cells (4.6 $\pm$ 0.3% and 5.2 $\pm$ 0.6%) and of PDL2<sup>hi</sup>CD80<sup>hi</sup>CD73<sup>hi</sup> (1.2 $\pm$ 0.1; 3.6 $\pm$ 0.3 and 1.8 $\pm$ 0.1%) B cells at days 6, 13 and 22 post Py NL infection were detected for both cell types, and the profile of the mean percentage B cell curve was similar to the Py NL infection parasitemia curve with maximal cell frequencies seen at the

day of peak parasitemia. Mice that were challenged with PyNL (malaria immune) had a rapid increase in the frequencies in PD-L2<sup>lo</sup>CD80<sup>lo</sup>CD73<sup>hi</sup> and PDL2<sup>hi</sup>CD80<sup>hi</sup>CD73<sup>hi</sup> cell subsets at 4 days post-infection. Most importantly, passively transfer of CD73+ B-cells from mice that had recovered from a primary PyNL infection protected naïve mice from a PyNL challenge. In conclusion, in these studies we characterized immune B cell subsets using PD-L2, CD80, and CD73 surface markers over the course of a primary infection and showed that passive transfer of CD73+ B cells generated nearly sterilizing immunity against a PyNL re-challenge.

## 1052

### NATURALLY ACQUIRED IMMUNITY TO *PLASMODIUM FALCIPARUM* GAMETOCYTE ANTIGENS

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The recent emergence and spread of drug-resistant malaria has accelerated the need for an effective vaccine, including vaccines that block malaria transmission. However, exceptionally little is known about host immune responses to gametocytes to reduce transmission. We have evaluated acquired antibodies to gametocytes, gametocyte-infected erythrocytes (gam-IEs) of different stages, and transmission-blocking vaccine candidates. We also assessed the functional activity of antibodies to fix complement, believed to be important in transmission-blocking immunity. We used genetically-modified parasites with suppressed PfEMP1 expression, the major surface antigen of asexual-stage IEs. We found little antibody response to the surface of gam-IEs, similar to that seen for asexual-stage IEs lacking PfEMP1. Significant antibody reactivity to the surface of gametocytes (without the host erythrocyte membrane) was also observed, including antibodies to the major gametocyte antigen and vaccine candidate, Pfs230. Some individuals had functional antibodies that fix complement, which may contribute to transmission-blocking immunity. Our findings suggest that effective immunity to gam-IEs is limited and do not effectively target these stages to mediate transmission-blocking immunity. In contrast, antibodies are acquired to gametocyte antigens, which likely contribute to transmission-blocking immunity. These insights will help inform vaccine development and the assessment of transmission-blocking immunity in endemic populations.

## 1053

### GLUCOSE AND IRON METABOLISM IN MONOCYTES EXPOSED TO MALARIA

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Malaria parasites are still one of the world's most significant infectious agents. Both the morbidity and mortality of malaria is in great measure a consequence of inflammation. Monocytes/macrophages are well-recognized sources of inflammation as they are amongst the most effective cells that can find and eliminate parasites. As such monocytes are key players in the development of malaria pathogenesis. We propose that a deeper understanding of the metabolic responses in monocytes during malaria can pave a new way to comprehend how the immune response against the parasite is generated and can be therapeutically targeted. The aims of this study were to expose blood-monocytes from naïve donors (n=10) to malaria parasites and measure changes in glucose metabolism (glucose transporter 1 (Glut1) levels, glucose uptake ability), and iron metabolism (levels of surface Ferroportin and intracellular Ferritin). Surface expression of Glut1 and cellular uptake of the fluorescent glucose analog 2-(N-(7-nitrobenz-2-oxa-1, 3-diazol-4-yl) amino)-2 deoxyglucose were analyzed by flow cytometry, using a method previously validated by us on samples from HIV-positive individuals, and coupled with immunofluorescence, and western blots to measure the levels of the iron metabolism proteins Ferroportin and Ferritin in the monocyte

compartment after co-culture with malaria parasites. Additionally we related these levels to the functional ability of the monocytes. Preliminary data shows that upon exposure to malaria parasites monocytes increase their expression of surface Glut1, which suggests a switch from oxidative to glycolytic metabolism. We discuss our results in the context of what are the known clinical outcomes observed in malaria-exposed individuals. Understanding the balance between the beneficial and harmful effects of monocyte-induced inflammation in malaria infection is key to successful clinical outcomes and reducing the global illness and death due to malaria.

## 1054

### A SINGLE NUCLEOTIDE POLYMORPHISM IN AN AP2 TRANSCRIPTION FACTOR ENCODED IN THE MALARIA CAUSING *PLASMODIUM BERGHEI* ALTERS THE DEVELOPMENT OF HOST IMMUNITY

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The Apetela2 (AP2) family of transcription factors is a major regulator of Plasmodium parasite gene expression. Twenty seven members of this family have been identified since their discovery in 2005. However, our understanding which AP2 members regulate key features of Plasmodium biology is incomplete. Here we describe the impact of a single nucleotide polymorphism (SNP) in the active site of the AP2 protein, PBANKA\_011210, on the outcome of infection in mice. *Plasmodium berghei* Anka (PbA), the only rodent parasite that causes experimental cerebral malaria (ECM), codes for a phenylalanine at position 1823 whereas all other Plasmodium parasite strains, none of which cause ECM in mice, including PbNK65, have a serine in this position. Using CRISPR, we modified the PbNK65 parasite (PbNK65-WT) to encode the PbA SNP (PbNK65-PbA). The course of infection with PbNK65-WT or PbNK65-PbA were similar and showed no ECM pathology indicating that the PbA AP2 SNP is not sufficient to cause ECM. However, mice infected with PbNK65-PbA had dramatically higher serum levels of IFN-gamma and TNF alpha and they generated more persistent, stronger germinal center responses as well as higher titers of parasite-specific antibodies. When infected mice were treated with sub-therapeutic doses of the anti-malarial, chloroquine, to lower the parasitemias PbNK65-PbA-infected mice cleared the infection whereas mice infected with PbNK65-WT were unable to control the infection resulting severe anemia and death. These results reveal a novel role of AP2 proteins in controlling host immunity to Plasmodium infections.

## 1055

### LIVER-RESIDENT MEMORY T CELLS CAN BE HARNESSSED FOR UNPRECEDENTED PROTECTION AGAINST MALARIA

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T cell memory allows for the rapid generation of effective immune responses to previously encountered pathogens. Although most memory T cells recirculate through the body, a recently discovered subset, the

tissue resident memory T cells (TRM), remains in the affected tissue after infection is cleared. By staying in the organ most likely targeted by the pathogen in subsequent reinfections, TRM have the potential to elicit faster, more focused responses than circulating memory T cell subsets. We found that following vaccination with radiation-attenuated *Plasmodium berghei* ANKA sporozoites (RAS), a population of TRM cells formed in the liver. These cells constantly surveyed the hepatic sinusoids and were essential for protection, as their depletion rendered vaccinated mice fully susceptible to sporozoite infection. Based on these results, we hypothesized that an immunization strategy aimed at maximizing liver TRM formation might be highly protective. By combining dendritic cell priming and antigen recognition on hepatocytes we were able to induce the formation of vast numbers of liver TRM and achieve high levels of sterile protection against live sporozoite challenge. Vaccination aimed at the induction of liver TRM cells may be a more effective way to control liver-stage malaria than traditional strategies generating circulating memory T cells.

## 1056

### IMMUNOBIOLOGY OF THE KUPFFER CELL-SPOROZOITE INTERACTION

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Malaria is a devastating disease, causing over 400,000 deaths annually. The *Plasmodium* parasite responsible is transmitted from the mosquito vector to the vertebrate host during a blood meal. After *Plasmodium* sporozoites are inoculated into the skin, the sporozoites make their way to the liver and traverse the sinusoidal barrier, which contains Kupffer cells (KCs) (liver-resident macrophages), before invading hepatocytes. The job of KCs during a typical infection is to detect, phagocytose, and present foreign antigens, while producing cytokines to activate an immune response. It has been shown that sporozoites are able to traverse KCs without being phagocytosed or killed, suggesting that sporozoites are altering the normal KC response to pathogens. However, the mechanism by which this occurs is not understood. It is well-documented that the parasite can evade the immune system during the blood stages through various mechanisms, including direct suppression of immune responses. To test whether similar phenomena occur in the liver stage, we analyzed the primary rat KC cytokine response to *P. berghei* sporozoites *in vitro*. We hypothesized that KC responses would be skewed towards a tolerant, or anti-inflammatory, phenotype. Here, we report that KCs up-regulate cytokines of both the M1 (pro-inflammatory/classically activated) and M2 (alternatively activated) variety in response to live sporozoites, but not in response to sporozoite lysate. The cytokine response is rapid and short-lived, with cytokines found in the supernatant just 10 minutes after exposure, and levels dropping below the limit of detection after 2 hours, suggesting sporozoite silencing of KC cytokine secretions. Additional work focuses on the interaction of KCs with a sporozoite secreted protein, the *P. berghei* homolog of macrophage migration inhibitory factor, which is involved with the disruption of immunological memory during the blood stages and may play a similar immune evasion role during the liver stage. Taken together, our work sheds new light on the mechanism by which sporozoites modulate KC activity to remain hidden from the host immune system during traversal.

## 1057

**INFLAMMATORY CYTOKINE RESPONSES IN MALARIAL ANAEMIA AMONG MANGALORE RESIDENTS, INDIA**

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In 2016, Mangalore alone accounted for malaria 6404 number of cases. Anaemia is a common complication, yet its severe form i.e, severe malarial anaemia (SMA) is of a major concern due to its high mortality rate. The present work aimed to evaluate the degree of anaemia (Haemoglobin levels, Hb) and levels of Pro and anti-inflammatory cytokines (TNF- $\alpha$ , IL-6, IL-10, IL-12 and IFN- $\gamma$ ) during malarial anaemia among residents of Mangalore, Karnataka, India during 2013 to 2015. Among 692 individuals, 138 were healthy controls (HC) while 351 patients suffered from *P.vivax*, 149 from *Plasmodium falciparum* and 54 with mixed infections. 63 patients with *Pv*, 37 with *Pf* and 15 patients with mixed infections had severe malaria. 70.1% were Non-anemic (NA) where as 20.2% had Mild anaemia, 5.1% with moderate and 4.6% have SMA. Among patients with SMA, *Pv* infections were the highest (56.3%), *Pf* (34.4%) and mixed infections (9.4%). The levels of Hb fell in mixed (11.19 g/dL), *Pf* (11.22 g/dL), *Pv* (12 g/dL) in comparison with HC (12.35 g/dL). Hb levels between NA and Anaemic (A) groups fell significantly across various infection types, mixed infections (13.28 vs 8.141), *Pf* (13.11 vs 8.252) *Pv* (13.02 vs 8.585) g/dL. The parasitemia (%) increased between NA and A groups in patients with *Pv* and *Pf* infections. With an increase in parasitemia(%), the levels of cytokines such as TNF- $\alpha$  increased in *Pv* ( $r=0.1708$ ,  $p=0.0040$ ) and *Pf* ( $r=0.3931$ ,  $p<0.0001$ ), where as IL-12 ( $r=0.1933$ ,  $p<0.0027$ ) and IFN- $\gamma$  levels ( $r=0.1624$ ,  $p<0.0154$ ) increased in only *Pv* groups. The plasmatic cytokines levels were significantly higher in infected patients compared to HC. In comparison with NA and A groups, TNF- $\alpha$  increased in *Pv* ( $P=0.030$ ), and *Pf* ( $P=0.0189$ ); IL-10 levels increased in *Pv* ( $P=0.076$ ); IFN- $\gamma$  increased in *Pv* and *Pf* ( $P=0.0095$ ); A non-significant increase in IL-12 levels was observed in all groups where as IL-6 levels remained same across all infection groups. In conclusion, the present study reveals that an imbalanced inflammatory response such as TNF- $\alpha$ , IL-12 and IFN- $\gamma$  levels may lead to malarial anaemia.

## 1058

**IDENTIFYING RIFIN AND STEVOR EPITOPES ASSOCIATED WITH MALARIA EXPOSURE USING PEPTIDE AND PROTEIN MICROARRAYS**

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An erythrocyte infected with *Plasmodium falciparum* expresses adhesive variant surface antigens (VSAs) on its surface that play a critical role in sequestration and evasion of host immune responses. These VSAs possess

extraordinary genetic diversity, posing a challenge to understanding their role in immunity and designing an effective vaccine. In regions with high malaria transmission, adults and older children develop immunity to clinical disease, which is believed to be associated with the development of antibodies to particular VSAs. The best studied and largest VSA family is comprised of *P. falciparum* erythrocyte membrane protein-1s. The other major VSA families, the repetitive interspersed family of polypeptides (RIFINs) and subtelomeric variable open reading frame (STEVORs), have also been implicated in malaria pathogenesis, but their genetic diversity and role in immunity have not been well characterized. We aimed to identify RIFIN and STEVOR epitopes involved in protective natural immunity by using protein and ultra-dense peptide microarrays based on VSAs in the *P. falciparum* reference genome 3D7. Using sera from malaria-exposed Malian children and adults before, during, and following a malaria season, we determined seroreactivity against three RIFINs and six STEVORs, with each VSA expressed on an array as a single protein, and divided into 16-amino acid (aa) peptides, with 12-aa overlap. All three RIFINs and six STEVORs were recognized by sera from Malian adults and children as compared to sera from malaria-naïve controls. In addition, sera from malaria-exposed adults reacted more intensely to four STEVORs than sera from malaria-exposed children. Although protein array results did not demonstrate overall differential seroreactivity of RIFINs in adults versus children, adult sera reacted more intensely than pediatric sera to a subset of RIFIN peptides that spanned the length of the PF07\_0134 RIFIN, predominantly in the variable domain. Using this approach, we plan to map critical RIFIN and STEVOR epitopes associated with short-term and long-term protective immunity to clinical manifestations of malaria.

## 1059

**UNDERSTANDING THE DECLINE AND REBOUND IN IMMUNITY TO SYMPTOMATIC MALARIA DUE TO INTERVENTION DISRUPTION IN MALARIA TRANSMISSION**

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High-level parasitaemia by *Plasmodium falciparum* is associated with the onset of symptomatic malarial disease and mortality. Individuals build partial immunity to symptomatic malaria with increasing age as a direct result of exposure to *P. falciparum* parasite. This immunity results in a subpopulation of asymptomatic carriers that remain a barrier to establishing and maintaining elimination. What happens to individual and population immunity to malaria when transmission decreases due to long-duration targeted interventions? Little is known about how immunity wanes as transmission decreases, and understanding the dynamics of immunity is critical to informing effective surveillance systems needed for elimination. Strong and long-lasting immunity may help populations reach elimination more rapidly; conversely, rapid return of symptomatic malaria after interruption could make elimination a "sticky" state where outbreaks can be quickly detected and addressed. We use longitudinal data from two distinct settings where malaria transmission was temporarily interrupted and subsequently reestablished: a high-transmission site in Tororo, Uganda subject to three rounds of indoor residual spraying with carbamate over 14 months; and a high-transmission site in Garki, Nigeria, with highly seasonal transmission subject to spraying over 18 months. We use an agent-based mathematical model and statistical methods to describe the effects of disrupted transmission on susceptibility to symptomatic disease among individuals in these sites. We demonstrate the dynamics of

individual-level acquired immunity during shifting transmission intensities among both child and adult cohorts to help guide strategies for resurgent epidemics of malaria amidst campaigns towards elimination.

## 1060

### ANTIBODY IN THE SKIN: DO ANTIBODIES HAVE THEIR GREATEST IMPACT AT THE INOCULATION SITE?

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Malaria-causing *Plasmodium* parasites are deposited into host skin as infected *Anopheles* mosquitoes search for blood. In order for *Plasmodium* to establish infection in the liver, sporozoites need to exit the inoculation site, which they do by moving in the skin to find blood vessels and enter the circulation. This stage of the *Plasmodium* lifecycle (the pre-erythrocytic stage), at which parasite numbers are the lowest, has been recognized as a bottleneck for the parasite. RTS,S, the only vaccine candidate to have shown efficacy in Phase III clinical trials, targets the pre-erythrocytic stages of the parasite. Indeed further studies have shown that antibodies targeting the major surface protein of sporozoites (circumsporozoite protein or CSP) are critical for RTS,S-mediated immunity. We hypothesized that since sporozoites are extracellular for a significant period of time at the inoculation site, that antibodies in the skin could contribute significantly to decreasing sporozoite infectivity. Using rodent malaria parasite *Plasmodium berghei*, we standardized the dose of sporozoites delivered intravenously and by mosquito bite that result in comparable liver infection. We then compared the efficacy of two different doses of a monoclonal antibody (mAb) specific for the P. berghei CSP repeats (50 µg & 25 µg mAb 3D11; IgG1) in their ability to inhibit infection when sporozoites were inoculated intravenously versus by mosquito bite. Our data shows that both concentrations of antibody have greater efficacy when sporozoites are inoculated by mosquito bite. These results have important implications for malaria vaccine development, and provide further insight into host-pathogen interactions in the skin.

## 1061

### MEMORY IL-4<sup>+</sup> CD4 T CELL RESPONSES AS A POTENTIAL SURROGATE OF PROTECTION INDUCED BY *PLASMODIUM FALCIPARUM* RADIATION ATTENUATED SPOROZOITES

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Immunization with radiation attenuated sporozoites (RAS) is considered the gold standard of protection against malaria. Various immune responses including proliferating and cytokine producing CD4 and CD8 T cells, increased prevalence of TCRγδ<sup>+</sup> cells and circumsporozoite protein-specific antibodies have been observed in RAS-immunized subjects. Despite the recent successful induction of lasting protection by intravenous delivery of *Plasmodium falciparum* (Pf) RAS, we still do not have complete understanding as to which of these immune responses underlie Pf RAS-induced lasting sterile protection. Previously, we have observed that subjects protected by exposure to Pf RAS delivered by a bite from an infected mosquito have increased numbers of peripheral memory (CD45RO<sup>+</sup>) CD4 T cells, that upon *in vitro* stimulation with parasitized red blood cells (pRBC) undergo further differentiation into CD4<sup>+</sup>CD27<sup>-</sup> T cells and secrete IL-4, a cytokine required for B cell maturation including Ig isotype switching, as well as memory CD8 T cell maintenance. We have also demonstrated induction of CD4 T cells expressing memory phenotype and producing IL-2 in persons protected by vaccination with RTS,S against homologous controlled human malaria infection (CHMI). A recently conducted study based on Pf RAS delivery by a mosquito bite, IMRAS, was designed to achieve sterile protection in 50% of the enrolled subjects against homologous CHMI. The availability of PBMCs from a cohort of protected and non-protected subjects prompted us to investigate whether pRBC-responding CD4 T cells that express memory phenotype and

produce IL-14 could distinguish protective from non-protective subjects. On the basis of the available evidence we are currently evaluating the possibility that memory IL-4<sup>+</sup>CD4 T cells may be considered as surrogate markers for lasting protective immunity against malaria.

## 1062

### ENHANCEMENT OF THE IMMUNE RESPONSE TO *PLASMODIUM YOELII* CIRCUMSPOROZOITE PROTEIN BY PD-1 INHIBITORS

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In malaria-infected individuals, PD-1 is highly elevated on CD4<sup>+</sup> T cells suggesting that PD-1-mediated immune dysfunction may limit protective immunity against the parasite. Further, in a rodent malaria model, PD-1 was shown to severely dampen CD8<sup>+</sup> T cell proliferation and prolong chronic infection. These data argue that blocking PD-1 inhibition may enhance protection from malaria after immunization. We identified several PD-1 peptides that bind both human and mouse PD-1. Their effect on the immunogenicity of a recombinant replication-defective adenovirus expressing *P. yoelii* circumsporozoite protein (AdPyCSP) was determined using a rodent malaria model. BALB/c mice were immunized with AdPyCSP and then injected with either PD-1 peptide blockers or anti-PD-L1 monoclonal antibody (mAb) on days 1, 3, 5 and 7. At day 12, a 3-fold increase in splenic PyCSP-specific, IFN-γ<sup>+</sup> CD8<sup>+</sup> T cells was seen in mice treated with PD-1 peptide blockers as compared to AdPyCSP only. Comparable splenic PyCSP-specific, IFN-γ<sup>+</sup> CD8<sup>+</sup> T cells numbers were detected between mice treated with PD-1 peptide blockers and anti-PD-L1 mAb. Similar studies are ongoing in preclinical challenge models to assess the immunomodulatory efficacy of the identified PD-1 peptide inhibitors with other malaria immunogens.

## 1063

### USING MULTI CRITERIA EVALUATION TO IDENTIFY PRIORITY AREA FOR INDOOR RESIDUAL SPRAYING, MADAGASCAR

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Despite sustained efforts to control malaria in Madagascar, the Central Highlands remain threatened by unstable malaria transmission. Indoor Residual Spraying (IRS) for malaria vector control mostly depends on external funding and relies on costly logistical implementation. We aimed to map malaria risk gradients using a multi-criteria evaluation (MCE) decision tool to help the Malagasy Ministry of Public Health (MPH) to prioritize areas for indoor residual spraying. We completed annual risk modeling for inhabited locations using MCE by weighted linear combination method. We performed multiple comparisons to assess area changes according to malaria risks using data per two consecutive years during the 2014 to 2016 period. A semi-automatic workflow combining risk modeling processes was developed. To estimate a best-fit risk magnitude probability per commune (administrative entity), we used per-pixel values for inhabited locations, and calculated an adjusted mean. The Jenks Natural Breaks algorithm was used to classify the

obtained malaria risk gradient. Risk models were obtained for 2014, 2015 and 2016. Between 2014 and 2015, we observed areas of malaria transmission risk changes as follows: a decrease in 35.3%, an increase in 29.1% and no change in 35.6%, respectively. Between 2015 and 2016, area changes were as follows: a decrease in 61.9%, an increase in 26.9% and no change in 11.2%, respectively. Environmental and climatic factors determined the changes in the models. The malaria risk maps per commune showed the priority areas for selective IRS. A plugin named "Full Multi Criteria Evaluation for Public Health" is currently available and works on the open-source program QGIS. Malaria epidemiological and intervention data will be integrated in the models for the annual IRS decision-making of the MPH. Running the process is cost- and time- saving for low income countries such as Madagascar. Prioritizing the interventions will optimize the IRS implementation and management. The tool may complement existing surveillance systems. It could be valuable for other infectious diseases of Integrated Disease Surveillance and Response system.

## 1064

### ARE PLANTATIONS A HOTSPOT OF MALARIA TRANSMISSION IN CAMBODIA? AN ECOLOGICAL STUDY AND MATHEMATICAL MODEL

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Malaria is a leading cause of morbidity in Cambodia with an estimated incidence of 120,000 cases in 2015 according to the WHO. Transmission is highest in rural areas where a large proportion of the population live and work. Plantations are hypothesised to be "hotspots" of malaria transmission due to the proximity of forests and the vectors therein. To assess this hypothesis, malaria incidence data collected by plantation malaria workers from August 2013 to March 2016 in north-eastern Cambodia was compared with incidence data from health facilities and village malaria workers in surrounding villages. A deterministic compartmental model was developed based on the malaria life cycle and interaction of plantation workers with the ecosystem to explore malaria transmission dynamics on plantations. Plantation malaria incidence was found to follow a seasonal pattern with a peak in cases at the end of the wet season. The mean annual incidences of cases reported by plantation malaria workers in Monduliri and Stung Treng provinces were 15.8 and 13.9 cases per 1,000 population respectively, consistent with a hypoendemic transmission setting. There was no statistical difference in malaria incidence between plantation and village settings which is probably due to similar risk factors and proximity to mosquito breeding sites. The model predicted that importation of malaria by migrant workers was not a contributing factor to its transmission on plantations. Plantation workers with immunity and asymptomatic malaria were predicted to act as a reservoir enhancing malaria transmission and thus need to be targeted in elimination efforts. As the plantations approach elimination, the transmission pattern will shift from a stable endemic to unstable epidemic pattern with declining immunity which may result in an increase in clinical and severe malaria cases. Plantation workers are often mobile, migrant, and hard to reach, rendering provision of healthcare services challenging. The results highlight the role ministry of health and civil society have in improving access to and uptake of mobile malaria worker services in order that malaria cases are treated early.

## 1065

### MODELLING THE POTENTIAL OF IVERMECTIN TREATED CATTLE AS A NOVEL MALARIA VECTOR CONTROL TOOL: IMPLICATIONS OF KILLING ZOOPHILIC MOSQUITOES

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Current malaria vector control interventions target anthropophilic, indoor-biting mosquitoes, leaving zoophilic, exophilic vectors to sustain residual transmission of malaria, even in areas with high vector control coverage. It has been suggested that targeting zoophilic vectors by injecting cattle with the multi-purpose mosquitocidal drug ivermectin (IVM) could ameliorate this. However, applying such a strong selection pressure against zoophilic vectors could shift vector populations towards being more anthropophilic which would increase the human biting rate. Here, a mathematical model of vector dynamics and malaria transmission are linked with an existing ecological larval model and extended to simulate administration of IVM to cattle. When this framework is used to investigate the effects of this intervention in a homogenous vector population of mosquitoes with identical Human Biting Indexes (HBIs), the administration of an annual dose of IVM to cattle is predicted to considerably reduce malaria infection, particularly when given immediately prior to the peak of the rainy season in a seasonal transmission setting. However, if two competing species with different HBIs are present, the intervention may cause an increase in annual malaria infections by changing the composition of the vector population to include a larger proportion of anthropophilic mosquitoes. If the two vector species occupy entirely overlapping breeding sites (complete competition), a single dose of IVM is predicted to cause a permanent increase in malaria prevalence. In reality, competition is more likely to be partial, in which case a single dose of IVM is predicted to only increase malaria prevalence temporarily when the level of competition is above a certain threshold. These findings highlight the importance of considering the local vector species before planning administration of IVM to cattle.

## 1066

### ESTIMATING THE MALARIA ATTRIBUTABLE FEVER FRACTION ACCOUNTING FOR PARASITES BEING KILLED BY FEVER AND MEASUREMENT ERROR

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A characteristic symptom of malaria is fever. The fraction of fevers that are attributable to malaria, the malaria attributable fever fraction (MAFF), is an important public health measure for assessing the effect of malaria control programs and other purposes. Estimating the MAFF is not straightforward because there is no gold standard diagnosis of a malaria attributable fever; an individual can have malaria parasites in her blood and a fever, but the individual may have developed partial immunity that allows her to tolerate the parasites and the fever is being caused by another infection. We develop a causal inference perspective on the MAFF by defining the MAFF using the potential outcome framework for causal inference and show what assumptions underlie current estimation methods. Current MAFF estimation methods rely on an assumption that the parasite density is correctly measured. However, this assumption does not generally hold because (i) fever kills some parasites and (ii) the measurement of parasite density has measurement error. In the presence of these problems, we show current estimation methods do not perform well. We propose a novel maximum likelihood estimation method based on exponential family g-modeling. Under the assumption that the measurement error mechanism and the magnitude of the fever killing effect are known, we show that our proposed method provides approximately unbiased estimates of the MAFF in simulation studies. A sensitivity analysis can be

used to assess the impact of different magnitudes of fever killing and different measurement error mechanisms. We apply our proposed method to estimate the MAFF in a study in Kilombero, Tanzania.

## 1067

### UNDERSTANDING THE MALARIA TRANSMISSION PROCESS IN NEAR-ELIMINATION SETTINGS

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In 2016 the WHO identified 21 countries for whom it would be realistic to eliminate Malaria within the next five years. However, as countries approach elimination targets, changes in geographic clustering, the influence of imported cases, *Plasmodium vivax* relapse dynamics, and the shifting demographics of infected individuals pose major challenges to reaching zero cases. An in-depth understanding of these changes is essential to design optimal elimination strategies. For outbreaks of numerous other diseases, surveillance data is used to reconstruct transmission chains and estimate transmission dynamics, providing useful information for decisions about disease control and intervention. These methods are difficult to apply to endemic malaria, which has long generation times, complexities in immunity and large numbers of unobserved cases. In near elimination countries, with strong surveillance systems, these complexities are mitigated and such methods can be applied to understand the malaria transmission process. We analysed novel health surveillance data from El Salvador. This dataset contains detailed geographic, epidemiological, and demographic information about all reported and confirmed cases from 2010 to 2015, (87 cases, of which 29 were imported). We apply a continuous diffusion process on discrete networks to reconstruct the most likely transmission chains between 2010 and 2015 and quantified heterogeneities in transmission over time, space and between individuals. Our results suggest that between 2010-2015 transmission intensity waned, however importation continues to sustain transmission in border regions. We found the mean value of  $R_c$  is below 1 between 2010 and 2015 suggesting that, in isolation, El Salvador would be likely to sustain elimination. However, following importation from neighbouring countries, time varying reproduction numbers often surpassed 1. This highlights the need for cross-border collaboration to achieve elimination and the potential value of the Elimination of Malaria in Mesoamerica and Espanola Initiative (EMMIE).

## 1068

### MODELING THE RELATIVE ABUNDANCE OF MAJOR MALARIA ANOPHELES SPP. ACROSS GHANA USING A BAYESIAN APPROACH

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Understanding the ecology and spatial distribution of dominant malaria vectors is essential to the design of effective and sustainable strategies for malaria control and elimination. However, rarely is spatial distribution of the dominant vectors used in planning at country-level and there have been fewer attempts to understand the link between spatial composition and behavioral bionomics. Previous studies have tried to define ecological niches and vector bionomics, but at larger-scales. These have been important for general understanding of vector dominance and distribution at the continental scale, but they lack the ability to define the density of each species at a finer resolution. In this study, we aim to build on these earlier approaches and assess the determinants of vector abundance, so as to produce better predictive maps of the relative abundance of major vectors of malaria in Ghana. Vector count data were modeled using an over-dispersed Poisson model within a Bayesian framework to evaluate key environmental drivers and to predict spatial distribution.

Environmental variables were derived from remote sensing applications. A total of 334 survey sites were collated for the species *Anopheles gambiae* and *An. funestus*. Model validation was done on 10% of these locations prior to final predictions. We find that, although mapping of these vector abundance patterns shows promise, there still the need for more systematic and consistent data to be able to help with vector management.

## 1069

### PREDICTING NEW YORK CITY COMMUNITIES AT RISK FOR IMPORTED MALARIA: METHODS AND APPLICATIONS

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In the United States, most malaria cases are acquired by immigrants visiting friends and relatives (VFRs). Methods for identifying, mapping, and predicting communities at risk for imported malaria now and in the future, can help inform intervention strategies and focus resources. Building upon previous work and using census and routine surveillance data for 2010-2014, our objective was to build and validate a model which could be used to accurately predict the distribution and case burden of imported malaria in New York City over a five-year period. To this end, we: 1) adapted and applied a multivariable negative binomial regression model previously derived from a study of Minnesota data to census data for New York City to predict number and location of malaria cases for ZIP Codes as a proxy for communities, and 2) attempted to validate and improve upon this model for the New York metropolitan area based on actual case data obtained from the New York City Department of Health. Our initial model for NYC census data from 2010-2014 suggested that, when compared to a similar map for the overlapping 2004-2012 period, findings were qualitatively similar in terms of the geographic distribution, but that even if doubled to reflect a longer reporting period, expected case counts (0 to 7) for a five-year period were substantially lower than actual cases (0 to >22) over a nine-year period. Testing our model with NYC case data for 2010-2014 resulted in a better fit and improved ability to predict case burden. Combined analysis of routine surveillance and census data can improve understanding of where and which communities may be at risk for imported malaria. Our methods can be extended to other reportable emerging infectious diseases to inform targeted interventions, as well as to predict malaria case distribution and burden for an expanded geographic area. Potential impacts include increased coverage of travel medicine services and improved pre- and post-travel care for high-risk communities.

## 1070

### COSTING MALARIA ELIMINATION IN THE ASIA-PACIFIC

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The Asia Pacific region has attracted between 12-21 percent of global malaria funding from 2006-2010. However, since 2009, there has been steady decline in external financing for malaria particularly in the low-burden and eliminating countries. In order to achieve the goal of a malaria-free Asia Pacific, financial resources will need to be increased and sustained over the medium to long-term. Without intensive support and evidence to understand and articulate the cost requirements to help

mobilize adequate and sustained resources, and/or reprogram existing funds to be spent as efficiently as possible, these countries will be unable to reach their elimination goals. The study aims to use mathematical modelling to project rates of decline to elimination by 2030 and determine the costs for (and maintaining) elimination in the Asia Pacific region. Non-linear differential equations were used to develop a mathematical model to capture the dynamics of *Plasmodium falciparum* and *Plasmodium vivax* malaria for the 22 countries in the Asia-Pacific region. The models accounted for health system interventions, immunity, G6PD deficiency and mobility. All 22 countries are predicted to achieve malaria elimination by 2030 through a mixture of interventions such as universal coverage of bednets and drug therapy, increased surveillance, indoor residual spraying and mass drug administration. Economic costing for elimination was conducted to assess the potential for efficiency savings on the path to malaria elimination by 2030. A web-based application was created for the users of the model.

## 1071

### THE RELATIONSHIP BETWEEN *PLASMODIUM FALCIPARUM* PARASITEMIA FROM MIS DATA AMONG PREGNANT WOMEN AND CHILDREN AND ASSESSING THE USE OF ANC DATA FOR ESTIMATING MALARIA PREVALENCE

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Malaria control programs often target vulnerable populations such as children and pregnant women. The Bioko Island Malaria Control Project (BIMCP) has implemented population specific control programs with aim of reducing the burden of malaria among such groups on Bioko Island, Equatorial Guinea. Through the annual Malaria Indicator Survey (MIS), the BIMCP is able to monitor malaria prevalence, but as most surveys this is an intensive and time-consuming process. Although the BIMCP has observed significant reductions in *P. falciparum* prevalence among children ages 2-14 years (From 45% in 2004 [95% CI, 40%-50%] to 12.1% in 2016 [95% CI, 11.2%-13.3%]), more efficient and timely methods of monitoring malaria prevalence will be needed to continue its progress. During antenatal care (ANC) visits, pregnant women are routinely screened for parasitemia, thus providing monthly variation of malaria incidence among this sub-population. If a relationship exists between malaria prevalence among pregnant women and children, monthly monitoring of ANC data could prove effective and efficient in estimating malaria trends in other subpopulations, such as children. The Pearson's product-moment correlation (PPMCC) will be used to assess the linear correlation between parasitemia prevalence among pregnant women and children using MIS data from 2008-2016 at a sentinel site level and island wide data from 2015-2016. Previous results indicate a strong relationship between the two groups. This study will continue to monitor the strength of the association of parasitemia between the two sub-populations, and therefore, the ability to use prenatal care attendees as an indicator for malaria prevalence estimation on Bioko Island.

## 1072

### USING MODELED *PLASMODIUM VIVAX* PREVALENCE DATA TO ESTIMATE THE MARKET SIZE FOR GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6PD) DEFICIENCY TESTS TO SUPPORT DECISION-MAKING ON SERVICE DELIVERY MODELS

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*Plasmodium vivax* is estimated to be responsible for over 100 million clinical infections annually. Drugs in the 8-aminoquinoline family, such as primaquine and tafenoquine, can completely clear *P. vivax* parasites by killing malaria gametocytes and thus blocking vector-borne transmission. For patients with reduced activity of the G6PD enzyme, however, 8-aminoquinoline drugs can cause severe hemolysis, and the World Health Organization therefore recommends identifying the G6PD status of patients infected with *P. vivax* malaria prior to administering these drugs. Point-of-care (POC) tests that can rapidly and affordably identify G6PD status are needed to support safer and wider use of the 8-aminoquinoline family of drugs. During the time that PATH has been supporting development and market entry of POC G6PD quantitative and qualitative tests, we also developed a computer program, GeoDX model, to estimate *P. vivax* burden per health facility (i.e., the number of patients positive for *P. vivax* who visit each health facility) in selected countries. GeoDX is a computer program written in an open-source programming language, R. Inputs to this model are *P. vivax* prevalence and population per geographic area, geospatial facility data, and certain assumptions around facility catchment. Outputs from GeoDX are ported to a secondary program geared toward leveraging GeoDX data to estimate G6PD diagnostic demand. Calculating diagnostic demand is achieved by considering different treatment algorithms, country diagnostic adoption characteristics, malaria elimination scenarios, and product costs as well as product shelf life and shipping characteristics. The output of the diagnostic demand software is a ten-year demand forecast of the total number of qualitative and quantitative G6PD diagnostics and their associated aggregate and unit costs. National malaria control programs and other program implementers can use the outputs of our model to evaluate which use-case scenario would most likely attain the desired target coverage in the most effective manner.

## 1073

### A CLUSTER-RANDOMIZED TRIAL TO TARGET SUBSIDIZED ARTEMISININ COMBINATION THERAPY (ACT) IN THE RETAIL SECTOR USING A COMMUNITY-BASED TESTING AND VOUCHER SCHEME

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Private drug retailers are a major source of antimalarials in malaria-endemic areas and yet dispensing in these shops is largely divorced from diagnostic testing. This leads to poor targeting of antimalarials; both under-consumption among those with malaria and over-consumption among those without. Several countries, including Kenya, offer publicly subsidized ACTs through the private sector. Poor targeting reduces the public health effectiveness of these subsidies. The objective of this study is to evaluate the impact of targeting an antimalarial subsidy through a partnership between Community Health Workers (CHWs) and private drug shops. Free, community-based malaria diagnostic testing was made available through CHWs. The subsidy is offered in the form of a voucher

from the CHW conditional upon a positive malaria rapid diagnostic test (RDT). The voucher is redeemed for a WHO-qualified ACT at a reduced price at a participating shop. We randomized 32 clusters in western Kenya between intervention (CHW testing&vouchers) and comparison arms (normal CHW activities). In the intervention arm, we trained 275 CHWs serving approximately 100,000 people to offer RDTs in their communities. 36 shops in intervention areas agreed to redeem vouchers. ACT cost for voucher-holders was \$0.10-0.40 compared to \$1.20 without a voucher. Over 15 months, >26,000 participants sought testing and 30% were RDT-positive (2.5% - 47%, by cluster). 90% of the vouchers issued were redeemed at shops. Community-based surveys of households with a malaria-like illness in the preceding four weeks were used to estimate the impact of the intervention. Surveys were conducted prior to the intervention and at six-month intervals during the 18 month intervention period. >7,000 households participated in the surveys. The primary outcome is the percent of fevers captured by the survey that received a malaria diagnostic test. Secondary outcomes include the proportion of ACTs consumed by individuals with a positive test, a negative test, no test, and the proportion of patients receiving an adequate dose of ACT. Primary and secondary outcomes will be compared between the study arms.

## 1074

### CONTRIBUTION OF COMMUNITY-BASED HEALTH WORKERS (CBHWS) TO IMPROVING PREVENTION OF MALARIA IN PREGNANCY: PROCESS FOR IMPLEMENTING A FEASIBILITY STUDY

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The Ministry of Health of Burkina Faso with the support of its partners initiated a study on the feasibility of increasing provision of Intermittent Preventive Malaria Treatment in pregnant women (IPTp) with sulfadoxine-pyrimethamine (SP) by involving existing community-based health workers (CBHWS). As Burkina Faso adopted the WHO recommendations for more doses of IPTp during pregnancy, it was proposed that the challenge of achieving coverage of third, fourth and additional doses could be met using CBHWS. The approved protocol calls for CBHWS to refer pregnant women to antenatal care (ANC) to receive their first IPTp dose. Subsequent doses at one-month intervals would be provided by trained CBHWS, who would report back to supervising midwives at the ANC clinics. Several steps were taken to gain approval and set up the intervention. First, IPTp data from the health information system was gathered. IPTp coverage based on ANC registration in the 6 intervention clinics was 69% IPTp1, 68% IPTp2, 56% IPTp3, and 1% IPTp4. Similar information was obtained from the 6 control clinic catchment areas. Situation analysis found that while CBHW curriculum stresses the importance of ANC, it does not address IPTp at community level. In response updated training materials have been developed. The study team also collected information on village size and availability of CBHWS, especially females. Among the villages in the catchment of the 6 intervention ANC clinics, 33 were found to lack female CBHWS. As a result, the team needed to recruit additional female CBHWS, as revised national recruitment guidance stressed attainment of primary school certificate over gender, meaning mainly men had been hired previously. Two institutional review boards were involved and suggested the need to address the potential rare side effects of SP and concerns that community IPTp would not detract from ANC clinic attendance. Since district and clinic level health staff will be involved in implementing the program using the national CBHW program, lessons learned from this effort to expand the work of CBHWS in preventing malaria in pregnancy should be applicable/adaptable nationwide.

## 1075

### STRENGTHENING MALARIA SUPPLY CHAINS LEADS TO IMPROVED MALARIA CASE MANAGEMENT

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Strong supply chains help ensure crucial access to malaria diagnostics and medicines. The significant progress that has been made towards Significant progress achieving the global targets of reducing malaria-related morbidity and mortality. would not have been possible without the strengthening of the supply chains that manage and move malaria products, improving product availability which support improvements in malaria case management and outcomes. Analyses of data on malaria product availability and malaria case management demonstrate the importance of product availability. In Burkina Faso, the percentage of health facilities with ACTs available improved from 15% to 85% between 2012 and 2015. During the same time period, the malaria mortality rate in children under 5 decreased by 50%. Facilities stocked out of RDTs decreased from >90% to less than 5%. In Ghana, in 2010 only 15% of the malaria cases were diagnosed with an RDT or microscopy. By mid-2016, this had improved to almost 90%. During this time, the percent of facilities stocked out of RDTs declined by 50%. In Malawi, in 2011 around 50% of facilities were stocked out of ACTs; by 2016, less than 10% of facilities were stocked out of ACTs. From 2010 to 2015, the percent of children under 5 that received an ACT increased by 42%. Also in Malawi, analysis of survey data and supply chain data showed that the peak of stockouts of sulfadoxine-pyrimethamine (SP) resulted in the lowest percentage of eligible pregnant woman who received any SP, a critical intervention to preventing malaria in pregnancy. In Zambia, the decline in stockout rates over the last five years, from more than 30% to less than 5% have been accompanied by significant increases in the percent of children under 5 that received an ACT (more than a 50% increase). Despite these positive country examples, there are still an estimated 212 million cases of malaria worldwide. Investments in supply chain strengthening contribute to greater product availability, to preventing, diagnosing, and treating malaria, and ultimately in malaria case outcomes. Supply chain strengthening remains a critical component to the global malaria agenda.

## 1076

### CLINICAL CONSEQUENCES OF SUBMICROSCOPIC MALARIA PARASITEMIA IN UGANDAN CHILDREN

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Submicroscopic malaria parasitemia remains common in both high- and low-endemicity settings in Africa. Submicroscopic parasitemia impacts mosquito infectivity and disease transmission, but its clinical consequences are unclear. We performed repeated cross-sectional surveys measuring parasite prevalence from August 2011 – June 2016 in a cohort of children (n=364) and adults (n=106) in Tororo District, Uganda, where malaria transmission intensity has historically been high, but has declined after implementation of indoor residual spraying (IRS) in December 2014. Participants were followed using active and passive surveillance. Those who presented with fever (tympanic temperature > 38.0°C or history of fever in the previous 24 hours) and a positive blood smear were treated for malaria. Participants also presented to the clinic every 90 days for routine visits (n=9,075); a subset of patients was followed every 30 days. Blood smears and dried blood spots (DBS) were collected at every routine visit, and all patients with a negative blood smear had loop-mediated isothermal amplification (LAMP) performed to test for submicroscopic parasitemia. Submicroscopic parasitemia was common, with an overall prevalence of 25.8% and 39.2% in children and adults, respectively. In children, but not adults, having submicroscopic parasitemia at a routine

visit, compared to having no detectable parasitemia, was associated with increased risk of fever (adjusted risk ratio [CI], 1.67 [1.37 – 2.03]) and an increased risk of clinical illness, a composite outcome comprised of eleven signs and symptoms (1.32 [1.15 – 1.51]). The risk of fever in children with submicroscopic infection was maintained after excluding individuals with a malaria episode within the preceding or following 14 days, to limit effects of recent symptomatic malaria, and after adjusting for other variables that may be associated with fever, including age, housing type, and socio-economic status (1.68 [1.39–2.03]). Although we cannot establish causation, our analyses suggest that submicroscopic malaria infection has clinical consequences in children.

## 1077

### IMPROVING QUALITY OF DATA TO ADVANCE MALARIA IN PREGNANCY INDICATOR COVERAGE IN EBONYI STATE, NIGERIA

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Quality data are crucial for informed decision-making to address health challenges and improve malaria service delivery among countries on the pathway to malaria elimination. This emphasis on better data quality was reflected in the World Malaria Day theme of “Counting Malaria Out” in 2009 and 2010. In Nigeria, improving malaria data quality has been difficult due to critical health system challenges including poor coordination across different departments, institutional complexities, and a shortage of medical record officers and service providers sufficiently trained in data visualization and use of data for decision-making. In response, the Maternal and Child Health Survival Program (MCSP) in Nigeria embarked on the implementation of key activities to improve quality of malaria data in Ebonyi State. These activities included training on record keeping and use of data for decision-making; post training follow-up; dash boards at the frontline for better data visualization; monthly data collation meetings; improved synergy among service departments; and quarterly data quality assurance visits. As a result, more than 75% of facilities graphed malaria indicators thereby increasing data visualization and use of data for decision-making. An example of data improvements leading to service increases was Intermittent Preventive Treatment for malaria in pregnancy (IPTp). IPTp1 service statistics in MCSP-supported facilities improved from 54.1% in Oct-Dec 2015 to 81.3% by Jul-Sept 2016 compared to 54.7% to 67.8% in the same periods for non-MCSP facilities. Similarly, IPTp2 service statistics in MCSP-supported facilities improved from 52.8% to 70.5% compared to 46.5% to 58.0% in the same period for non-MCSP facilities. Data quality improvement interventions such as monthly data collation and validation meetings prior uploading data to DHIS can contribute to improved quality of malaria performance indicators, better coordination between antenatal care, outpatient and pharmacy departments and increased IPTp coverage.

## 1078

### ASSESSMENT OF BEHAVIOR CHANGE COMMUNICATION (BCC) INTERVENTIONS IN SUPPORT OF MALARIA CONTROL ACTIVITIES CONDUCTED IN BENIN BY PMI'S ARM3 PROJECT

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In Benin, from 2012 to 2014, PMI ARM3 project conducted Behavior Change Communication (BCC) activities in 28 of 34 health zones (HZs). In 2015, we assessed the project's BCC contributions to improving malaria preventive and treatment-seeking attitudes and behaviors among pregnant women and children <5. We selected two HZs in Oueme-Plateau region as the intervention group and two HZs in Mono-Couffo region as the control group. Both groups shared similar socio-economic characteristics. We sampled 16 health centers, 528 women (321 pregnant and 207 mothers of children <5) and assessed in both areas using the Roll Back Malaria 2014, Malaria BCC Indicator Reference Guide. Associations among variables were tested at the 5% threshold using the ANOVA *F*-test. Dissemination of malaria messages was mainly conducted through social mobilization (34% of women recalled participating in the intervention group vs. 9% in the control group), community radio spots (32% of participants in the intervention group reached/recalled having heard a message versus 14% control group,  $p < 0.001$ ) and flyers distribution through household visits (76% intervention group recognize an image from a flyer vs. 54% control group). 93% of the women in the intervention group knew what to do (use mosquito nets, preventive medication taken during pregnancy) to prevent malaria compared with 84% in the control group ( $p < 0.05$ ). 90% of children <5 in the intervention group had slept under an LLIN the night before the assessment compared to 82% in the control group ( $p < 0.01$ ). The level of LLIN use by pregnant women in the intervention group was higher compared to the control group (87% against 76%,  $p < 0.05$ ). 66% of women in the intervention group who had a live birth during the past two years received two or more doses of sulfadoxin/pyramethamin during antenatal care visits versus 42% in the control group ( $p < 0.01$ ). 48% of women in the intervention group stated that ACT is the most effective drug against malaria compared to 28% in the control group ( $p < 0.01$ ). BCC activities had a positive effect in improving behaviors related to the prevention of malaria in pregnant women and children <5.

## 1079

### IMPROVEMENTS IN QUALITY OF MALARIA CASE MANAGEMENT THROUGH COUNTY REFERRAL HOSPITAL MEDICINES AND THERAPEUTICS COMMITTEES IN KENYA: THE MIGORI COUNTY EXPERIENCE

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Hospital medicines and therapeutics committees (HMTc) are used to assure administrative and departmental coordination of clinical hospital outcomes in the developing world. MalariaCare has supported revival of HMTcs in several counties in western Kenya in order to strengthen implementation of high quality malaria case management services. This report focuses on improvements in malaria case management indicators in Migori County Referral Hospital (MCRH), after initiation of an HMTc in April 2016. The MCRH HMTc held four meetings between May and December 2016. Participants included the hospital superintendent, pharmacist, surgeon, pediatrician, gynecologist, laboratory in charge, clinicians, MalariaCare regional coordinator and technical advisors. Meetings focused on reviewing gaps identified during the most recent malaria case management supervision visit, conducted by MalariaCare in coordination with the National Malaria Control Program and Ministry of Health (MOH). The HMTc developed actions plans for implementation

by various responsible departments to address these gaps which included several activities: dissemination of national guidelines for the diagnosis, treatment and prevention of malaria in Kenya to all hospital departments; developing facility specific clinical flow charts for malaria case management; organizing continuing medical education (CME) sessions for relevant hospital staff; and sharing of feedback to all key departments on supervision performance indicators. The hospital has seen a dramatic improvement in key indicators assessed during three supervision visits from the first visit in November 2015 to the most recent in December 2016. The overall clinical performance score improved from 83% to 96%, the percentage of suspected malaria cases with a corresponding test improved from 85% to 100% (20 records sampled), and percentage confirmed malaria cases treated with ACTs increased from 50% to 100% (10 records sampled). Given these successes, MalariaCare is working with the MOH to continue reviving HMTCs in one referral hospital in each of the eight MalariaCare-supported counties in Kenya.

## 1080

### INCREASING CARE SEEKING BEHAVIOR AMONG CAREGIVERS OF CHILDREN UNDER FIVE YEARS OF AGE WITH FEVER IN BENIN USING BEHAVIOR CHANGE COMMUNICATION: RANDOMIZED TRIAL

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Increasing the utilization of malaria prevention and control efforts in Benin is challenging although simple and effective diagnosis and medicines for malaria exist. We investigated whether an effective behavior change communication (BCC) strategy and related trainings for caregivers can increase the proportion of children less than five years of age suffering from fever or illness for whom care is sought by the caregiver at the community level. We conducted operational research using a randomized trial in Benin, West Africa. We compared care seeking behavior of two groups: the reference group used integrated management of childhood illness plus rapid diagnostic tests (IMCI+RDT) and the intervention group applied IMCI+RDT+BCC. Eighteen arrondissements (in the north and the south) were assigned in two groups by block randomization, based on preliminary surveys. Within those 18 arrondissements, there were 46 intervention and 54 control villages. The primary endpoint was the proportion of children less than five years of age suffering from fever or illness for whom care is sought by the caregiver at the community level. Information was obtained using cross-sectional household surveys from May 2015 to July 2016. During 14 months of observation, 2,691 households were considered for data collection and 67% met the three inclusion criteria of (1) consenting to the survey, (2) having the caregiver at home and able to answer the questions, and (3) having a child less than five years of age, who had fever or illness in the previous two weeks. With a power of 80%, there was no statistically significant difference between intervention and comparison groups at baseline (27% versus 22% respectively), ( $p=0.112$ ). At the end line, the care seeking behavior was significantly higher in the intervention group (22%) compared to control (14%), ( $p<0.001$ ). So, although the intervention group is higher than the control group, both are worse. The lessons learned from this study can be used to refine as well as inform the national BCC strategy within the National Malaria Control Program and could serve as a foundation to improve BCC message that influences care seeking.

## 1081

### USING RAPID TASK ANALYSIS TO STRENGTHEN PRE-SERVICE EDUCATION (PSE) LEARNING AND PERFORMANCE OF CRITICAL MALARIA INTERVENTIONS BY REGISTERED MIDWIVES (RMS) AND MEDICAL LAB TECHNICIANS (MLTS) IN LIBERIA

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Health worker task analysis helps human resource planners and managers update pre-service education (PSE) curricula and plan needed in-service training. In Liberia, a task analysis was conducted focusing on Liberia's midwives' and Medical Laboratory Technicians' (MLT) work practices. Task lists were developed using curricula, job descriptions and professional scope of practice, and validated by key stakeholders for each cadre. Responses from 25 MLTs and 26 midwives were examined that addressed the following questions: How often do you do the task (frequency)? 2) Where did you learn to do the task (location)? 3) How well do you think you are able to perform the task (performance)? 4) How critical is the task in terms of patient and/or public health outcomes (criticality). Eligibility criteria included those currently practicing between 6 months and 5.5 years following graduation. Midwives were assessed for five tasks relating to malaria service provision, including provision of preventive treatment for malaria in pregnancy, management of vector borne diseases, diagnosis and management uncomplicated malaria in adults and children (respectively), and provision of malaria preventive services. Lab technicians were assessed for one malaria task, performance of parasitological tests. On average 61% of midwives learned these malaria tasks in PSE, 74% said they performed these tasks daily, 80% felt proficient in performing the tasks, and 82% rated the tasks moderate to high in criticality. For MLTs, 88% learned malaria testing in pre-service education, 100% performed this task daily, 77% felt they were proficient and 93% said the task was of moderate to high criticality. The results from this rapid task analysis are being applied to the current curricula review. Courses that could be updated or strengthened have been identified. Malaria Case Management Technical Update and Effective Teaching Skills Training are being organized for tutors at the training schools. Finally, integrated supportive supervision tools are being strengthened to improve performance of these malaria tasks by midwives and lab technicians.

## 1082

### UNDERSTANDING THE ENVIRONMENT OF MALARIA-RELATED BEHAVIORS ON BIKO ISLAND, EQUATORIAL GUINEA

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Indoor Residual Spraying (IRS) and Long Lasting Insecticide Treated Nets (LLIN) are the two largest vector control interventions used by the Bioko Island Malaria Control Project (BIMCP) on Bioko Island, Equatorial Guinea. The BIMCP has been successful in achieving optimal coverage with IRS in targeted communities and completed a mass distribution of LLINs in 2015, with targeted top-off campaigns in 2016 and 2017. In 2016, the BIMCP conducted a malaria indicator survey (MIS) in a representative sample of households. The MIS collects data regarding malaria prevention methods practiced at home, socio-economic status, and general health, and LLINs observed in use in household sleeping spaces were recorded. Additionally, participants were asked a series of knowledge-based questions related to malaria transmission, symptoms, and treatment methods. Clustering, linear regression, and association techniques will be used to determine how malaria knowledge, socio-economic status, and geographical location correlate with behaviors at the household level. Dependent variables

used in the analyses are: whether the survey participant slept under a net the night before, ratio of people to LLINs, whether a household desires to participate in upcoming IRS spray rounds, and if the household has participated in past rounds of IRS. Explanatory variables will be used to find data patterns to help better understand the household environment of the 38.4 % of individuals that slept under a net the night before versus the 55% who did not, the 38.5% of household that have sufficient bed nets for members versus 61.5% that did not, or the 81.9% of households that want to receive in IRS versus the 10.8% who do not. These explanatory variables will include a household malaria knowledge score, a socio-economic score, and geographical clusters. Scatter plots, classification trees and maps will be used to visually explain the correlation of the dependent and explanatory variables, and will shed light on which factors may be most influential in the uptake of key malaria control interventions.

## 1083

### USE OF CAP380 AS A MARKER FOR *PLASMODIUM FALCIPARUM* OOCYST DEVELOPMENT IN VIVO AND IN VITRO

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The sporozoite stage of *Plasmodium* first develops inside the oocyst; then, sporozoites invade and mature in mosquito salivary glands before transmission to humans. Oocysts develop in between the midgut epithelial cell plasma membrane (PM) and the basal lamina of the mosquito. The outside of the oocyst is composed of two parts, including the oocyst capsule, which is the outermost layer comprised of mosquito-derived and parasite components, including laminin from the mosquito and the parasite capsule protein, CAP380. Directly under the oocyst capsule is the PM, which contains the parasite-derived circumsporozoite protein (CSP). Both CAP380 and CSP are essential for normal oocyst development in the mosquito. To study *Plasmodium falciparum* (*Pf*) oocyst development of mosquito-derived (*in vivo*) and culture-derived (*in vitro*) oocysts, we raised an antibody against *Pf*CAP380. We monitored *in vivo* oocyst development, by performing immunofluorescence assays (IFAs) on *Pf*-infected midguts using anti-*Pf*CAP380 to detect oocysts. To study *in vitro* oocyst development, we first cultured gametocytes, and then promoted *in vitro* mosquito stage development through the following stages: gamete → zygote → ookinete → oocyst. For *in vivo* oocysts, we found that *Pf*CAP380 is expressed as early as 2 days after mosquito infection and persists through day 11. We also found that CSP is expressed ~6 days after mosquito infection. Using *in vitro* methods to produce mosquito stages, we achieved high conversion of gametocyte → ookinete (24%) and ookinete → early oocyst (85%). After screening basal lamina components, we found that the combination of collagen IV, entactin, and laminin allows for greatest conversion to early oocysts. Similar to the IFA results using *in vivo*-derived oocysts, we detect *Pf*CAP380 from *in vitro*-derived oocysts as early as day 2 and persistence up to day 11. We conclude that we can produce mosquito stages of *Pf* using *in vitro* methods through the oocyst stage as determined by oocyst marker expression of *Pf*CAP380. Further *in vitro* studies focused on sporozoite production will support manufacturing of live-attenuated sporozoites for malaria vaccines.

## 1084

### ASSESSING THE IMPACT OF MALARIA AND MALARIA CONTROL INTERVENTIONS ON THE WELFARE OF THE POPULATION ON BIKO ISLAND, EQUATORIAL GUINEA

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The Bioko Island Malaria Control Project (BIMCP) has conducted intensive malaria control interventions on Bioko Island, Equatorial Guinea since 2005. The BIMCP has had significant success in reducing *P. falciparum* parasite prevalence in 2-14 year olds from 45% (95% CI 40%-50%) in 2004 (baseline) to 12.1% (95% CI: 11.2%-13.3%) in 2016 based on annual Malaria Indicator Survey (MIS) data. During MIS surveys, the BIMCP assessed, every 5 years, the impact of malaria and malaria control interventions on the welfare of the island's population and its effect on the poor, as measured by socio-economic status (SES) indicators. Most often, SES is calculated as a proxy using principal components assessment (PCA) which enables ranking of surveyed households. However, using PCA to measure SES provides several limitations such as: the inability to measure the absolute level of poverty in a community or changes in poverty level over time (20% of houses always remain the poorest without any knowledge of how vulnerable they are). Inter-country and inter-temporal analysis is only possible if SES indices are derived from the same asset combination (which may depend on context). Using data on expenditure and savings questions on a subset of households from 2004, 2009, and 2013 MIS data, regression analysis will be used to impute incomes for all surveyed households which also provided information on assets and ownership. Per capita income will be calculated as a proxy for household welfare, and used to rank the households into deciles. This study will examine self-reported incidence of malaria and prevalence of infection across welfare groups, and establish whether the malaria control interventions have an effect on health improvement, as well as welfare-enhancing (pro-poor), on the population of Bioko Island in Equatorial Guinea.

## 1085

### IMPLEMENTING THE GLOBAL TECHNICAL STRATEGY AT THE DISTRICT LEVEL: INDIVIDUAL CAPACITY BUILDING IN MALARIA SURVEILLANCE IN SENEGAL

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Malaria surveillance is now a globally recognized intervention strategy to achieve pre-elimination and elimination. Senegal has adopted this strategy and recently intensified its malaria surveillance system by strengthening the capacity of the National Malaria Control Program (NMCP) to conduct surveillance supervision, case detection, case investigation, and response. In 2015, the NMCP, with support from MEASURE Evaluation, conducted a national surveillance and monitoring and evaluation (M&E) workshop to train subnational NMCP personnel in fundamental M&E concepts and practical surveillance approaches. As more districts in Senegal move toward pre-elimination and elimination, a robust malaria surveillance system, and personnel to lead it, will be essential. To date, 49 NMCP staff have completed this three-week training, learned to develop M&E plans, conducted surveillance supervision, and led case investigations. Surveillance supervisions and case detection took place in four health

facilities in the Niakhar and Fatick regions, followed by case investigations at the household level, using the focal test-and-treat and focal screening test-and-treat methods. In the coming years, the NMCP plans to train all relevant surveillance and M&E staff from the 76 health districts in malaria surveillance best practices. The NMCP anticipates leading five sessions, with two sessions per year. Success from this program may inform scale-up of capacity for malaria surveillance systems in other countries in low-transmission settings where malaria surveillance as an intervention would be beneficial.

## 1086

### CONCORDANCE OF QPCR AND MICROSCOPY FOR ENDPOINT ANALYSIS IN CLINICAL TRIALS OF ANTIMALARIAL DRUGS AND VACCINES

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While microscopy remains the gold standard for diagnosis of malaria and for quantitation of parasite counts, it is well recognised to be less sensitive compared to qPCR (limit of detection (LOD) of ~10 vs 0.02-0.10 parasites/μL). Low sensitivity is particularly problematic in studies where the key endpoints for evaluation of candidate blood stage vaccines and antimalarial drugs are the parasite growth and clearance curves respectively, as many of the values are typically below the LOD of microscopy. Using two 18s rDNA qPCR assays, both of which had undergone external validation, we compared the equivalence of parasite counts for 357 blood samples analysed by thick film microscopy in a previously published trial undertaken in Vietnam, where parallel laboratory analyses by qPCR had been independently undertaken in Vietnam and Australia. The median and interquartile range for parasite counts by microscopy were 813 and 40-10,715 parasites/μL. Excellent intra-class correlation (>0.97) of log-transformed parasite counts for microscopy and qPCR were observed and no systematic or proportional differences by Passing-Bablok regression were observed, indicating equivalence of microscopy and the two qPCR methods. Intercepts were close to zero [0.06 (95%CI -0.18, 0.28) and -0.01 (95%CI -0.27, 0.23)] and slopes close to one [1.01 (95%CI 0.97, 1.04) and 0.97 (95%CI 0.93, 1.00)] for Vietnamese and Australian qPCR, respectively. There was a tendency for higher discrepancies between methods at low counts, where microscopy is known to be less reliable. There was close agreement in mean log-transformed parasite counts by paired t-test (difference 0.04, 95%CI -0.01, 0.10, p=0.09) between microscopy and Vietnamese qPCR, but disagreement (difference -0.25, 95%CI -0.30, -0.20, p<0.001) between microscopy and Australian qPCR, indicating lower mean parasite counts by Australian qPCR after storage, transport and additional processing of samples. We conclude that qPCR gives equivalent parasite counts and is a viable alternative method to quantitate malaria parasite counts and to estimate parasite growth and clearance rates.

## 1087

### CAPACITY BUILDING FOR VECTOR BORNE DISEASE CONTROL PROGRAM FOR THE SUB DISTRICT LEVEL SURVEILLANCE AND RAPID RESPONSE TEAM (SRRT) IN KRABI PROVINCE, THAILAND

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Vector borne diseases are illnesses caused by pathogens and parasites in human populations such as malaria, dengue and chikungunya fever, etc. The Bureau of Vector Borne Disease works together with partners and many different government sectors in Koh Lanta District, Krabi Province to strengthen and enhance the capacity building in the proactive surveillance system including case detection, case investigation and vector control measures for the sub district health officers called as Surveillance and Rapid Response Team (SRRT) which includes 3 sub district health promoting hospitals, 2 sub district municipality administrative organizations, 10 village health volunteers, 5 village malaria volunteers and 5 NGO workers in the sub district areas. The objective of the surveillance training program is to provide basic knowledge on malaria and vector borne diseases and to enable health personnel at sub district level to do malaria and vector borne disease surveillance, investigation, prevention and control. The results have shown that knowledge of malaria and vector borne diseases among 25 observations were in moderate level having the good practice and attitude about the vector borne disease prevention and control. The capacity building for SRRT team at sub district level is very helpful for malaria elimination and vector borne disease control program. This will be useful for the SRRT to do case investigation and control diseases effectively and also transfer the knowledge to health personnel and villagers for effective prevention and control of vector borne diseases in the near future.

## 1088

### LESSONS LEARNED: MALARIA CASE MANAGEMENT TRAINING COMMUNITY IN MADAGASCAR

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In Madagascar the scaling up of malaria management through the IMCI approach, complementary to that applied in health facilities, stipulated in the National Strategic Plan, was effective in 2012. Community health workers have been trained. To help improve the quality of service and care at the community level, the Malaria Control Branch with the support of OMS to upgrade these community workers in district of Vatomandry in 2017. A training day for community health workers on the management of malaria cases was conducted in the six targeted communes: Tsiangina, Ifasina, Niarovana, Ilaka East, Ambodivoananto and Ampasamadinika. The training focused on the quality of diagnosis with the practice of TDR and treatment with Artesunate-Amodiaquine. Participants were assessed using pretests and posttests to assess knowledge gains on case management. Of the 134 participants, 66.5% were women and 33.5% were men. Overall, 89% of participants stated that the training met their expectations. 72% of participants in Tsiangina improved their knowledge and skills after training - with an average increase of 13 percentage points between pretest and posttest. Similarly, 70% of participants in Ifasina increased their scores by an average of 8 percentage points, 50% of participants in Niarovana increased their scores by an average of 6 percentage points, 50% of participants in Ilaka Est increased their scores by an average of 6 increased their scores by an average, 71% of participants in Ambodivoananto increased their scores by an average of 11 percentage points and 40% of participants in Ampasamadinika increased their scores by an average of 4 percentage points. Average pre and posttest scores were 54% à 67% pour Tsiangina, 56% à 64% for Ifasina; 58% à 64% for Niarovana et 58% à 64% for Ilaka Est. 53% à 64% for Ambodivoananto. 62% à 66% for Ampasamadinika. Participants will use the knowledge gained from this training to improve the quality of diagnostic and treatment in their communities, aimed at reducing malaria related morbidity and mortality rate in remote areas of Madagascar.

## 1089

### CLINICAL AND LABORATORY PREDICTORS OF DEATH IN AFRICAN CHILDREN WITH FEATURES OF SEVERE MALARIA: A SYSTEMATIC REVIEW AND META-ANALYSIS

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The criteria for defining severe malaria have evolved over the last 20 years. We aimed at assessing the strength of association of death with features currently characterizing severe malaria by using a systematic review and meta-analysis. Electronic databases (Medline, Embase, Cochrane Database of Systematic Reviews, Thomson Reuters Web of Knowledge) were searched to identify publications including African children with severe malaria. PRISMA guidelines were followed. Selection was based on: design (epidemiological, clinical and treatment studies), setting (Africa), participants (children <15 years with severe malaria), outcome (survival/death rate), and prognostic indicators (clinical and laboratory features). Quality assessment was performed following the criteria of the 2011 Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2). Odds ratios (ORs) were calculated for each study and prognostic indicator, and when a test was assessed in at least two studies, pooled estimates of ORs were computed using fixed- or random-effects meta-analysis. 601 articles were identified and screened and 30 publications were retained. Features with the highest pooled ORs were: renal failure (5.96 [2.93-12.11]), coma score (4.83 [3.11-7.5]), hypoglycemia (4.59 [2.68-7.89]), shock (4.31 [2.15-8.64]), and deep breathing (3.8 [3.29-4.39]). Only half of the criteria had an OR > 2. Features with the lowest pooled ORs were: impaired consciousness (0.58 [0.25-1.37]), severe anemia (0.76 [0.5-1.13]), and prostration (1.12 [0.45-2.82]). The findings of this meta-analysis show that the strength of association between the criteria defining severe malaria and death is quite variable from one to the other clinical and/or laboratory features (OR ranging from 0.58-5.96). This ranking allowed identifying features weakly associated with death, such as impaired consciousness and prostration, which could assist in refining case definitions, and hence optimize antimalarial treatment.

## 1090

### POVERTY CONSTRAINS BED NET USE AMONG ETHNIC MINORITIES IN CENTRAL VIETNAM

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Malaria in impoverished ethnic groups in remote forested areas is a challenge for malaria elimination in Vietnam. It is important to understand what factors influence their actual usage of long-lasting insecticidal nets (LLINs). This study aimed to assess constraints to LLIN use in an ethnic minority. Between April 2009 and January 2011, an anthropological study, using a mixed-method exploratory design, was carried out in four remote villages of the M'nonng and Ca Dong ethnic minority in central Vietnam. The qualitative strand was carried out to assess structural socio-cultural factors impacting on the bed net use and exposure to malaria. The quantitative strand was administered in one of the four villages to quantify

the ownership and the utilization of LLINs. One-hundred-forty-one people from 80 households participated in the quantitative strand. Poverty and the related harsh living conditions were the important constraints to the regular use of LLINs. (i) Residence patterns. 71% of households had multiple residences, consisting of a village house and a plot hut on their farmland. Homes located at farms in the forest are not taken into consideration in the LLIN distribution program, leading the reported shortage of LLINs. (ii) Housing and the social use of space. LLINs get easily torn due to bamboo construction materials, children using them to play and chickens kept indoors. Due to harsh climate conditions and poor housing structure, they traditionally sleep around the kitchen fire (60% of households), leading to low use of LLINs as nets can catch fire. The humid cold and the lack of good support to hang nets led to the use of nets as blankets (92%) especially during the rainy season despite 83% stating to use the nets. Perceived etiology. Effective bed net use was further limited by a lack of understanding of malaria etiology. In addition to the relevance of knowledge on malaria etiology and other sociocultural factors, poverty-related factors were the main constraints to bed net use. They cannot be overcome by traditional campaigns and require more trans-disciplinary approaches to health care.

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### ANALYSIS OF LLIN USE IN INFORMAL KORANIC RESIDENTIAL SCHOOLS OF DAROU MOUSTY HEALTH DISTRICT, SENEGAL

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The use of long lasting insecticidal nets (LLIN) has not achieved targets in Senegal despite the efforts made by the Government through National Malaria Control Program (NMCP) and partners to ensure availability, including campaigns of mass distribution to target every sleeping space, availability through routine channels, and communication campaigns. Students (talibés) of the residential Koranic schools (dahras) are a population at high risk for severe malaria, and have particularly poor LLIN access and use. The living conditions in dahras are often favorable to malaria transmission. We studied the determinants associated with the use of LLIN in the dahras of one health district. A cross-sectional, descriptive and analytical study of talibés and their teachers (marabouts) was conducted in the health district of Darou Mousty, in the North, which hosts many dahras. The data were collected at the level of the dahras using a questionnaire for individual interview after informed consent, for both talibés and marabouts. The data included socio-demographic, environmental, knowledge of malaria, LLINs, and other protective measures. They were entered and analyzed using EPI INFO software version 3.5.3. A total of 400 talibés and 40 marabouts were enrolled. The mean age of the talibés was 10.7 +/- 2.6 years; males outnumbered females by 15.7 fold. The proportion of talibés not schooled in french was 90.2%. The rate of utilization of the LLIN of 33.9%. The use of the LLIN was positively correlated to knowledge of the talibés of the utility of LLIN (OR = 2.7, CI = [1.5 - 4.6]), knowledge of the mode of malaria transmission (OR = 1.8; CI = [1.0 - 3.3]), the age of the talibés in the dahra (p = 0.03) and female sex (OR = 10, CI = [1.4-25], P = 0.0029), and negatively correlated with the number of talibés per sleeping space (OR = 0.5; CI = [0.3 - 0.8]). The use of LLIN in dahras requires improving the knowledge of the talibés and marabouts on malaria and LLIN, ensuring that dahras are included in mass LLIN distributions for equitable access throughout Senegal. This strategy require the support of all partners intervening with the vulnerable population.

## ASSESSMENT OF DATA USE FOR MALARIA PROGRAM DECISION MAKING IN THE DEMOCRATIC REPUBLIC OF THE CONGO

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The use of data and information to guide policy and program implementation is critical to successfully reaching intervention targets. However, most national malaria control programs and departments of health information systems in malaria endemic countries face challenges to data use, particularly at the subnational level. The USAID-funded MEASURE Evaluation project conducted an assessment in the Democratic Republic of the Congo (DRC) to identify how data are being used for decision making and how future interventions can be designed to promote data demand and use for decision making. The assessment was conducted at the national level and at the provincial level in Haut Katanga, Kinshasa, and Lualaba. The methodology comprised individual interviews, a group assessment, and a site visit checklist to look at technical, organizational, and behavioral determinants of data use. The assessment gave insight into the main barriers preventing data use for decision making among the national and provincial teams. For example, most of the 38 participants acknowledged that they are not using data for decision making, because of data quality concerns. These findings paved the process for strategic action planning to overcome these barriers and, ultimately, to improve the culture of data use. Participants prioritized three key programmatic questions to answer with data in the two months following the assessment. They were also encouraged to use action plans to delegate tasks and responsibilities and to produce timelines for these activities to ensure completion. The assessment process increased participants' commitment to the data use cycle—in which data use improves quality, which in turn leads to further use. These activities will launch the data use cycle in the DRC and are anticipated to lead to better quality data.

## CYP2D6 POLYMORPHISMS INVOLVED IN PRIMAQUINE TREATMENT OUTCOME OF MALARIA PATIENTS

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Malaria is life-threatening disease caused by *Plasmodium* parasites. *Plasmodium vivax* is the second common cause of malaria after *Plasmodium falciparum*. The presence of hypnozoites is reported to cause relapse after the initial attack for up to about 2 years. Primaquine, which is the FDA approved drug, has been used for treatment relapse and against hypnozoite. However, relapse of *P.vivax* due to drug failure still occurs. Human cytochrome P450 isoenzyme 2D6 (CYP2D6) is a highly polymorphic gene involved in the metabolism of numerous drugs. Common CYP2D6 genetic variants affect enzymatic function resulting in a wide range of enzyme activity from ultrarapid to no metabolism. Recent reports have shown that CYP2D6 may be a key enzyme involved in metabolizing primaquine into redox-active metabolites to against hypnozoite. To determine the relationship between CYP2D6 genotypes and relapse of *P. vivax* in patients in Vietnam, the CYP2D6 genotypes were determined by Luminex assay and using the activity score to predict CYP2D6 phenotypes. Data was available on 92 patients treated with primaquine, in which there were 28 recurrent cases. Preliminary results have shown that CYP2D6\*10, which is a reduced function allele, have a high frequency at about 52.6%, and frequency of the non-functional alleles represent about 8% with CYP2D6\*4, \*5. Among the 92 patients, 38 (41.3%) were classified as intermediate metabolizer (IM), 47 (51.1%) as extensive metabolizer (EM), 1 (1.1%) as poor metabolizer and the last one was ultra-rapid extensive metabolizer. Out of 28 recurrent patients, CYP2D6 phenotype was observed at frequency with 42.9% IM and 50% EM. However, the sample size in the clinical trial was limited. So there

was not significantly different between non-relapsed group and relapsed group with the level of the CYP2D6 enzyme activity on primaquine treatment. Larger sample size is necessary to explain primaquine treatment failure cases. The CYP2D6-Primaquine interaction will help to understand the rates of primaquine failures and to improve the overall effect of anti-relapse therapies such as the different primaquine dosages and combination of other drugs.

## FACTORS INFLUENCING UTILIZATION OF ANTIMALARIALS IN NIGERIA

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Globally, the use of artemisinin - based combination therapy (ACT) has contributed significantly to the reduction in malaria-related morbidity and mortality. In 2005, Nigeria adopted ACT as the first line treatment for uncomplicated malaria and the availability of ACTs in both public and private sectors has increased over time. It is assumed that the effectiveness of ACTs coupled with its increased availability would result in reduced utilisation of non-recommended antimalarials (NRA). This outcome is most desired as NRA act as potential triggers for development and spread of antimalarial drug resistance. As mothers and health providers play a crucial role in the treatment provided to children under- five (U5), this paper seeks to determine mothers' individual factors, influence of source of antimalarials and information on the use of ACTs and NRA in Nigeria. Secondary data analysis of 2013 Nigeria Demographic Health Survey was conducted. The outcomes were use of ACTs and NRA for malaria treatment in U5 in both public and private sectors. Explanatory variables include mother's age, marital status, education, and residence. Overall, 49.1% of mother sought antimalarial treatment from the private sector, 39.9% in public sector while 9.9% administered antimalarials without seeking treatment. Overall, 27.8%, 25.6% and 17.8% of mothers administered Chloroquine (CQ), Sulphadoxine-pyrimethamine (SP) and ACTs respectively. Mother of U5 living in the rural area, married and younger mothers were less likely to use ACTs in the public sector while mothers with secondary (OR: 2.1; 95% CI: 1.5-5.0) and higher level of education (OR:4.3; 95% CI: 1.4-13.0) were more likely to use ACTs in the private sector. Watching television was significantly associated with use of ACT in the private sector (OR:2.3;95% CI:1.2-4.3). This study revealed low use of ACTs especially in rural areas, and increased use of CQ and SP. Ensuring availability of affordable ACTs for rural populace and phasing out NRA in Nigeria are recommended. Mothers who do not seek care before antimalarial treatment should be targeted for media messages on ACT.

## REAL-TIME COMMUNITY SURVEILLANCE FOR MALARIA CONTROL IN MADAGASCAR

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While malaria is endemic in Madagascar, the potential for outbreaks is high. Early identification of these outbreaks is critical for initiating an effective response, including case management and supply forecasting to prevent shortages of commodities for diagnosing and treating cases. However, the traditional surveillance system relies on hand-written reports, which results in delays. In order to improve timely reporting, the USAID Mikolo Project created a real-time community surveillance tool. Beginning in 2014, the Project customized District Health Information System (DHIS2) database software for epidemiological surveillance data collected

by Community Health Volunteers (CHVs). Technical assistants support CHVs in the 8 project regions to collect their monthly paper reports, and enter their data into DHIS2 using tablets. Data includes case information (location, demographics, rapid diagnostic test [RDT] results) and the availability of RDTs and artemisinin-based combination therapy (ACT). The data is reviewed by Project staff monthly; when a surge in malaria cases, or shortages of RDT and/or ACT are observed, the Ministry of Health's (MOH) malaria unit is notified. In March 2016, the tool enabled Project staff to identify a surge of confirmed (RDT-positive) cases in children under 5 (CU5)-- from 14,410 in December 2015 to 18,340 in March 2016 across 8 regions. MOH staff were notified, and resources were mobilized. As a result, the number of confirmed malaria cases treated with ACTs by CHVs increased from 71% in March 2016 to 82% by September 2016, and stock-outs of ACTs decreased from 23% to 9% during the same time period. There were 202,520 confirmed malaria cases in CU5 throughout 2015, compared with 109,028 cases in 2016, which represents a 45% decrease in malaria cases. USAID Mikolo's DHIS-2 data collection system allowed for real-time community surveillance which was used to alert the MOH about an increase in malaria cases, enabling them to mobilize partners and respond to the outbreak. Rapid response facilitated stock management, and may have resulted in more rapid treatment of confirmed cases and limiting of the outbreak impact.

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### REPORTED COMMUNITY-LEVEL INDOOR RESIDUAL SPRAY COVERAGE FROM 2-STAGE CLUSTER SURVEYS IN SUB-SAHARAN AFRICA

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Malaria is an important cause of morbidity and mortality in malaria-endemic areas. Indoor residual spray (IRS) is an effective intervention to control malaria, but high community-level coverage is needed to maximize its impact. We used lot quality assurance sampling to estimate IRS coverage at the enumeration level across sub-Saharan Africa since 2010. Thirty-four 2-stage cluster surveys were identified and included in the analysis. IRS coverage was defined as <50%, 50-75%, 75-85% and >85%. For enumeration areas receiving IRS a logistic regression predicted whether enumeration-area coverage exceeded 50% or not. Household-level coverage with IRS was equitable both in terms of wealth and urban/rural, with poorer and rural houses more likely to be sprayed than richer and urban houses. Coverage of indoor residual spray at the community level, however, was poor across the continent, with 54% of enumeration areas reportedly receiving the intervention not reaching 50% coverage threshold. Having > 50% coverage at the enumeration area was not associated with increased number of houses sprayed in the country. Implementation and monitoring of indoor residual coverage at small geographical scales need to improve greatly to receive maximum benefit of this costly intervention.

## 1097

### PROVIDER ORIENTATION TO MALARIA CASE MANAGEMENT GUIDELINES IN REGIONAL HOSPITALS

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Malaria remains the leading cause of consultations, hospitalization and death in health facilities in Burkina Faso. In 2015, 23,634 cases of severe malaria were recorded in hospitals with 1,634 deaths, a mortality rate of 7% at this level compared to 1% nationally. Since April 2014, 1,819 providers from 49 districts have been trained in malaria case management, specifically at the first level (health center - CSPS). Conversely, at referral centers - medical centers with surgical units (CMA), regional hospitals (CHR) and university hospitals (CHU) - providers are not well educated on the new WHO guidelines for malaria prevention and case management. This situation led the United States Agency for International Development-

supported Improving Malaria Care (IMC) project and the National Malaria Control Program (NMCP) to organize orientation sessions for providers in 8 CHR in September 2016. The sessions were conducted by trainers at the national level, supported by clinicians from hospitals including pediatricians and gynecologists. A total of 298 health workers were oriented, including 24 physicians, 157 nurses, 56 midwives, as well as pharmacists and laboratory technicians. 39% of participants were female and 43% have less than 5 years of service in these hospitals. The sessions have provided participants with an opportunity to familiarize themselves with the new guidelines for malaria prevention and case management. The orientations have also made it possible to identify the difficulties encountered by referral structures in malaria case management, which include: insufficient staff, inadequate capacity building, no blood bank in some hospitals, reagent stock-outs, inadequacies in the referral system, and insufficient equipment. To address these difficulties, staff redeployment, internal supervision, development of tools to monitor reagents stocks have been proposed. To move forward, response plans for the period of high malaria transmission is expected to be developed for these referral facilities.

## 1098

### SAFETY, TOLERABILITY, IMMUNOGENICITY AND EFFICACY OF PFSPZ VACCINE VERSUS PFSPZ-CVAC IN EQUATOGUINEAN YOUNG ADULTS

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PfSPZ Vaccine is a candidate pre-erythrocytic malaria vaccine composed of radiation-attenuated, aseptic, purified, cryopreserved *Plasmodium falciparum* (Pf) NF54 sporozoites (SPZ). In trials in the U.S. and Africa, PfSPZ Vaccine administered by direct venous inoculation (DVI) provided durable protection against heterologous strains and heterogeneous populations of Pf for at least 24 to 33 wks. A second PfSPZ-based vaccine approach - administration of low doses of non-irradiated, infectious NF54 PfSPZ (PfSPZ Challenge) under chloroquine chemoprophylaxis (PfSPZ-CVac) - protected against homologous strains of Pf in the U.S. and Europe for at least 10 wks, but had not been tested in Africa. We conducted a randomized, double blind placebo-controlled trial comparing tolerability, safety, immunogenicity and efficacy against controlled human malaria infection (CHMI) of PfSPZ Vaccine versus PfSPZ-CVac in healthy malaria-exposed Equatoguinean 18 to 35-year-old men and women. We randomized 26 subjects to receive 3 doses of 2.7x10<sup>6</sup> PfSPZ (PfSPZ Vaccine) or placebo at 0, 8 and 16 wks, and 24 subjects to receive 3 doses of 1x10<sup>5</sup> PfSPZ (PfSPZ Challenge) or placebo at 0, 4 and 8 wks after an oral dose of chloroquine (CQ) 600 mg base then CQ 300 mg base weekly (PfSPZ-CVac), followed in both groups by homologous CHMI at 10-13 wks post final vaccine dose. Adverse events (AEs) were solicited for 7 days and surveillance for unsolicited AEs done for 28 days after each vaccination. Monitoring for serious AEs was done throughout. Hematologic, renal and hepatic tests were done at baseline, 2 and 14 days after each vaccination, and prior to CHMI. Blood samples for humoral and cellular immunology were taken at

baseline and 14 days after each vaccination. Both vaccine approaches were well-tolerated, and DVI was typically straightforward with only mild pain associated with injection. Safety, immunogenicity and efficacy data will be presented. This comparison of PfSPZ Vaccine and PfSPZ-CVac will provide more information as to which product could be used in mass vaccination programs aimed at regional elimination of malaria. (ClinicalTrials.gov number, NCT02859350)

## 1099

### SAFETY, TOLERABILITY AND IMMUNOGENICITY OF PFSPZ VACCINE IN EQUATOGUINEAN CHILDREN AND OLDER ADULTS

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PfSPZ Vaccine is a candidate pre-erythrocytic malaria vaccine composed of aseptic, purified, live (metabolically active), radiation-attenuated, cryopreserved *Plasmodium falciparum* (Pf) NF54 sporozoites (SPZ). In trials in the U.S. and Africa, PfSPZ Vaccine administered by direct venous inoculation (DVI) to young adults proved safe and well-tolerated, and provided durable protection against homologous and heterogenous populations of Pf for at least 24 to 33 weeks. PfSPZ Vaccine has undergone limited testing in children in Tanzania and an ongoing trial in Kenya, but has not previously been tested in older adults. Since the eventual goal is to use PfSPZ Vaccine in mass vaccination programs for malaria elimination in specified geographical areas, it is necessary to test the vaccine in all age groups. As part of a larger randomized, double blind placebo-controlled trial, we evaluated the safety, tolerability, and immunogenicity of PfSPZ Vaccine in 62 healthy malaria-exposed Equatoguinean children and older adults. We randomized 15 adults age 36-65 years to receive 3 doses of 2.7x10<sup>6</sup> PfSPZ of PfSPZ Vaccine, and 16 children age 11-17 years, 16 children age 6-10 years, and 15 children age 1-5 years to receive 3 doses of 1.8x10<sup>6</sup> PfSPZ, or placebo at 0, 8 and 16 weeks. A sentinel group of 3 subjects was vaccinated one day prior to the rest of each age cohort. Age de-escalation was done sequentially in children, with safety data evaluated by an external data and safety monitoring board before progressing to the youngest group. Adverse events (AEs) were solicited for 7 days and surveillance for unsolicited AEs done for 28 days after each vaccination. Monitoring for serious AEs was done throughout. Hematologic, renal and hepatic tests were done at baseline, 2 and 14 days after each vaccine dose. Blood samples for immunologic assays were taken prior to and 14 days after each vaccine dose, and at 4 and 20-24 weeks after the final vaccine dose. The vaccine was well-tolerated in all age groups, and DVI was generally straightforward with only mild pain associated with injection. Safety and immunogenicity data will be presented. (ClinicalTrials.gov number, NCT02859350)

## 1100

### SAFETY, FEASIBILITY AND TOLERABILITY OF RADIATION ATTENUATED *PLASMODIUM FALCIPARUM* SPOOROZOITE (PFSPZ) VACCINE ADMINISTERED BY DIRECT VENOUS INOCULATION TO HEALTHY CHILDREN AND INFANTS 5 MONTHS THROUGH 9 YEARS OF AGE IN WESTERN KENYA

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A highly effective vaccine is needed to reduce the burden of malaria among African children. PfSPZ Vaccine is a pre-erythrocytic vaccine candidate composed of radiation-attenuated, aseptic, purified, cryopreserved *Plasmodium falciparum* sporozoites administered by direct venous inoculation (DVI). We conducted an age de-escalating, dose escalating, randomized, double-blind, placebo-controlled trial to assess safety, tolerability, and feasibility of one or two doses of PfSPZ Vaccine in children residing in Siaya County, western Kenya. From July to November 2016, we enrolled children in three age groups (5-9 years, 13-59 months, 5-12 months), and vaccinated with doses of 1.35/2.7/4.5/9.0/18.0 x 10<sup>5</sup> PfSPZ or placebo. Each age-dose group consisted of 8 vaccine and 4 placebo recipients. Two vaccinations, 8 weeks apart, were administered to those receiving the two highest doses. DVI success rate was recorded. Safety and tolerability were evaluated through daily visits for 5 days post vaccination and on days 8 and 29. We performed 233 vaccinations on 165 participants, including 9 partial vaccinations in children <5 years. There were no Grade 3 related adverse events (AEs), laboratory abnormalities, or significant electrocardiogram changes post vaccination. We noted possibly to definitely related solicited local and systemic AEs (Grade 1/2) during 7 days post vaccination in 20.6% and 10.3% of participants, respectively, and possibly related unsolicited AEs (Grade 1) during 28 days in 6.1% of participants; all resolved. All new day 8 post-vaccination Grade 1/2 laboratory abnormalities resolved. Three serious AEs were related to incident malaria infection, and one grade 3 AE to a blood draw; all resolved. 156 children received the full vaccine dose: 91.5%, 81.6%, and 75.3% in the 5-9 year, 13-59 month, and 5-12 month age groups, respectively, were vaccinated on first injection. Unblinded data will be presented. DVI with PfSPZ Vaccine was tolerable, safe and feasible in children and infants. These data provided the foundation for an ongoing phase 2 trial of PfSPZ Vaccine to assess safety, immunogenicity, and efficacy in 5-12-month-olds in Siaya.

## 1101

### DOSE DEPENDENT INFECTIVITY OF DIRECT VENOUS INOCULATION OF ASEPTIC, PURIFIED, CRYOPRESERVED *PLASMODIUM FALCIPARUM* 7G8 SPOOROZOITES IN MALARIA-NAIVE ADULTS IN BALTIMORE, USA

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Direct venous injection (DVI) of aseptic, purified, cryopreserved *Plasmodium falciparum* (Pf) NF54 sporozoites (SPZ) (Sanaria® PfSPZ

Challenge (NF54) mimics controlled human malaria infection (CHMI) by mosquito bite. Development of additional strains for CHMI by DVI would facilitate vaccine and therapeutics testing against representative parasitic strains circulating in nature that are heterologous to vaccine strains. Pf7G8 is a Brazilian clone with a divergent genomic sequence from PfNF54. We conducted a double blind, randomized, dose escalation study to assess the infectivity PfSPZ Challenge (7G8) administered by DVI. We randomized 30 malaria-naïve adults to five groups. Four groups received escalating doses of PfSPZ Challenge (7G8) (800, 1600, 3200 and 4800 PfSPZ) and one group received standard dose PfSPZ Challenge (NF54) that infects 100% of malaria-naïve individuals (3200 PfSPZ). We monitored participants daily for symptoms. Daily quantitative polymerase chain reaction (qPCR) testing for parasite RNA and DNA determined malaria patency. Participants tolerated injections well. Only mild local post-injection reactogenicity was recorded (12/30 participants), and only one participant (NF54 group) experienced fever due to malaria before diagnosis and treatment initiation with atovaquone/proguanil. Infectivity of PfSPZ Challenge (7G8) was dose-dependent: 43% (800 PfSPZ; n=7; 95% CI 10-82); 57% (1600 PfSPZ; n=7; 95% CI 18-90); 89% (3200 PfSPZ; n=9; 95% CI 52-100); and 100% (4800 PfSPZ; n=2; 95% CI 48-100); Pearson correlation coefficient = 0.98. All five participants who received PfSPZ Challenge (NF54) developed Pf parasitemia. PfSPZ Challenge (7G8) administered by DVI was well tolerated by malaria-naïve adults. Higher doses of PfSPZ may be required to ensure 100% infectivity of Pf7G8 compared to PfNF54. Additional studies of other Pf strains are needed to confirm the infective dose for each. The availability of multiple Pf strains of diverse geographic origin will greatly aid clinical development of candidate malaria vaccines to inform heterologous efficacy and dose optimization.

## 1102

### SAFETY AND TOLERABILITY OF A METABOLICALLY ACTIVE, NON-REPLICATING, WHOLE ORGANISM MALARIA VACCINE IN MALARIA-EXPERIENCED ADULTS IN BURKINA FASO

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*Plasmodium falciparum* sporozoite (PfSPZ) Vaccine is a metabolically active, non-replicating, whole malaria sporozoite vaccine with an extremely favorable safety profile that is protective against *P. falciparum* controlled human malaria infection in malaria-naïve individuals. A recent clinical trial demonstrated 52% protection by time to event and 29% protection by proportional analysis against naturally occurring infection in Malian adults receiving five doses of 2.7 x 10<sup>5</sup> PfSPZ. We aimed to assess the safety and protective efficacy of higher doses of PfSPZ Vaccine against naturally acquired Pf in malaria-experienced adults in Burkina Faso. We conducted an open-label dose escalation study in four cohorts of eight participants who received two vaccinations at 12-14 week intervals. Vaccine dose increased from 4.5 x 10<sup>5</sup> to 9 x 10<sup>5</sup> to 1.8 x 10<sup>6</sup> to 2.7 x 10<sup>6</sup> PfSPZ in a step-wise manner after safety and tolerability assessments. Data Safety and Monitoring Board (DSMB) members conducted reviews after the first dose of 2.7 x 10<sup>6</sup> PfSPZ, and after the first four cohorts received dose two. All 32 participants received two vaccinations except for one participant in the 1.8 x 10<sup>6</sup> PfSPZ dose group who was lost to follow-up. No participant experienced local solicited symptoms after either vaccination. Only two clinical laboratory abnormalities were noted after vaccination, both deemed related to other causes—mild ALT elevation in one participant and moderate anemia in another. No related grade two or three adverse events were recorded through Day 56 following second vaccination, and only eight mild grade 1 adverse events were noted. The vaccine demonstrated a favorable safety and tolerability profile at all doses tested. At the DSMB's

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recommendation, recruitment and vaccination of a fifth cohort of 80 individuals occurred in early 2017. This double-blinded part of the study randomized participants to receive three doses of either 2.7 x 10<sup>6</sup> PfSPZ Vaccine or normal saline placebo. We will assess overall and strain-specific efficacy against malaria infection during the malaria transmission seasons in 2017 and 2018.

## 1103

### HOMOLOGOUS AND HETEROLOGOUS PRIME BOOST VACCINATIONS WITH DISTINCT VARIANTS OF PLASMODIUM VIVAX CIRCUMSPOROZOITE PROTEIN (CSP) PROTECTS MICE AGAINST TRANSGENIC PB/PV SPOOROZOITE CHALLENGE

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Vaccine development against *Plasmodium vivax* malaria lags behind that for *Plasmodium falciparum*. To narrow this gap, we administered recombinant antigens based on *P. vivax* circumsporozoite protein (CSP) to mice. We expressed two novel soluble chimeric proteins by merging the three central repeat regions of different CSP alleles (VK210, VK247, and *P. vivax*-like). The first construct (yPvCSP-AllFL) contained the fused repeat regions flanked by N- and C-terminal regions. The second construct (yPvCSP-AllCT) contained the fused repeat regions and the C-terminal domain only. We generated replication-defective adenovirus vectors expressing CSP of human serotype 5 (AdHu5) and chimpanzee serotype 68 (AdC68). Mice were vaccinated with three doses of yPvCSP in adjuvants Poly (I:C) or Montanide ISA720, or with a regimen of a primary AdHu5 or AdC68 immunization followed by two immunizations with yPvCSP in adjuvant. Regardless of the adjuvant used, mice immunized with yPvCSP-AllFL or yPvCSP-AllCT generated high IgG titres specific to all CSP alleles. After challenge with *P. berghei* ANKA transgenic parasites expressing Pb/PvVK210 or Pb/PvVK247 sporozoites, significant time delays for parasitemia were observed in all vaccinated mice. These vaccine formulations should be clinically tried for their potential as protective universal vaccine against *P. vivax* malaria.

## 1104

### PFSPZ VACCINE INDUCES T CELL RESPONSES TO SPOOROZOITES AND FOUR MALARIA ANTIGENS

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T cell responses to multiple *Plasmodium falciparum* (Pf) antigens are thought to mediate protection induced by PfSPZ Vaccine. PBMCs from subjects immunized with 3- and 5-doses of PfSPZ Vaccine were tested in a FLUOROSpot assay to measure single IFN- $\gamma$ , single IL2, and double (IFN- $\gamma$  + IL2) responses to NF54 Pf sporozoites (PfSPZ) and PfCSP, PfAMA1, PfTRAP/SSP2 and PfCeITOS peptide pools. The 5-dose regimen induced 92% protection against homologous (Pf3D7) controlled human malaria infection (CHMI) and 80% protection against heterologous (Pf7G8) CHMI 3 weeks after final immunization; the 3-dose regimen induced 87% efficacy to homologous CHMI. At 24 weeks after final immunization, the 5-dose regimen induced 70% efficacy against homologous CHMI but only 10% against heterologous CHMI; the 3-dose regimen induced

57% efficacy to homologous CHMI. Responses to PfSPZ peaked post-1st, and occurred in all subjects. In the 5-dose regimen, IFN- $\gamma$  and IL2 responses dropped significantly post-3rd and post-4th and rose post-5th. In the 3-dose regimen, IFN- $\gamma$  and IL2 responses were similar to those induced by the 5-dose regimen post-1st, significantly dropped post-2nd and remained unchanged post-3rd. IFN- $\gamma$ +IL2 responses were lower than IFN- $\gamma$  and IL2 but had similar kinetics. While the kinetics of IFN- $\gamma$  and IL2 activities against peptide pools was similar to PfSPZ, the magnitude of response was lower compared to PfSPZ responses (peptides range: 1-319 sfc/m; PfSPZ range: 1-1344 sfc/m). In both regimens, IFN- $\gamma$ +IL2 response against any peptide was rare. The most frequent activity was against PfTRAP/SSP2. Most subjects were positive to one or two antigens but 25% were negative to all antigens, consistent with MHC-restriction, and with the concept that other antigens may contribute to protection. Responses to PfCSP, PfCeITOS and PfTRAP/SSP2 were more frequent in protected subjects at the 3-week CHMI and were largely absent at the 24-week CHMI. Responses to PfAMA1 increased at the 24-week CHMI; the significance of this is not clear. Overall, these results show that the Pf specific T cell responses in subjects immunized with the 3- and 5-dose regimens were similar.

## 1105

### PROTECTIVE EFFICACY OF DIRECT VENOUS INOCULATION OF ESCALATING DOSES OF PFSZ VACCINE AGAINST CHMI BY DIRECT VENOUS INOCULATION OF PFSZ CHALLENGE IN TANZANIAN ADULTS

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*Plasmodium falciparum* (Pf) Pf sporozoite (SPZ) Vaccine administered by direct venous inoculation (DVI) has been extremely well tolerated and safe in studies with volunteers from malaria naïve and endemic populations. PfSPZ Vaccine induces durable, sterile protection against homologous and heterologous controlled human malaria infection (CHMI) with Pf in naïve volunteers (U.S.), and durable protection against heterogeneous Pf parasites in the field. Previously available data indicated a dose response relationship between increasing the numbers of PfSPZ/dose and improving protective efficacy, and an indication that the vaccine could be administered in 3 doses as opposed to 5 doses. This relationship has not been assessed in malaria-exposed volunteers from endemic areas. To address this deficit, between 2014 and 2017, we conducted two double blind, normal saline placebo-controlled trials at the Bagamoyo Clinical Trials Unit of the Ifakara Health Institute in a total of 94 male and female Tanzanian adults aged 18 to 45 years. These trials, in addition to determining the tolerability and safety, were aimed at demonstrating dose response immunogenicity and protective efficacy against CHMI at 3 and 24 weeks, following either 5 doses (each with  $1.35 \times 10^5$  PfSPZ or  $2.7 \times 10^5$  PfSPZ given at 4 week intervals with last dose at 8 weeks) or 3 doses (each with  $9.0 \times 10^5$  PfSPZ or  $1.8 \times 10^6$  PfSPZ given at 8 week intervals). The last CHMI was administered in March 2017. Preliminary results indicate that the two 3-dose regimens with a higher total number of PfSPZ gave better protection against CHMI at 3-11 weeks after last dose (8/12=67%) as compared to the 5-dose regimens with a lower total number of PfSPZ (5/39=13%) ( $p=0.006$ , Fisher's exact test-2 tailed), but still have not achieved significant long term (24 weeks) protection against CHMI. The complete un-blinded results of immunogenicity and protective efficacy through this spectrum of PfSPZ doses will be presented, as will plans for the next IHI study designed to determine the safety and protective efficacy of an accelerated regimen with a single boost in Tanzanian adults including HIV infected individuals.

## 1106

### A PHASE 1, BLINDED, RANDOMIZED, DOSE ESCALATION TRIAL OF PFSZ CHEMOPROPHYLAXIS VACCINATION (PFSZ-CVAC) ON AN ACCELERATED SCHEDULE IN HEALTHY MALARIA-NAÏVE ADULTS IN THE UNITED STATES

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A highly effective malaria vaccine is urgently needed to protect at-risk populations, travelers and the military. Such a vaccine could accelerate efforts to eliminate malaria globally. Direct venous inoculation (DVI) of PfSPZ Vaccine (radiation attenuated aseptically, purified, cryopreserved *Plasmodium falciparum* (Pf) sporozoites (SPZ)) protects volunteers against short and long term homologous and heterologous controlled human malaria infection (CHMI) and natural exposure to Pf in Mali. For the PfSPZ-CVAc (chemoprophylaxis vaccine) approach, subjects taking antimalarial chemoprophylaxis are administered aseptically, purified, cryopreserved infectious PfSPZ (PfSPZ Challenge). Three doses at 28-day intervals of  $5.12 \times 10^4$  PfSPZ administered to volunteers taking chloroquine (CQ) chemoprophylaxis protected 100% (9/9) against CHMI with homologous PfSPZ 10 weeks after last dose of PfSPZ. When the vaccine was administered at 5-day intervals protection was reduced to 63%. To further define an optimal dose and dosing interval for PfSPZ-CVAc, we are assessing the safety, tolerability, immunogenicity and protective efficacy of PfSPZ-CVAc with CQ chemoprophylaxis administered 3 times at 7 day intervals. There are 3 dose escalation cohorts ( $5.12 \times 10^4$ ,  $1.0 \times 10^5$  or  $2.0 \times 10^5$  of PfSPZ Challenge) with 12 subjects per cohort (9 vaccinees and 3 placebo controls each). Subjects will undergo CHMI with PfSPZ Challenge by DVI 10 weeks after last dose of vaccine. A *Plasmodium* 18S rRNA qRT-PCR is being used to detect blood-stage infections and initiate treatment in unprotected subjects. Adverse events, safety laboratory data, parasite growth kinetics, pharmacokinetics of CQ and humoral and cellular immune responses to PfSPZ will be assessed. The first cohort has undergone CHMI, and the next cohort is being enrolled. Data from all cohorts will be presented.

## 1107

### EXCEPTIONAL TOLERABILITY OF CHLOROQUINE WHEN ADMINISTERED AS CHEMOPROPHYLAXIS WITH ASEPTIC, LIVE, CRYOPRESERVED NON-ATTENUATED WHOLE PLASMODIUM FALCIPARUM SPOOROZOITES (PFSZ-CVAC) IN HEALTHY EQUATOGUINEAN YOUNG ADULTS

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PfSPZ-CVAc is a candidate pre-erythrocytic malaria vaccine which involves the direct venous inoculation of aseptically, purified, live, cryopreserved, chloroquine (CQ)-sensitive *Plasmodium falciparum* (Pf) NF54 sporozoites (PfSPZ Challenge) under antimalarial chemoprophylaxis. Using CQ as the partner drug, this approach provided high levels of protection against

homologous strains of Pf in the U.S. and Europe, but had not been tested in Africa. Among the known side effects of CQ, pruritus has been reported in up to 52% of dark-skinned individuals, and can be severe. The viability of CQ as a partner drug for use in PfSPZ-CVac in Africa will depend on its tolerability. As part of a larger double blind placebo-controlled trial, 24 healthy malaria-exposed Equatoguinean 18 to 35-year-old men and women were randomized to receive 3 doses of 1x10<sup>5</sup> PfSPZ (PfSPZ Challenge) or placebo at 0, 4 and 8 weeks. All subjects were given CQ diphosphate 1000 mg (620 mg CQ base) orally 2 days prior to PfSPZ Challenge, followed by 500 mg CQ diphosphate (310 mg CQ base) orally weekly. CQ administration was directly observed. Previous testing had verified the quality of the CQ, and whole blood drawn 2 days after the initial CQ dose contained >15 ng/mL in all subjects. Malaria parasitemia was ruled out by blood smear and qPCR prior to each vaccination. Fifteen potentially CQ-related adverse events (AEs) (GI symptoms, neurologic and psychiatric symptoms, pruritus, fatigue, and myalgia) plus 10 general AEs were solicited daily for 12 days, then at 14, 19 and 26 days post-vaccination. Surveillance for unsolicited AEs was done for 26 days after the vaccine dose. After the first four weeks, 1 (4.2%) subject had nausea, 1 (4.2%) had tinnitus, 1 (4.2%) had myalgia, 2 (8.3%) had fatigue, 2 (8.3%) had pruritus, and 4 (16.7%) had headache and/or dizziness. All symptoms were mild, transient and required no treatment. There were no related unsolicited AEs. The extremely low incidence of pruritus was unexpected and encouraging; it may be related to the chemoprophylaxis versus treatment dose of CQ used. (ClinicalTrials.gov number, NCT02859350)

## 1108

### IMMUNIZATION OF NON-HUMAN PRIMATES WITH A *PLASMODIUM FALCIPARUM* WHOLE PARASITE VACCINE INCLUDING PARASITE SEXUAL AND MOSQUITO STAGES INDUCES ANTIBODIES THAT BLOCKS PARASITE TRANSMISSION TO MOSQUITOES

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A vaccine that interrupts malaria transmission (VIMT) would be a valuable tool for malaria control and elimination. Sanaria is exploiting its capacity to manufacture *Plasmodium falciparum* (Pf) whole parasite vaccines by producing sexual and mosquito stage (SMS) parasites under cGMP conditions. Compared to subunit vaccine approaches, a whole parasite (WP) Pf SMS-WP-VIMT would increase the diversity of antigens presented to the immune system and negate the need for complex post-translational processing of recombinant antigens. Starting with mature stage V gametocyte cultures, Pf SMS parasite development was initiated by a decrease in temperature and exchange into an exflagellation medium for 2 hours. The parasites were then introduced into ookinete medium for 1 or 22 hours to obtain parasites of different stages. The parasites were then purified using a magnetic column to eliminate uninfected erythrocytes and non-parasite material. Non-human primates (NHPs) (rhesus macaques) were immunized intramuscularly on days 0, 21, and 42 with 1.5 × 10<sup>7</sup> purified Pf SMS/dose in GLA-LSQ (glucopyranosyl lipid adjuvant in liposomes with QS-21), and boosted on day 85 with 3.0 × 10<sup>7</sup> purified Pf SMS. Anti-sera from immunized NHPs were collected 2 weeks after the third and fourth immunizations and fed with mature Pf NF54 gametocytes in a standard membrane feeding assay. Transmission blocking activity at a 1:9 dilution was equivalent to that of an anti-Pfs25 monoclonal antibody (a leading transmission blocking vaccine candidate antigen) at 125 µg/mL concentration. A Pf SMS-WP-VIMT will be an ideal complement to Sanaria's PfSPZ-based vaccines to dramatically reduce transmission by preventing blood stage infection with the pre-erythrocytic stage vaccine and preventing transmission of any breakthrough parasites with the SMS vaccine.

## 1109

### BETWEEN FILL-FINISH AND THE CLINIC: THE SUPPLY CHAIN FOR DISTRIBUTION OF PFSPZ VACCINES

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The PfSPZ platform products' (PfSPZ Vaccine, PfSPZ-CVac, PfSPZ-GA1 Vaccine) immunogen is *Plasmodium falciparum* (Pf) sporozoites (SPZ), which are eukaryotic organisms, ergo stabilization is by cryopreservation. Distribution uses a liquid nitrogen (LN2) vapor phase (LNVP) cold chain that maintains temperature below -150 oC. Critical to distribution is the product container. Standard cryovials have screw-cap with O-ring or gasket seal closures and are not suitable for many reasons, most importantly because upon opening, product sterility is compromised. We have created a new cryovial with co-molded septum for access by needle/syringe, enabling use in any location in a manner similar to typical pharmaceutical vials. During manufacture, 96-place SBS array boxes of cryovials are filled using an automated 12-channel filling machine, caps are pressure-applied forming an impervious vial-cap seal and the caps' tops are protected by applying a mat of individual heat-annealed foil seals. Boxes of cryovials are batch cryopreserved and placed into LNVP storage. For distribution, boxes are loaded into LNVP cryoshippers and air/road freighted to clinical sites. Each cryoshipper remains on-site up to one month as the vaccine store. The cryoshipper returns to base for LN2 recharging and vaccine re-stocking. Cryoshipper temperature is continuously monitored. For immunizations, vaccine vials are retrieved, thawed using an automated dry thawing device, the foil seal removed and a syringe/needle injects diluent and withdraws mixed diluted vaccine for injection. Over 380 shipments supplying >30 trials have used this LNVP cold chain to date and experience gained is being translated into expanded distribution network designs for Phase 3 clinical trials and into post-launch distribution models for travelers and focal elimination campaigns.

## 1110

### CONSISTENCY OF INFECTION AFTER CONTROLLED HUMAN MALARIA INFECTION WITH PFSPZ CHALLENGE OF DIFFERENT AGE AND LOTS

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Sanaria® PfSPZ Challenge is composed of aseptic, purified, cryopreserved infectious *Plasmodium falciparum* (Pf) sporozoites (SPZ) that are used to conduct controlled human malaria infection (CHMI) studies to assess anti-malarial vaccines and drugs, and naturally acquired immunity. Thus far, 661 volunteers in the USA, the Netherlands, Germany, Spain, the UK, Tanzania, Kenya, Gabon, and Mali have undergone CHMI with PfSPZ Challenge. Three hundred fifty of those volunteers were in Africa. An additional 134 volunteers have undergone immunization with a total of 390 doses of PfSPZ Challenge as part of Sanaria® PfSPZ-CVac. PfSPZ Challenge is stored in liquid nitrogen vapor phase (LNVP) at below -150 degrees C, and the stability of PfSPZ Challenge is assessed at Sanaria by

PfSPZ cell membrane integrity tests (viability assay) and *in vitro* growth and development in hepatocytes (potency assay). However, the most powerful indicator of the stability of PfSPZ Challenge over time has been the infectivity of PfSPZ Challenge held in LNVP for different periods of time. For the majority of studies, volunteers are injected by direct venous inoculation (DVI) with 3,200 PfSPZ derived from the NF54 strain of Pf. The infection rates and time to parasitemia vary considerably from country to country in Africa depending on the levels of naturally acquired and innate immunity in recipients. However, they are similar in non-immune subjects in the USA and Europe. To assess the potential for changes in infectivity from lot to lot and over time, we used the results of CHMI in 54 non-immune, non-immunized control volunteers in the USA, Germany, and Spain who were injected with 3,200 PfSPZ (NF54) from two different lots of PfSPZ Challenge that had been manufactured from 9 to 33 months previously. All 55 subjects developed parasitemia (100% infection rate), and the pre-patent periods after CHMI were similar over time and between lots. CHMI has been an enormously important tool to establish the lot to lot consistency and stability of infectivity of PfSPZ Challenge and by analogy of all of Sanaria's PfSPZ-based products.

## 1111

### DEVELOPMENT AND EXECUTION OF A REGULATORY PROGRAM FOR A MALARIA VACCINE TO BE LICENSED ON THREE CONTINENTS

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Sanaria's mission is to develop highly effective malaria vaccines that can be used to immunize the entire population, including pregnant women, in geographically defined areas to halt transmission and eliminate malaria, and to prevent malaria infection in individuals. In collaboration with partners, Sanaria has conducted 32 clinical trials of its *Plasmodium falciparum* (Pf) sporozoite (SPZ)-based products in the USA, the Netherlands, Germany, Spain, the UK, Tanzania, Kenya, Mali, Burkina Faso, Gabon, and Equatorial Guinea, and anticipates initiating trials in Ghana and Indonesia within a year. All of these clinical trials are conducted under Investigational New Drug (IND) applications with the U.S. FDA (n=10 INDs) and with the appropriate regulatory filings in each of the other countries internationally. The regulatory process generally starts with a pre-IND meeting with FDA at which specific questions are asked of the FDA, the responses to which facilitate successful IND preparation. In addition, the IND submission is generally preceded by submission of a Biologics Master File (BMF) to FDA on a specific PfSPZ-based product that provides critically important information that supports the specific IND. The PfSPZ-based products are all aseptic, purified, and cryopreserved in liquid nitrogen vapor phase (LNVP). They include Sanaria® PfSPZ Challenge (fully infectious), Sanaria® PfSPZ Vaccine (radiation attenuated), Sanaria® PfSPZ-CVac (PfSPZ Challenge administered under chemoprophylaxis) and PfSPZ-GA1 Vaccine (genetically attenuated). Sanaria has moved from conducting pre-clinical IND-enabling studies to phase 1 and now phase 2 clinical trials. The goal is to initiate phase 3 clinical trials in next few years. Sanaria's leading and most advanced product, PfSPZ Vaccine, was granted FDA's Fast Track Designation in 2016. The specifics and pitfalls of this complicated regulatory process, including plans for submission of a Biologics License Application (BLA) and the equivalent internationally will be presented.

## 1112

### PERIPHERAL CELLULAR RESPONSES OF HUMAN SUBJECTS IMMUNIZED VIA MOSQUITOES WITH RADIATION ATTENUATED SPOOROZOITES (IMRAS)

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Protection from malaria following immunization with radiation attenuated sporozoites (RAS) is well established, yet our understanding of the immune mechanisms of protection is incomplete. To facilitate further understanding of protective immunity induced by RAS, a phase I clinical trial was recently completed (IMRAS). Two consecutive cohorts, consisting of twelve true- and four mock-immunized subjects, received approximately 1000 infectious mosquito bites over five sessions four weeks apart, followed by controlled human malaria infection (CHMI). Protection was 55% and 90% respectively in the two cohorts. Peripheral blood mononuclear cells (PBMCs) were collected by leukapheresis at several time points throughout the study, including pre-immunization, day 14 post 3rd immunization, day 5-6 post-CHMI, and day 112 post-CHMI. A subset of four subjects received a second set of immunizations (hyper-immunization) and PBMCs were collected after the boost immunizations and second CHMI. PBMCs were stimulated for 18h with *P. falciparum* sporozoites and infected red blood cells (PfRBCs). Surface and intracellular staining were performed, and data were acquired by flow cytometry. Responding populations of CD4 and CD8 T cells, NK cells, and gamma-delta T cells were identified and production of IFN- $\gamma$ , TNF, IL-2, MIP1 $\beta$ , CD154, granzyme B, and CD107a was evaluated in response to parasite stimulation. We will present a comprehensive analysis of these cellular responses, including a comparison of responses to sporozoites and PfRBCs, and association with protection.

## 1113

### ANALYSIS OF LIVER PARASITE BURDEN FOLLOWING IMMUNIZATION WITH NOVEL MALARIA ANTIGEN PYE140 AND PLASMODIUM YOELII SPOOROZOITE CHALLENGE

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Recently we identified a novel *Plasmodium yoelii* antigen, PyE140, which induces a high level of sterile protection following immunization, as measured by the emergence of blood-stage parasites on daily thin smears. Mice immunized by DNA-prime/adenovirus serotype 5 (Ad5)-boost expressing PyE140 develop high titer antibodies which correlate with sterile protection from sporozoite challenge. High frequency CD8+ T cells were also detected in both spleen and liver following immunization, but depletion of neither CD8+ nor CD4+ T cells had any effect on sterile protection. PyE140 and its *P. falciparum* ortholog PfE140 are expressed in pre-erythrocytic (sporozoite and liver) as well as blood-stage parasites. Therefore, it is not known at which stage the parasite is targeted by E140 antigen-specific immunity. In the present study, mice were immunized against PyE140 by DNA-prime/Ad5-boost, and separate groups of mice

were challenged with 100 and 10,000 sporozoites. Livers were harvest 42 hours after sporozoite challenge, homogenized, and total RNA was isolated. RT-qPCR was performed to measure the copies of Py18S and murine GAPDH RNA. The resulting data improve our understanding of the mechanism of protection conferred by the PyE140 antigen, and will guide subsequent vaccine development approaches to develop a PFE140-based subunit vaccine against *P. falciparum* malaria.

## 1114

### REMOTE AND OBJECTIVE MONITORING OF ANTI-MALARIAL BEDNET USE IN RURAL UGANDA: INSIGHTS FROM A PILOT STUDY

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Insecticide-treated bednets (ITNs) are a mainstay of malaria prevention, recommended by the World Health Organization (WHO) for nightly use by over 3 billion people worldwide. ITNs are accepted as a cost-effective means of preventing malaria, but our tools for measuring ITN use limit our ability to examine exactly how ITNs are used. The most common tool for assessing ITN use is based on inquiring into a household's self-reported use the prior night. While effective for obtaining a general sense of ITN coverage, these measures are inadequate for relating specific ITN use behaviors to important malaria outcomes. SmartNet is a remote ITN-use monitoring system designed to provide objective measures of ITN use over time. SmartNet is integrated into a WHO-approved ITN and uses battery power to log at 15 minute intervals the use of that ITN on a memory card for later analysis. To date, we have performed lab testing, a proof of concept study among volunteers in the United States and a pre-deployment acceptability study in Uganda. SmartNet is currently undergoing a pilot study in 10 Ugandan households over six weeks to demonstrate the feasibility of objective remote ITN use monitoring. The results provide novel insights into ITN use behaviors in households at risk of malaria infection, including exact quantification of ITN coverage, daily records of the times of unfurling, and the ability to capture identifiable episodes of interruptions in use. We also relate ITN use behaviors to perceptions of malaria risk and detailed household and individual characteristics that increase the risk of malaria infection, such as night work or early rising. Remote objective ITN use monitors, such as SmartNet, provide precise measures of ITN use over extended periods of time at a far greater resolution than current methods. These tools improve our understanding of how ITNs are used in practice and greatly enhance our ability to quantify how actual ITN use relates to the risk of malaria infection.

## 1115

### URBAN LONG LASTING INSECTICIDAL NETS MASS CAMPAIGN DISTRIBUTION IN MADAGASCAR

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The Madagascar National Strategy 2013-2017 is sharing by the facies epidemiological. In the high area transmission, there are the LLIN distributions. In the otherwise, there are the localized IRS and the daily epidemiological surveillance of the low transmission. In the LLIN mass campaign 2015, we have 85% of the population so 19 112 163; 3 981 826 households and 46 217 community health workers. By the LLIN geographic coverage, the 92 districts is divided in 17 urban, 85 rural. We have distributed 10 600 750 LLINs. The universal coverage with mass free distribution of LLINs by the end of 2015 is doing in the 92 per 112 health districts targeted in LLIN. The objective is 1 LLINs to 2 persons. Involving the LLIN Mass Campaign Distribution in Urban Cities, Tamatave city is a town that is located on the east coast of Madagascar

astmh.org

and 298 000 populations. Most people work on Port is related activities and big mining company. 75% of the population lives below the poverty line. The climate is subequatorial type and the city is exposed frequent to cyclone disaster and high malaria transmission. The LLIN distributed are 146 750. The successes in campaign methodology, which is being improved in urban city based on 2013 lessons learned, have improved the training of local distribution teams, standardized guidelines and training materials, developed guidelines on waste management for urban cities, extended campaign period for 2 weeks instead of one. Then, we improved the supervision teams participation to resolve problems with the local authorities. We have emphasized BBC by the community health workers with NMCP. We have produced communication plans, mass media in the different dialects. The logistic tools are simplified. In the end, the challenges are the household registration, the late registration completion in some villages and health center caused by confused messages from other health activities taking place at the same period, some people not at home during the household registration or not willing to have their house visited. For the hang up, some people refused CHW's visit and the CHW are not recognized by the urban population.

## 1116

### FEASIBILITY ASSESSMENTS FOR ITN CONTINUOUS DISTRIBUTION IN TWO SETTINGS: KENYA AND GUINEA

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Recent pilots of continuous distribution of insecticide-treated nets (ITNs) through the education sector, health facilities, and community mechanisms show that ITN access can be sustained between universal coverage campaigns, and potentially function in lieu of mass campaigns. Kenya and Guinea provide two disparate environments to demonstrate how aspects of health systems, education systems or social structures can affect feasibility and potential effectiveness of implementation. VectorWorks conducted feasibility assessments in both countries, consisting of four weeks of interviews with stakeholders from health, education and community sectors to discern factors impacting the success of currently implemented continuous distribution channels and to examine what structures could be used to optimize new channel implementation. Kenya possesses a reliable supply chain that ensures ITNs reach health facilities as needed, making community ITN distribution by community health workers connected to health facilities feasible in endemic areas. In contrast, health facility distribution in Guinea is currently transitioning to a data driven requisition system that must be improved before community-distribution is possible. Guinea's Ebola response created a strong collaboration between health and education sectors that could serve as an effective backbone for school distribution. Both countries have strong cross-sectoral partnership between health and education offices, but due to factors like quality of roads and available human resources, would need to approach school distribution in a slightly different manner. Contextual, structural, and logistical considerations must be paired with modeling tools to determine which channel or combination of channels is ideal in a given context to provide adequate population access. This case study demonstrates that these factors determine which continuous distribution channels are viable, and explores how these nuances would shape planning and implementation of distribution.

## STREAMLINING OPERATIONS AND REDUCING COSTS IN SCHOOL ITN DISTRIBUTION IN TANZANIA: 2013-2017

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In 2011, the Ministry of Health and National Malaria Control Program of Tanzania developed a Keep Up Strategy with the goal of maintaining the population's access to an ITN at or above 80%, by using school-based distribution as an innovative distribution channel. Over the last five years, improved coordination between government offices has streamlined operations, and this has led to significant cost-savings for implementation. School distribution of ITNs was first piloted in the Southern Zone in 2013, when the NMCP and the Tanzanian Red Cross Society distributed ITNs in 2,302 schools in 19 districts in Lindi, Mtwara, and Ruvuma, a total of 421,285 ITNs, to classes 1,3,5,7 in primary school and Form 2 and 4 in secondary schools. SNP2 was implemented in 2014 by NMCP and Research Triangle Institute, delivering 489,099 ITNs to school children, and adding classes 2 and 4 in Lindi. In the third round in 2015, NMCP with JHUCCP's VectorWorks project delivered 494,407 ITNs to 1,919 schools in the 19 districts, targeting classes 1-3, 5, and 7 in primary school in Ruvuma and Mtwara, and classes 1-5 and 7 in Lindi. In 2016, SNP4 expanded to four additional regions in the Lake Zone; 1,152,715 ITNs were delivered to 5,242 schools in a total of seven regions. SNP5 in 2017 will expand to 14 regions in total. The President's Office of Regional Administration and Local Government now coordinates the majority of activities at the regional level and below. Over the last five years, enrolment data collection has been streamlined and digitized, using the Basic Education Monitoring Information System. Training requirements have been reduced (Ward Education Coordinators and teachers will no longer receive training in SNP5), and transport and storage have been optimized in collaboration with the private-sector logistics partner, who will deliver nets from the port directly to the school level. The improvements in operations have resulted in significant cost-savings, allowing scale up from 9 to 14 regions within the original budget envelope. Cost analysis is underway, and will be discussed in further detail.

## 1118

### EXPLORING MELANIN-BASED ANOPHELES GAMBIAE IMMUNE RESPONSE TO MALARIA PARASITE

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Malaria parasite transmission is dependent on the survival of *Plasmodium* during its cycle in the mosquito. The major bottleneck for parasite development occurs during traversal of the midgut epithelium, where it is exposed to human-blood derived factors, mosquito immune defenses, and gut microbiota. Indeed, melanin-based cellular immune response and midgut microbiota are responsible for the refractoriness of certain mosquito strains to *Plasmodium* infection. Several studies have suggested that parasite killing precedes the melanization step; nonetheless, molecular mechanisms that govern these processes are poorly understood. We hypothesize that key mosquito factors that associate with the parasite to

mediate killing and melanization will be trapped in the melanotic capsules. Thus, *An. gambiae* L3-5 refractory line was infected with *P. berghei* to enrich the melanotic capsules for proteomics. Melanotic capsules were successfully recovered using a protocol for fungal melanin isolation. We identified a subset of *Anopheles* proteins associated with the melanin matrix by performing a pilot proteomic analysis using a filter-aided sample preparation (FASP) procedure followed by tandem MS/MS. In parallel, a confrontational assay between *Cryptococcus neoformans* (fungal biosensor) and mosquito midgut-derived bacteria was performed to detect melanin-precursors released by the bacteria. We identified dopaminergic bacterial strains that induced fungal melanization. Accumulative evidence has shown that catecholamines regulate the crosstalk between microbes and the immune system. Currently, we are validating *Anopheles* proteins implication in parasite killing, encapsulation, and association with the parasite, as well as identifying melanin-precursors released by mosquito intestinal microbiota. This project will provide mechanistic insights on the vector-parasite interactions while enhancing our knowledge of mosquito vectorial capacity and malaria parasite transmission, possible contributing to the development of potential novel malaria control strategies to make these mosquitoes more resistance to *Plasmodium*.

## 1119

### CAN AGE AND GENDER DIFFERENCES IN THE RISK OF MALARIA BE EXPLAINED BY BEHAVIOR RELATED TO MOSQUITO EXPOSURE?

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In our studies in rural Odisha, India, as part of an NIH-funded International Center of Excellence in Malaria Research, children and men were found to be at higher risk of malaria than older people and women. Using surveys of individuals and households in the same villages, we examined if **behavior** could be identified that may impact on malaria acquisition by gender and age. Adjusting for clustering at the household level, preliminary analysis of 171 participants from 73 households shows that there were no differences by age and gender in bedtime (median 9 pm, range 5.30 pm to 11 pm); however, adults were up earlier (median 5 am, range 3-8 am) than children (<18 years, median 6 am, range 4-7 am,  $p < 0.001$ ), and females (median 5 am, range 3-7) were up earlier than males (median 5 am, range 3-8,  $p = 0.004$ ). Adult males were more likely to be outside in the evening (26%), when sleeping (21%), and in the early morning (81%), compared to adult women (7%, 1%, and 13%, respectively,  $p < 0.01$  for all comparisons) and children (9%, 2%, and 10%, respectively,  $p < 0.05$  for all comparisons). Insecticide treated net use in the previous night was lower among adults (33%) compared to children (51%,  $p = 0.005$ ), but not different by gender ( $p = 0.9$ ). More women (21%) than men (10%) said they tried to cover up exposed skin during morning and evening hours ( $p = 0.03$ ). There were no differences in time before health unit visits for fever by gender or age (median 1 day, range 0-6 days). At the household level, coils (5%), mats (1%), vaporizers (10%), insecticide spray (12%) and window screening (4%) were infrequently used. Clearing bushes (64%) and stagnating water (59%) around the house, burning leaves or dung (26%), and keeping doors/windows closed in the evening (70%) were common methods to decrease mosquito burden, and bedrooms in 26% of households were reported to have been sprayed with insecticide the year before. Outdoor **behavior** may play a role in gender differences in malaria prevalence in rural Odisha, where exophilic early biting mosquitoes are more common than in Africa. Additional protection methods to reduce outdoor exposure to mosquitoes in early morning and evening may be useful.

## 1120

**QUANTIFYING GAPS IN ITN USE TO BETTER PLAN AND TARGET MALARIA INTERVENTIONS IN MADAGASCAR**

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Though Madagascar is experiencing a significant scale-up of key malaria interventions, intervention coverage remains below the targets set by the National Malaria Control Program (NMCP). There is a need to quantify these gaps in coverage and identify specific populations with low coverage to better target interventions, particularly as Madagascar considers subnational pre-elimination strategies. Data from the 2016 Malaria Indicator Survey (MIS) were compared to the NMCP's 2016 targets for selected indicators. The target for use of insecticide-treated nets (ITNs) among children under the age of five years (U5) was 90%, and the target for pregnant women (PW) was 88%. The overall gaps between the targets and ITN use were 17 percentage points among U5 and 19 percentage points among PW. In an analysis of subgroups among U5, the gaps in ITN use for place of residence were 17 percentage points in rural areas and 10 percentage points in urban areas. Similarly, the gaps in ITN use among PW vary between rural areas (20 percentage points) and urban ones (12 percentage points). Regarding socioeconomic status, the gaps in ITN use among U5 ranged from 8 percentage points in the lowest wealth quintile to 15 percentage points in the highest. Among PW, the gap in ITN use was also lower for the lowest quintile (3 percentage points) compared to the highest wealth quintile (26 percentage points). There were additional variations by malaria epidemiological zones in ITN use among both U5 and PW. Overall, substantial progress has been made in reducing the gaps in ITN use among U5 and PW; however, there are significant variations in subpopulations. Identifying groups with large gaps allows the NMCP to target resources better during program planning and implementation. Further analyses could identify factors driving these gaps in coverage of malaria control interventions and improve program performance.

## 1121

**EMPOWERMENT EVALUATION TO ENGAGE COMMUNITY FOR MALARIA PREVENTION AND TREATMENT IN ETHNIC MINORITY POPULATIONS ALONG THE THAI MYANMAR BORDER**

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In moving toward global malaria elimination, community engagement is one important strategy to achieve a malaria free zone. The ethnic minority populations who are at higher risk of malaria need to be equipped with appropriate tools in activities related to their socio-ecological situation and lifestyle. Empowerment evaluation is an evaluation approach designed to help communities monitor. The objectives were to explore the socio-ecological situation among ethnic populations and empower the community to identify the gaps in their way of life and find solutions in preventing malaria. We conducted the study in malaria endemic villages of selected provinces in the western part of Thailand. A variety of qualitative methods were used as appropriate. The results obtained were as follows: the ethnic communities' livelihood activities were the same as before which was related to the time of malaria peak season. Some elderly villagers experienced malaria infection over 10 times in the past 10 years. But nowadays occurrence of malaria cases have slowed down. On the other hand, some aspects of their lifestyle changed from the past due to the availability of solar cells and television. Because the

electricity was limited, but they preferred to watch at night time without using neon lights which coincides with the anopheles mosquitoes biting time. Most of ladies and children watched Thai drama without protection against mosquitos. After receiving feedback, we gave 3 suggestions and empowered them to decide on the suggestions. Some villagers volunteered to use Insecticide Treated Clothes (ITC) for covering the exposed parts of their bodies. After using for 6 months, it was found that acceptability to the use of ITC was high, and they mostly preferred to cover their lower part of body. But for children, ITC was used as a blanket to cover their bodies and they also preferred for other activities. No side effects were detected. In conclusion, the empowerment evaluation will be useful for increasing and reaching the community engagement in preventing malaria outdoor transmission in the remote areas of border communities in Thailand.

## 1122

**COMPARISON BETWEEN AGE ESTIMATES OF WILD ANOPHELES ARABIENSIS USING NIRS CLASSIFICATION MODEL AND OVARY DISSECTION (DETINOVA'S METHOD)**

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Different studies have demonstrated that near-infrared spectroscopy (NIRS) accurately classifies the age of lab-reared mosquitoes with accuracy greater than 80%. Despite the ability of near-infrared spectrometry to classify the age of lab-reared mosquitoes, it is unknown if NIRS can classify the age of wild mosquitoes because we lack age-labeled wild mosquitoes with which to train a model. Training a model using labels from ovary dissection yields a model with poor accuracy. Applying a model trained on spectra from lab-reared mosquitoes to estimate the age of wild mosquitoes was unjustifiable until Milali study ran a cluster analysis on a mixture of spectra collected from lab-reared and wild mosquitoes of the same species. They found no clear difference between spectra collected from lab-reared mosquitoes and those collected from wild mosquitoes. Referring to the Milali study, we applied a classification model trained on lab-reared *Anopheles arabiensis* to classify the ages of wild *An. arabiensis*. Because we lack age labels of wild *Anopheles arabiensis*, we cannot directly validate the accuracy of the model. Alternatively, we indirectly validated our model by comparing the number of mosquitoes in each age class obtained when classification was done using our model and when done using Detinova ovary dissection. Our model classified 86% of the total 927 wild mosquitoes as young (less than seven days old) and 14% as old (greater or equal to seven days old). Detinova ovary dissection classified 72% of the same number (927) of mosquitoes as young (not laid eggs) and 28% as old (laid eggs). A Jaccard similarity analysis comparing Detinova ovary dissection and our model trained on lab-reared mosquitoes shows there is a 67% chance that the two methods will classify a mosquito into the same age class and a 33% chance they will classify a mosquito into different age classes. Hence, a classification model trained on lab-reared mosquitoes and Detinova ovary dissection are more similar than they are different.

## 1123

**"SLEEP IS LEISURE FOR THE POOR" - UNDERSTANDING PERCEPTIONS, BARRIERS AND MOTIVATORS TO NET CARE AND REPAIR IN SOUTHERN TANZANIA**

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The Government of Tanzania has made substantial effort in achieving universal coverage of Long-Lasting Insecticidal Nets to its population. Personal responsibility to maintain nets provided is crucial to sustain gains in the fight against malaria. Therefore, this study aimed to understand local perceptions, barriers and motivators of net care and repair in Ruangwa district. Qualitative research methods were applied in Kilimahewa and Makanjiro villages to three groups of people; men, women, and women with children under five. We also conducted an assessment of the physical status of nets currently in use by the study participants. Most of the study participants reported to have received nets from the School Net Programme while others purchased theirs from local stores. Continued protection from mosquitoes and in turn malaria was the main motivation to net care. Net care also ensured cleanliness which prevented other dirt-related symptoms such as sneezing. Barriers to net care included it not being a priority in the day-to-day activities, lack of education and understanding of the importance of net care. Net repair was reported necessary as soon as a hole was identified; however, during the net assessment and participatory activity, most of the nets with smaller holes were less likely to be repaired than to be left as is for continued use. Protection against mosquitoes, malaria and cost saving were identified as motivators to net repair. Barriers to net repair included it not being a priority especially as holes at the bottom could be tucked under the mattress, lack of education on the how and the importance of repairing nets. Some participants also reported they did not have tools for net repair. In southern Tanzania, there seems to be readiness to care and repair for mosquito nets, especially among women who are the primary caretakers in the households. Targeted education through health facilities and community change agents were reported as potential means to overcome barriers to net care and repair that could be reinforced for implementation by the National Malaria Control Program.

## 1124

### MISSED OPPORTUNITIES FOR UPTAKE OF INTERMITTENT PREVENTATIVE TREATMENT FOR MALARIA IN PREGNANCY (IPTP): A CASE OF TANZANIA

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About 35 million people in Tanzania are at risk of malaria, with pregnant women and under five children being the most vulnerable. The Tanzania National Malaria Control Program's (NMCP) Strategic Plan for 2007–2012 reports that malaria accounts for 30% of the national disease burden, with about 1.7 million cases per year among pregnant women. To prevent the effect of malaria in pregnancy, the Tanzania Government adopted IPTp3+ therapy for pregnant women per the WHO recommendations for IPTp-SP. This study explores missed opportunities to deliver IPT by looking at predictors causing the drop between coverage of IPTp2 (34%) and IPTp3+ (7%). The study examined Tanzania Demographic and Health Survey (TDHS) 2015/2016 data on women aged 15–49 with a live birth in the two years preceding the survey and at least 2 doses or more of IPTp during ANC (n=4219) to identify factors associated with differences in IPTp uptake. Variables of interest were identified, recoded and generated as required. Data was analyzed using STATA v14, whereby frequency distributions were calculated and cross-tabs and logistic regressions were done comparing dependent and independent variables. The analysis shows the factors contributing to the drop of IPTp uptake include wealth (the richest people are 2.5 times more likely to take at least three doses of IPTp) and education (those with no education are less likely to take more doses of IPTp compared to those who are educated). Residency is

the largest contributing factor: 50% of pregnant mothers in rural areas are less likely to take three or more doses of SP. Clients living within 5 km of health facilities have higher uptake of IPTp3+ compared to their counterparts who live further from the health facilities (33% less likely). However, our analysis shows that there is no correlation between IPTp3+ uptake and number of ANC visits, health insurance or number of children. Based on these results, it is important to strategize to make health services and education more accessible to the population in order to increase IPTp uptake among pregnant women.

## 1125

### IMPLEMENTATION OF MOSQUITO-PROOF HOUSING: LESSONS LEARNED ON OPERATIONAL FEASIBILITY, COST AND COMMUNITY ENGAGEMENT IN NAMIBIA

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As Namibia works toward its 2020 elimination goal, the country strives to identify and operationalize effective interventions as part of an integrated malaria elimination strategy. The program conducted a small-scale implementation of housing improvements to assess operational feasibility and inform potential scale up. Communities were selected based on malaria risk, acceptability and need, diversity of housing type, number of structures and operational feasibility. Types of improvements include screening windows, eaves, and doors; sealing gaps in the wall; and patching roofs. Planning included a literature review, community surveys and focus groups, material selection, and household assessments. Local contractors were recruited from the National Youth Service and received a two-week technical training. Community health workers and local headmen were also trained on the project and maintenance for two days. Six communities were selected to receive housing improvements, targeting approximately 1,540 structures. Implementation is complete, and basic epidemiological and entomological indicators will be collected as indication of directional effect for impact, while additional questionnaires will capture data on maintenance and durability. The delivery of the project on budget and on time was supported by a comprehensive protocol and technical manual; piloting which allowed for recalibration of the designs; direct lines of communication with the implementers and regular supervision visits. It was found that early and frequent touch points with the community and recruitment of local labor resulted in high community acceptance. Careful planning around materials helped with maximizing effectiveness and efficiencies, with early estimates for cost per structure being \$38, \$31, \$29 for a modern, traditional and metal structure, respectively. Early results show that there is good uptake, and that with careful planning, local ownership, regular supervision and cross-ministerial engagement, housing improvement has the potential of becoming a cost-effective intervention for elimination.

## 1126

### SAFE INDOORS: CHEAP, SUSTAINABLE SPATIAL REPELLENTS TO COMBAT RESISTANCE AND KEEP MALARIA VECTORS OUT OF HOMES

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While there is increasing interest in the role of housing for malaria prevention, economic realities mean that neither ideal buildings, nor retrofit measures such as screening and eave-tubes, will likely be universally available over the short term. A reappraisal of the repellent

action of DDT in historic malaria control programs is generating interest in the use of spatial repellents as an alternative means to keep vector mosquitoes out of homes. We have suggested generating cheap, effective new spatial repellents by harnessing the same evolutionary processes which drive the spread of insecticide resistance. Combining existing IRS programs with use of an indoor repellent initially effective against only part of the vector population can generate selection for vectors susceptible to the repellent, modifying the target vector population and hence increasing efficacy of the repellent. In turn, the repellent protects the IRS insecticide against the rapid spread of insecticide resistance. We formalised this idea using models which explore vector population genetics and associated disease transmission metrics, and have now extended our analysis to consider location-specific scenarios and to explore how evolved repellence might contribute to protection of the crucial pyrethroid insecticides used on bed nets.

## 1127

### INCREASING THE TIME BETWEEN INCIDENT MALARIA EPISODES IN UGANDAN CHILDREN: REPEATED APPLICATION OF IRS

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Malaria is the leading cause of morbidity and mortality in Uganda, with some of the highest levels of malaria transmission intensity in the world. Indoor residual spraying of insecticide (IRS) is one control intervention that is used in targeted areas in Uganda. The goal of the study was to estimate the time between incident malaria episodes in individual children before and after several rounds of IRS application. A dynamic cohort of children was enrolled in Nagongera, Uganda starting in 2011. Household were randomly selection from enumeration surveys and all eligible children aged 0.5-10 yr were enrolled from 107 households. Cohort study participants received all medical care free of charge at a designated study clinic open every day. Malaria was diagnosed using passive surveillance and defined as a fever and the detection of parasites by microscopy. The first 3 rounds of IRS (December 2014-February 2015, June-July 2015, and November-December 2015) utilized a carbamate (bendiocarb) and a fourth round (June-July 2016) utilized an organophosphate (pirimphos-methyl). The analysis included data through December 2016 and involved using a multiple Poisson regression, with factor variables to represent the IRS rounds, to estimate the mean time between episodes of incident malaria. There were 376 children enrolled. In total, there were 2,857 cases of incident malaria. The mean number of malaria episodes per child was 9, with a range of 1 to 32. In the pre-IRS treatment period, there was an average of 100 days between malaria infections for each child. After IRS round 1, IRS round 2, IRS round 3, and IRS round 4, the average time between infections was 159 days, 224 days, 438 days, and 507 days, respectively. There was a consistent increase in time between malaria episodes post-IRS applications, with the largest increase in time occurring after rounds 3 and 4 of IRS. This suggests that IRS is effective at reducing the burden of malaria episodes these children experienced by increasing the time between each episode. Future work will include estimating the longevity of effect post-IRS application to determine the optimal timing for IRS application.

## 1128

### AN OBSERVATIONAL ANALYSIS OF THE IMPACT OF INDOOR RESIDUAL SPRAYING IN THE NORTHERN, UPPER EAST AND UPPER WEST REGIONS OF GHANA: 2011-2016

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The indoor residual spraying (IRS) of insecticides has contributed substantially to recent successes in malaria control, leaving IRS as one of the key components of vector control strategies moving forward. Ghana has seen a decline in malaria prevalence nationally since 2011, particularly in the northern Savannah where IRS has been implemented in a number of districts. Demographic and Health Survey and Multiple Indicators Cluster Survey data show consistent decreases in malaria prevalence from 2011 to 2016. In 2011, Northern, Upper East and Upper West regions presented high parasite prevalence in children under 5 (48%, 44%, and 51% respectively), but all were below 40% in 2016. Entomological surveillance has tracked the effect of IRS and the introduction of new IRS products on vector indicators, but there is a need for evidence demonstrating epidemiological impact as well. We conducted a retrospective, observational analysis of the epidemiological impact of IRS with varying insecticide classes, using routine surveillance data comparing malaria incidence from IRS and comparable non-IRS districts in the Northern, Upper East and Upper West regions. Preliminary results of district-level analysis in the Northern Region show that in districts that received IRS in 2016, rapid diagnosis test (RDT)-confirmed incidence rates fell 18% during the three months immediately after the spray campaign; incidence rates in non-IRS districts increased by 98% in the same timeframe. In addition, US President's Malaria Initiative (PMI)/Africa Indoor Residual Spraying (AIRS) project's entomological surveillance has shown lower indoor resting vector densities between IRS and non-IRS sentinel sites as well as overall lower entomological inoculation rates. These analyses illustrate the positive contribution of IRS in malaria control programs in the north of Ghana and the value of using routine surveillance and implementation data to help rapidly assess the impact of vector control interventions in operational settings. Further analysis will examine monthly trends in incidence over several years following IRS with pyrethroid and carbamate insecticides.

## 1129

### AN OBSERVATIONAL ANALYSIS OF THE IMPACT OF IRS IN THE SÉGOU REGION OF MALI: 2011-2014

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The widespread adoption of new products for indoor residual spraying (IRS) is hampered by gaps in the evidence used to evaluate their public health impact and cost-effectiveness across various malaria transmission settings. To help address this gap, and to foster evidence-based decision making, we present a retrospective, observational analysis of the epidemiological and entomological impact of IRS with two different non-pyrethroid insecticides, using routine indicators of malaria transmission in the Ségou Region of Mali. Ségou has had high access to and use of insecticide treated nets, documented pyrethroid and organochloride resistance, and a scale-up of seasonal malaria chemoprevention that began

in 2013. District level analysis of routine passive surveillance data shows substantial decreases in the number of rapid diagnostic test-confirmed symptomatic malaria cases following IRS campaigns from 2011 - 2014. In 2012 and 2013, in the Ségou Region, two districts (Bla and Barouéli) were sprayed with the carbamate bendiocarb and, together, reported 321 (2012) and 289 (2013) fewer malaria cases/10,000 person-months than those districts that did not receive IRS. Results were similar in 2014, when houses in both districts were sprayed with the organophosphate pirimiphos-methyl and subsequently reported 473 fewer cases/10,000 person-months than did non-IRS districts. In addition, data from the US President's Malaria Initiative Africa Indoor Residual Spraying project routine entomological surveillance show that reduced malaria incidence following each IRS campaign correspond to reductions in *An. gambiae* s.l. indoor biting rates (73% reduction post-spray in 2014, from 3.75 bites/night to 1.0 bites/night). This work shows the utility and importance of using quality assured and validated routine surveillance and implementation data to help rapidly assess the impact of vector control interventions in operational settings, even in complex implementation environments. Further evaluations of various malaria control interventions in different settings will develop a more robust evidence-base to help guide decision making.

### 1131

#### **TYPHOID FEVER CASE FATALITY RATE IN PATIENTS PRESENTING TO A LABORATORY NETWORK IN DHAKA, BANGLADESH**

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Case fatality rate estimates for typhoid fever are central to estimating disease burden, but are scarce. Estimates range widely from 0-15%, with active population based surveillance reporting lower rates presumably due to early detection and hospital based surveillance with higher estimates given their sicker patients. We measured the case fatality rate among patients who had blood culture confirmed typhoid in Dhaka Bangladesh. Between January and December 2010, we prospectively followed patients with blood cultures positive for *Salmonella typhi*, identified from six private laboratories utilized by both hospitals and outpatient private practitioners throughout Dhaka, Bangladesh. We collected antibiotic resistance information from the laboratories. 1425 patients were enrolled; 59% were male, 41% were female, with a median age of 14. 98% experienced fever, 26% required hospitalization and 4 patients died from *S. typhi* (0.3%, 95% CI 0.28-0.32%). There were no significant differences in demographics between patients who were hospitalized and those not hospitalized with typhoid. The four patients who died were all female and had ages ranging from 32-65 years old. 26% of isolates were resistant to chloramphenicol, ampicillin and co-trimoxazole; 38% were resistant to azithromycin. 47% started antibiotics prior to blood cultures, with another 17% starting antibiotics after cultures but before results were reported. Culture results caused 55% to switch antibiotics. This study found a 0.3% case fatality rate for blood culture confirmed typhoid fever among a mixed population of sicker, hospitalized patients and healthier outpatients in urban Bangladesh. This assessment did not capture the experience of people too poor to secure a blood culture, but offers a low-cost strategy to generate an empirical estimate and explore case fatality in other contexts. Future studies should track antimicrobial resistance and its impact on patient outcomes.

### 1132

#### **SALMONELLA BACTEREMIA IN HOSPITALIZED UGANDAN CHILDREN WITH FEBRILE ILLNESS**

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*Salmonella* is a known cause of acute febrile illness (AFI) among children in sub-Saharan Africa; however its contribution to bacterial blood stream infections is poorly defined due to limited diagnostic capacity. To address this gap and to inform accurate patient diagnosis and treatment, improved AFI surveillance and expanded diagnostic testing is needed. The Uganda AFI project conducts sentinel surveillance for causes of AFI in children <1 to 14 years old hospitalized at six regional hospitals. We evaluated preliminary demographic data, blood culture and antimicrobial susceptibility results of children hospitalized with a history of fever or documented temperature  $\geq 37.5^{\circ}\text{C}$  from the first three sentinel AFI sites during the first seven months of this ongoing surveillance project (July 1, 2016 – January 31, 2017). Over a combined total of 585 days at the three sites, blood cultures were performed and results were available for 1,169 (26%) of 4,545 hospitalized children. Overall, 1,059 (93%) yielded no growth; 21 (2%) yielded a likely contaminant, and 53 (5%) yielded a pathogen. Among positive blood cultures, *Salmonella* was found in 21 (48%) samples, including 10 (24%) identified as non-Typhi *Salmonella*, 5 (12%) as *Salmonella Typhi*, and 5 (12%) *Salmonella* whose serotypes are pending. *Salmonella* isolates were resistant to ampicillin (58%) and cotrimoxazole (37%), but all were fully susceptible to ciprofloxacin and ceftriaxone. Six (29%) of the children whose blood cultures yielded *Salmonella* had a positive malaria rapid diagnostic test (RDT) (2) or a positive blood smear (4), and one had both a positive malaria RDT and blood smear. The majority (86%) of *Salmonella* isolates were identified from the site with the highest malaria transmission intensity. *Salmonella* was an important cause of bacteremia in children hospitalized with fever, including those with a positive malaria RDT or blood smear. To improve detection and treatment, evaluation of sentinel AFI surveillance is ongoing to better characterize the serotypes of *Salmonella* causing bacteremia and their associated drug resistance patterns.

## 1133

### IDENTIFYING CLINICAL PROFILES TO DISTINGUISH ROTAVIRUS FROM OTHER ETIOLOGIES AMONG CHILDREN < 5 YEARS OF AGE SEEKING CARE FOR MODERATE-TO-SEVERE DIARRHEA IN RURAL WESTERN KENYA – 2008-2012

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Moderate-to severe diarrhea (MSD) in infants is caused by a myriad of enteric pathogens. In resource limited areas, rapid diagnostics are often not available to identify causative agents, thus complicating treatment decisions. We identified clinical profiles to distinguish rotavirus from other diarrheal agents in children <5 years old in Kenya (prior to introduction of rotavirus vaccination) participating in the Global Enterics Multicenter Study (GEMS). Demographic and clinical features were collected from caretaker interviews, and clinician assessment of children seeking medical care for MSD. Stool specimens were collected and tested for viral, bacterial and parasitic etiologies of diarrhea. Variables collected were considered as predictors of viral versus other enteric etiologies in tree-based classification models. Complementary logistic regression models were applied for validation. GEMS enrolled 1,778 children with MSD in rural western Kenya from Jan 31, 2008 to Sep 30, 2012. Enteric pathogens were identified in 1,436 (81%) children of which 719 (50%) had a single pathogen identified. Of those with a single pathogen, 90 (13%) had rotavirus, 54 (8%) had another virus, 412 (57%) had a bacterial and 163 (23%) a parasitic pathogen identified. The rotavirus weighted tree models partitioned rotavirus-positive children into groups based on four main predictors: age <18 months; vomiting  $\geq 3$  times/24 h; onset in warm-dry month; skin tenting. The largest group of children with MSD identified with <1% rotavirus positive (n=174) were  $\geq 18$  months without skin tenting. No rotavirus cases were identified in young children <18 months, without vomiting present, and without skin tenting. These results were supported by logistic regression models that identified age (Odds Ratio= 5.63; 95% confidence interval (CI): 2.78-11.41), vomiting  $\geq 3$  times/24 h (OR= 2.98; 1.85-4.81), and onset in warm-dry month (OR= 2.37; 95% CI: 1.45-3.87) as significantly associated with rotavirus. Tree-based models highlighted the order of clinical variable importance in predicting rotavirus infection in children and can assist in guiding treatment at point of care.

## 1134

### FEASIBILITY OF A COMPREHENSIVE TARGETED CHOLERA INTERVENTION IN KATHMANDU VALLEY, NEPAL

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Studies have demonstrated a much higher risk of cholera in the immediate area around a case and that this increased risk persists for about 2 to

3 weeks. A Comprehensive Targeted Intervention (CTI) was designed and deployed in the neighborhoods of cholera cases in the Kathmandu Valley during 2016 with the intent to reduce rates among neighbors of the case. This was a feasibility study to determine if clinical centers, laboratories and field teams were able to cooperate and coordinate a response within 48 hours of hospital admission. Daily line listings were requested from the 12 participating hospitals, and a single case initiated the CTI. Rapid diagnostic tests and culture were used for confirmation. The strategy included hospital and household investigation of cases, water testing, WASH intervention, and health education. A single-dose of oral cholera vaccine using a reactive ring strategy was included in the plan, but vaccine could not be obtained in time for the summer 2016 outbreak. An intervention coverage survey was conducted 8 months post-monsoon season. Between June 1 and December 1, 2016, 169 cases of *V. cholerae* O1 were confirmed by culture. Average time to result of hospital culture was 2.4 days. On average, the CTI Rapid Response Team (RRT) was able to visit the household 2 days after the culture result was received from the hospital. 90.4% of household water samples were found unsuitable for drinking and 3 sources were positive for cholera. Coverage of WASH and health behavior messaging campaigns was 30.2% in the target areas. Recipients were 2.3 times more likely to have knowledge of cholera symptoms, treatment, and prevention. This attempt at implementing CTI was successful in raising awareness and engaging stakeholders in both government and private sectors. While the RRT were able to investigate cases at the household within 48 hours of a positive culture result, the study identified several constraints which limited a truly rapid response. Using the information from this first year, we identified pathways that will be useful for future implementation of CTI which have been included in the country's national cholera control strategy.

## 1135

### ENTEROPATHOGENS AND GUT INFLAMMATION IN ASYMPTOMATIC INFANTS AND CHILDREN IN DIFFERENT ENVIRONMENTS IN SOUTHERN INDIA

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Children in poor environmental conditions are exposed early and often to enteric pathogens, but within developing countries heterogeneity is rarely addressed. We tested fecal samples from healthy infants and children from two different environments in the same Indian town for gut enteropathogens and biomarkers of gut inflammation. Fecal samples were collected from N=83 infants and children between 3 months to 4 years age residing in Chinnalapuram, a poor neighbourhood (formerly a slum) in Vellore town, south India and N=53 infants and children staying on the residential campus of the Christian Medical College, Vellore. Testing for gut enteropathogens was done using arrayed qPCR assays (Taqman array card assays) and levels of inflammatory biomarkers (fecal calprotectin and myeloperoxidase) were assessed using commercial ELISA assays. A significantly higher proportion of infants and children from the poor semi-urban neighborhood (93%) had one or more enteropathogens than those from a medical college campus (71.7%). Infants and children from the poor neighborhood had an average of 3.3 (95% confidence interval 2.9-3.7) enteropathogens compared to an average of 1.4 (95% confidence interval 1.0-1.7) enteropathogens in campus infants/children. Viral and bacterial infections, including enteroviruses, adenoviruses, *Campylobacter* spp and diarrheagenic *Escherichia coli* were more common and levels of fecal biomarkers of inflammation such as fecal calprotectin and myeloperoxidase were significantly higher among infants and children from the poor neighborhood, even after age-related adjustments. The findings demonstrate significant difference in the asymptomatic carriage of gut enteropathogens and gut inflammatory biomarker levels in infants and children from two different environments within the same town in

south India. Understanding this kind of heterogeneity within the same town enables measures for improvement and might have implications for environmental enteropathy and performance of oral vaccines.

## 1136

### A NOVEL MOUSE MODEL OF *CAMPYLOBACTER JEJUNI* ENTEROPATHY AND DIARRHEA

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*Campylobacter jejuni* is a gram-negative bacteria that is one of the leading recognized bacterial causes of food-borne illnesses worldwide. In the Global Enteric Multicenter Study (GEMS), *C. jejuni* was a leading bacterial cause of moderate to severe diarrhea burden at several South Asian sites. In addition, *Campylobacter* was the leading bacterial pathogen associated with diarrhea across all 8 sites in the MAL-ED study. Furthermore, "asymptomatic" *Campylobacter* infections (i.e. without overt diarrhea) were also the leading pathogen associated with significant growth failure, increased intestinal permeability, and inflammatory biomarkers. Lack of an inexpensive small animal model of enteric disease with *Campylobacter* has been a major limitation for understanding its pathogenesis, interventions or vaccine development. We describe a robust normal mouse model that can exhibit reproducible bloody diarrhea or growth failure as well as fecal biomarkers, depending on the zinc or protein deficient diet. Weaned C57BL/6 mice were fed a regular diet for 2 days, and then either standard rodent 'House Chow' (HC), a defined protein source diet without zinc (dZD), or protein (2%) deficient (dPD) diet. On day 10 of diet, mice were given 3 days on antibiotics to disrupt resident microbiota followed by 1 day on untreated water to clear antibiotics. *C. jejuni* (CVD 81-176) infected mice fed dZD diet had significantly greater weight loss than HC or dPD groups. While all HC or dZD fed mice developed diarrhea by day 3 post infection, all dZD mice had persistent bloody diarrhea for greater than 7 days post infection. Only dZD fed mice were colonized with *C. jejuni* for the duration of the experiment. The inflammatory biomarkers myeloperoxidase and lipocalin-2 measured in cecal contents at day 3 post infection were significantly elevated in dZD fed groups, with MPO being highest with dZD, while LCN-2 was also increased in HC and dPD groups as well. Finally, histology of mice infected with *C. jejuni* showed increased mucus secretion and flattened villi. This model opens new approaches to testing specific hypotheses regarding disease pathogenesis and vaccine development.

## 1137

### SYNERGISTIC AND ANTAGONISTIC EFFECTS OF DIARRHEAGENIC *E. COLI* CO-INFECTIONS IN A MURINE MODEL

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Diarrheal diseases are major causes of morbidity in children worldwide. Enteropathogenic *E. coli* (EPEC), Enteroaggregative *E. coli* (EAEC) and Enterotoxigenic *E. coli* (ETEC) are major causes of acute diarrhea; dual infections pathogens have been reported in different parts of the world. The goal of the study is to determine the role of diet and disease severity during single infections of EAEC, EPEC and ETEC and also during co-infections. Four-week old weaned C57BL/6 mice on standard chow diet were placed on antibiotic cocktail water for 3 days. These mice were administered orally (109 c.f.u./mouse) single infections (EAEC 042, EPEC E2348/69, ETEC H10407) and co-infections (EAEC/EPEC and EPEC/ETEC) (109 c.f.u./mouse per pathogen). During infection, mice were monitored for change in weight and stools were collected daily. At peak of infection; serum, intestinal tissue and cecum contents were collected. Bacterial DNA

was extracted from stools and tissue, and qPCR was used to determine shedding and tissue burden. Proteins lysates extracted from cecum contents and stools were used to determine inflammatory biomarkers myeloperoxidase (MPO) and lipocalin-2 (LCN-2). All mice developed diarrhea on the first day post-infection except EAEC/EPEC co-infections. There was significant weight loss on day 2 and 3 post-infection in mice infected with EPEC or ETEC alone or with EPEC/ETEC co-infections, but not for those infected with EAEC/EPEC. MPO and LCN-2 levels in EPEC/ETEC co-infections were significantly higher compared to EPEC or ETEC alone, while MPO levels were diminished during EAEC/EPEC co-infection. Disease outcome of EPEC/ETEC co-infection was worse compared to the single infections. In addition, EPEC/ETEC co-infection resulted in worse outcomes with mice experiencing severe disease outcome with increased weight loss, diarrhea and increased MPO and LCN-2. EAEC/EPEC co-infection resulted in antagonistic effects with no weight loss and reduced inflammatory LCN-2 and MPO. Future directions include assessing expression of virulence genes during co-infections to better understand these potentially important differences in outcomes.

## 1138

### PROTECTION INDUCED BY A SEROTYPE-INDEPENDENT VACCINE AGAINST SHIGELLOSIS: THE ROLE OF DENDRITIC CELLS

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Diarrheal diseases are a major cause of morbidity and mortality worldwide and are common in settings where there is inadequate sanitation, poor hygiene and contaminated water. In a multicenter study conducted in Africa and Asia, it was observed that infections with rotavirus, *Shigella* spp., *Cryptosporidium* spp. and ST-ETEC (*E. coli*) accounted for the majority of cases of moderate-to-severe diarrhea in children. No commercially available vaccine exists against shigellosis, and immunity to the pathogen is commonly serotype-restricted. The variety of serotypes (over 50 serotypes across 4 different species of *Shigella*), along with the geographical overlap between them highlight the need for a broadly protective vaccine. This need drives our efforts on the development of a subunit vaccine against this enteric disease. Our research group has previously shown the Type Three Secretion System (T3SS) proteins IpaB and IpaD are protective antigens in mouse models of infection. These proteins are highly conserved among all *Shigella* serotypes and are essential to virulence and pathogenesis. In order to optimize vaccine formulation we generated a fusion protein (DBF) that includes IpaD and IpaB in the same polypeptide chain and used dmLT as an adjuvant. This formulation was protective against *Shigella flexneri*, *Shigella sonnei* and *Shigella dysenteriae*. Testing of different administration routes showed the degree of protection conferred by our vaccine formulation did not correlate with antibody titers. We subsequently have studied the cellular response to intranasal immunization and found dendritic cell activation at this site. Dendritic cell activation was further confirmed *in vitro* along with its effect on T cell differentiation and proliferation. We have proposed a mechanism of protection based on participation of the antigen portion of our vaccine along the adjuvant in activating antigen presenting cells of the immune system and subsequent presentation to T cells. This proposed mechanism would allow bacterial clearance in the gut upon infection with *Shigella* spp.

## 1139

### EARLY ENTEROPATHOGENIC *E. COLI* INFECTIONS ASSOCIATED WITH GROWTH FALTERING AT 24 MONTHS OF AGE IN URBAN BANGLADESH

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*Escherichia coli* (*E. coli*) are among the first microbes to colonize the infant gut. *E. coli* pathotypes (heat labile (LT-) or heat stable (ST-) enterotoxigenic (ETEC), typical and atypical enteropathogenic (tEPEC, aEPEC), enteroaggregative (EAEC), enteroinvasive (EIEC), and Shiga-toxin producing (STEC)) are among the leading causes of child diarrhea globally. Early life enteropathogen exposure has been associated with child stunting in low-resource settings, however the contribution of pathogenic *E. coli* has not been well characterized. We sought to describe the frequency of pathogenic *E. coli* infection from birth to 6 months of age in urban Bangladesh, and to identify strains associated with linear growth at 24 months of age. Healthy newborns from Dhaka, Bangladesh enrolled in MAL-ED cohort were visited every other day for 2 years for morbidity and health behavior assessment. Length was measured monthly, and stools collected monthly through 1 year of age from children without diarrhea, and each time a child had diarrhea. *E. coli* spp. were identified using conventional stool culture, and virulence genes were detected by multiplex PCR. Linear regression was used to model the relationship between detection of *E. coli* pathotypes in the first 6 months and length for age z-score (LAZ) at 24 months of age, controlling for LAZ at birth, exclusive breastfeeding in the first 100 days, and number of diarrhea episodes before 6 months. Among 210 children enrolled, 89% had at least one *E. coli* pathotype identified in their stool in the first 6 months of life. The most commonly detected pathotypes were EAEC (79%) and tEPEC (22%). Accounting for potential confounders, the mean LAZ at 24 months was 0.37 lower in children with tEPEC detected in any stool (95% CI: -0.64, -0.09,  $p=0.009$ ) and 0.39 lower in children with LT-ETEC than those without (95% CI: -0.71, -0.06,  $p=0.022$ ). There were no associations among other pathotypes and linear growth at 24 months. Early tEPEC and LT-ETEC infections were significantly associated with growth faltering in children in urban Bangladesh. Early tEPEC infections may be influencing child growth even in the absence of diarrhea.

## 1140

### LONGITUDINAL COHORT STUDY OF ACUTE GASTROENTERITIS AMONG U.S. MILITARY PERSONNEL DEPLOYED TO HONDURAS FROM 2014-2016

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The need for field sites to monitor infectious disease burden among deployed military personnel and for evaluation of treatment and preventive interventions, including vaccines is a strategic need for the U.S. military. We established a field site at Soto Cano Air Base for clinical trials among military deployed to Honduras complete with an on-site laboratory

for stool cultures, in-house BioFire FilmArray GI pathogens panel, and reference capacity at NAMRU-6 in Peru for enhanced workup. With this framework, we developed a pilot longitudinal cohort study for acute gastroenteritis (AGE) during two deployment cycles from June 2015-March 2016 and March-November 2016. Participants were given a description of the study during their medical in-brief and allowed to individually meet with the civilian study coordinator for consent, completion of data forms, and submission of baseline stool samples. A total of 39 persons were enrolled with 1 loss to follow-up. Subjects were contacted weekly to track illness and if ill were asked to complete a case report form and submit a stool sample for testing. Additionally, 10 subjects were recruited into a nested gut microbiome study to explore changes associated with deployment and illness. In this subset, participants submitted stool samples weekly regardless of illness events and completion of a daily diet log. During this period there were 16 episodes of AGE over 316.6 months at risk for an incidence rate of 5.1 per 100 person-months as compared to the incidence of respiratory illness of 2.8 per 100 person-months. Stool culture yielded only 1/16 (6%) isolate of *Shigella sonnei*, whereas the FilmArray was positive in 12/16 (75%) of cases with 9/12 (75%) found positive for multiple pathogens versus only 3/12 (25%) with a single pathogen. Diarrheagenic *E. coli* was found in all 12 cases with 7/12 (58%) EPEC-positive, 7/12 (58%) ETEC, and 6/12 (50%) with EAEC. These findings illustrate the complex etiology of diarrhea in deployed settings. Gut microbiome analysis and correlations with dietary and illness is pending. This study provides a foundation for future clinical trials for treatment regimens and vaccines at this site.

## 1141

### ASSOCIATION BETWEEN CONTINUED FEEDING DURING HOME TREATMENT AND EXTENDED CASE FATALITY 50-90 DAYS FOLLOWING A MODERATE-TO-SEVERE DIARRHEAL EPISODE IN LOW AND MIDDLE-INCOME COUNTRIES

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The global burden of diarrhea is high, particularly among children in low- and middle-income countries. Despite global recommendation of continued feeding, prior studies have reported inadequate adherence to this recommendation during diarrheal episode treated at home. We hypothesized that continued feeding (offering usual or more than usual amount of food) during home treatment of moderate-to-severe diarrhea (MSD) will reduce case-fatality. Using data from the 7 sites of the Global Enteric Multicenter Study, we conducted secondary analyses to estimate prevalence of continued feeding during home treatment of MSD and exclusive breastfeeding among babies less than 6 months old. We also estimated risk ratios (RR) and 95% confidence intervals (CI) for death, in relation to continued feeding at home before seeking care for the MSD episode. Prevalence of exclusive breastfeeding among babies less than 6 months old was low across the sites, ranging from 9.5% to 52.2%. Prevalence of continued feeding during MSD varied across sites ranging from 19.3 to 88.9%, with low pooled prevalence of 43.6%. In multivariable pooled analyses adjusted for age, sex and socioeconomic status, continued feeding was inversely associated with extended case-fatality at follow up visits 50-90 days after MSD episodes (0.47, CI 0.22-1.03), but the association was borderline statistically significant. Further analyses that examine the role of factors such as nutritional status, maternal education and concomitant treatments (oral rehydration and zinc) in this association are still needed. Future studies should address a broader range of potential confounders and evaluate impact of interventions to facilitate uptake of continued feeding on case-fatality in low-income settings.

## 1142

### DEVELOPMENT OF A *SALMONELLA ENTERICA* SEROVAR TYPHI TY21A STRAIN AS A MULTIVALENT VACCINE PLATFORM AGAINST SHIGELLOSIS, ETEC DIARRHEA AND TYPHOID FEVER

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Recent reports estimated that shigellosis, ETEC diarrhea, and typhoid fever caused a combined 448 million cases and close to 200,000 deaths annually. Sadly, there are no vaccines against shigellosis nor ETEC diarrheal disease and licensed vaccines against typhoid are only partially effective. We exploit the extensive safety and long-term efficacy record of the live, oral, attenuated Ty21a typhoid fever vaccine, which has been administered to more than 200 million individuals worldwide, by utilizing it as our vector for combination oral vaccines. We established an acid-stabilized Ty21a platform that carries stable, inducible expression of glutamate-glutamine based acid resistance genes and showed that this strain (Ty21a-AR) is acid resistant *in vitro*. This strategy allows for increased passage of vaccine organisms through the acid environment of the stomach, thereby optimizing immunogenicity and protective efficacy. Genes encoding serotype-specific O-antigens of *Shigella* spp. and a multi-epitope fusion

antigen (MEFA) of toxoided heat-labile toxin of ETEC were integrated into the chromosome of our innovative acid-stabilized Ty21a-AR strain. Our most advanced vaccine candidate, Ty21a-AR-Ss (TyOraSs), expressed *S. sonnei* form I O-antigen and *Salmonella* Typhi O-9,12-antigens simultaneously on the cell surface. Mice immunized with TyOraSs through a mucosal route produced IgG antibodies to both *S. sonnei* and *S. Typhi* O-antigens and were protected by a lethal infection of *S. sonnei* 53G. We have also constructed Ty21a-AR-Sf2a (TyOraSf2a) that expresses *S. flexneri* 2a O-antigen on Ty21a-AR cell surface and demonstrated immunogenicity of TyOraSf2a in mice. We have innovatively stabilized our Ty21a-vector platform at room temperature using foam dry technology. By proving the principle that our rTy21a shigellosis and ETEC vaccines are safe, well tolerated, and immunogenic and the shigellosis vaccine is protective, we will establish acid-stabilized rTy21a as a flexible, oral, rapidly administered (over 1 week), room temperature stable vector platform for rapid response to emerging and re-emerging infectious disease threats.

## 1143

### YERSINIA PESTIS SURVIVES AND REPLICATES IN PHAGOCYTOTIC AMOEBAS: THE CONTINUING SEARCH FOR AN ENVIRONMENTAL PLAGUE RESERVOIR

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Plague ecology is characterized by sporadic epizootics, followed by periods of cryptic dormancy. Building evidence suggests environmentally ubiquitous amoebae act as "feral macrophages" and permissive hosts to a wealth of competent intracellular pathogens. We performed environmental genetic surveys and laboratory co-culture infection experiments to assess whether plague bacteria were resistant to digestion by five common environmental amoeba species. We demonstrate: (1) *Yersinia pestis* is an amoeba-resistant microorganism, (2) *Y. pestis* survives and replicates intracellularly within the social amoeba, *Dictyostelium discoideum*, for at least 48h post-infection, (3) *Y. pestis* is transiently amoeba-resistant in four other free-living amoeba species, and (4) *Y. pestis* resides within amoeba structures visually synonymous to those found in infected human macrophages, for which *Y. pestis* is a competent intracellular pathogen. Evidence for the existence of plague reservoirs and these newly identified amoeba-resistance phenotypes in *Y. pestis* stress the importance of recognizing pathogen-harboring amoebae as potential threats to global health, agriculture, conservation, and biodefense.

## 1144

### PREVALENCE OF ACUTE CHORIOAMNIONITIS AMONG HIV INFECTED AND UNINFECTED PREGNANT UGANDAN WOMEN AND ITS ASSOCIATION WITH ADVERSE BIRTH OUTCOMES

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Preterm birth (PTB) is a leading cause of neonatal mortality and longer-term morbidity. Acute chorioamnionitis (ACA) is a common cause of PTB, however, there are limited data on the prevalence of ACA and its association with PTB in resource limited settings. Data and samples came from clinical trials evaluating novel strategies for the prevention of malaria in HIV infected and uninfected pregnant women in Uganda. Women were enrolled between 12-28 weeks gestation and followed through

delivery. For each placenta delivered, 3 placental tissue types (placental membrane roll, umbilical cord and fetal plate) were collected. Slides were assessed for histologic diagnosis of maternal and fetal ACA by microscopic evaluation of neutrophilic infiltration of the placenta using a grading scale. The primary outcomes were preterm birth (< 37 weeks) and low birth weight (LBW, < 2500gm). Biopsies were collected from a total of 486 placentas, with 483 included in the analysis (40.4% HIV infected, 59.6% HIV uninfected). Evidence of maternal ACA was seen in 44.3% of samples (21.2% mild, 12.3% moderate, and 10.8% severe). Evidence of fetal ACA was seen in 26.1% of samples (15.5% mild, 8.9% moderate, and 1.7% severe). There were no significant associations between HIV status or gravidity and maternal or fetal ACA. HIV infected women with evidence of moderate-severe ACA had a significantly higher risk of preterm birth (25.0% vs. 6.0%; RR=3.97, 95% CI 1.68-9.36, p=0.002) and LBW (22.7% vs. 9.4%; RR=2.14, 95% CI 1.03-4.43, p=0.04) compared to those with mild or no ACA after adjusting gravidity and maternal age. There was no significant association between evidence of maternal ACA and adverse birth outcomes among HIV uninfected women or between fetal ACA and adverse birth outcomes regardless of HIV status. Histological evidence of maternal ACA was associated with an increased risk of preterm birth and LBW among HIV infected women but not HIV uninfected women.

## 1146

### CHARACTERIZATION OF ANTI-*HELICOBACTER PYLORI* PEPTIDES PRESENT IN THE HEMOLYMPH OF *HERMETIA ILLUCENS* LARVAE

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More than half of the global population is infected by the gastric pathogen *Helicobacter pylori*, often since childhood. This waterborne bacteria is known to cause chronic gastritis, peptic ulcers, and is a risk factor for gastric adenocarcinoma development. It is highly prevalent in tropical developing nations. Chemotherapeutic eradication of *H. pylori* is necessary to treat the accompanying gastrointestinal disorders. However, resistance to first line antibiotics is increasing, especially for clarithromycin (17.5%) and metronidazole (34.9%). Therefore, we are searching for alternative chemotherapeutic agents, such as antimicrobial peptides (AMPs) from insects. AMPs are immune system molecules present in every living organism; indeed, some have been found with activity against multidrug resistant bacteria. Our research focuses on the Black Soldier Fly (*Hermetia illucens*), a saprophytic dipteran that can tolerate pathogen-rich environments, such as decomposed waste and manure. Due to its life cycle, we postulate that it is a rich source of useful AMPs. Hemolymph peptidome analysis of inoculated *H. illucens* larvae using centrifugal filtration and sequential reverse-phase preparative liquid chromatography revealed a fraction of 3-10 kDa that had a minimal inhibitory concentration of 31.3 µg/ml against *H. pylori* ATCC®43504. Mass spectrometry (MS) divulged that the active fraction contained four polycationic peptides with masses near 4.2 kDa (~36 aa). Production of these peptides is inducible, as their expression hardly occurred in uninoculated control larvae. These peptides have very similar sequences and no disulfide bridges. To our knowledge, this is the first time that an antibacterial effect against Gram-negative microorganisms has been demonstrated in *H. illucens*. We are now trying to elucidate the primary sequence of the active peptides using MS.

## 1147

### UTILIZING THE GENEEXPERT TESTING SYSTEM FOR SEXUALLY TRANSMITTED INFECTION DIAGNOSIS IN THE DEMOCRATIC REPUBLIC OF THE CONGO

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Sexually transmitted infections (STIs), excluding HIV, are the second most common cause of healthy life years lost by women 15 - 44 years old in Africa. More than one million STIs are acquired every day worldwide, and many are asymptomatic. In many resource-limited countries, including the Democratic Republic of the Congo (DRC), a syndromic approach for testing and treating STIs is used. This method is non-specific and often leads to misdiagnosis and mistreatment, which can generate serious reproductive health consequences and promote antibiotic resistance among vulnerable populations. From October 2016 to March 2017, we conducted a cross-sectional study in pregnant women receiving antenatal care in Kisantu, DRC to compare self-reported and clinically-observed syndromic diagnosis to a PCR-based GeneXpert® rapid testing technology (Cepheid, Sunnyvale, CA). Consenting participants were enrolled; completed a questionnaire on sociodemographic factors, sexual history, and STI symptoms; and had a vaginal swab collected during clinical observation of the genital area. Swab specimens were tested with the Xpert® CT/NG Assay and Xpert® TV Assay for *Chlamydia trachomatis*, *Neisseria gonorrhoea* and *Trichomonas vaginalis*, respectively. Among 352 pregnant women, 59 (16.8%) self reported vaginal symptoms (ulcer, vaginal discharge, genital warts). Of those women, 6.7% had clinically-observed symptoms or lesions, and 15.2% were found positive for an STI via GeneXpert®. Almost the same percentage of asymptomatic women (15.0%, n=44/293) were also found positive upon nucleic acid amplification testing, resulting in very low symptom reporting-based sensitivity and specificity (17.0% and 83.3%, respectively) when compared against the gold standard technology. These results suggest that syndromic STI management is insufficient, and that GeneXpert® technology may be appropriate for disease diagnosis in resource-limited areas. Rapid NAAT diagnosis allows for detection of STI-positive women who may have been missed by traditional syndromic testing and further identifies specific infective species to ensure proper and timely treatment.

## 1148

### FREQUENCY OF ANTIBIOTIC RESISTANCE AND ADHESION GENOTYPES IN *ESCHERICHIA COLI* STRAINS ISOLATED FROM VAGINAL INFECTIONS

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The purpose of this work was to establish the frequency of the genes encoding adhesins (*fimH*, *papA*, *papC*, *papEF*, *papGI*, *papGII*, *papGIII*, *iha*, *afa*, *sfa*, *sfaE*, *bmaE*, *focG*, and *gafD*), and antibiotic resistance genes [*aac3-IV* (gentamicin), *CITM* (betalactams), *cmla* (chloramphenicol), *tet(A)* and *tet(B)* (tetracycline), *dfrA1* (trimethoprim) and *qnr* (quinolones)] in a group of cervicovaginal *Escherichia coli* strains (CVEC). We analyzed 200 strains of *E. coli* isolated from patients with cervicovaginal infections from the outpatient clinic of two IMSS clinics in the State of Mexico.

Identification of *E. coli* was performed by biochemical tests and by PCR amplification of the 16S rRNA gene. Adhesion and resistance genes to antibiotics were detected by conventional PCR and by multiplex PCR. Eighty-nine percent (n = 179) of the *E. coli* strains were carriers of the *fimH* gene, 11% (n=22) of *papA*, 41% (n=82) of *papC*, 26.5% (n=53) of *papEF*, 33.5% (n=67) of *papGII*, 3% (n=6) of *papGIII*, 51.5% (n=103) of *iha*, 9% (n=18) of *afa*, 14.5% (n=29) of *sfa*, and 9% (n=18) of *focG*. The *papGI*, *sfaS*, *bmaE* and *gafD* genotypes were not identified in any of the strains. A total of 36% (n = 72) of the strains were carriers of the *dfra1* gene, 14.5% (n = 29) of *CITM*, 3.5% (n = 7) of *cmla*, 49.5% (n=99) of *tet(A)*, 33.5% (n = 67) of *tet (B)*, and 4.5% (n = 9) of *aac(3)-IV*. The *qnr* gene was not identified in any strain. The results obtained in this study showed that strains of *E. coli* responsible for cervicovaginal infections were carriers of multiple genotypes of adhesion and resistance to antibiotics, which could increase the seriousness of infectious processes. This results may be used to orient the doctors to prescribe the appropriate treatment.

## 1149

### COLONIZATION WITH ESBL-PRODUCING ENTEROBACTERIACEAE OF HOUSEHOLD MEMBERS AND NEIGHBORS TWO MONTHS AFTER DISCHARGE OF COLONIZED PATIENTS FROM HOSPITAL IN RWANDA

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At the University Teaching Hospital of Butare, Rwanda, we recently observed two thirds of patients to be colonized with ESBL-producing Enterobacteriaceae (ESBL-PE) at discharge. Two months later, we visited the households of previously colonized patients, screened them for ESBL-PE carriage as well as each two household members and one household animal. In parallel, three household members and one animal of the neighboring dwelling were screened for each index patient (group sizes, 43 to 66) as well as >1000 schoolchildren in the study area. Rectal swab samples were screened on chromogenic agar, and bacterial species were determined using the API-20E system. Two months after discharge, approximately one third of the previous patients and of the household members were ESBL-PE carriers. Among members of the neighboring households, this figure was ten percent lower ( $P = 0.03$ ), similar to the difference on the level of household animals. *Escherichia coli* was the predominant ESBL-PE species. ESBL-PE carriage in patients' and neighbors' household was positively associated ( $P = 0.03$ ) in addition to other risk factors to be presented. The community prevalence of ESBL-PE (schoolchildren) was 4.9%. The observed gradient of ESBL-PE carriage from patients' households to neighbors and schoolchildren suggests transmission of highly resistant pathogens from hospital to community. Prevention of hospital-related acquisition of ESBL-PE and hygiene counselling of colonized patients may help to contain further spread.

## 1150

### A ROBUST INCUBATOR TO FACILITATE MORE WIDESPREAD BACTERIAL CULTURE FOR LOW RESOURCE ENVIRONMENTS IN DEVELOPING COUNTRIES

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In order to address the limitations of operating currently available incubators in low resource environments, a new incubator design was developed and tested to meet the requirements of operation in laboratories without reliable power (power outages up to 12 hours) or

climate control (ambient indoor temperatures from 5°C to 45°C). This incubator is designed to broaden the reach of microbiological culture in poorly-resourced health systems, to guide case management and presumptive treatment of a community, and enable extension of antimicrobial resistance (AMR) and disease surveillance. The challenges of operation in low resource environments were met by a design that uses phase change materials (PCM) as a bi-directional thermal battery to maintain incubation temperature during prolonged power outages and periods of poorly regulated input power in ambient temperatures ranging from 5°C to 45°C. Five prototypes were built and tested in the laboratory using environmental test chambers and programmable power supplies. Three of the prototypes were then successfully field tested in Laos in conjunction with the Lao-Oxford-Mahosot Hospital-Wellcome Trust Research Unit (LOMWRU). The devices were operated for an extended period in representative target facilities by health technicians performing an interaction script designed to replicate the intended use of the device.

## 1151

### ADHESION AND FIBRIN CLOTTING INHIBITION BY LEPTOSPIRAL PROTEINS

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Leptospirosis is a global infectious disease of human and veterinary concern. The study of surface antigens may shed light to pathogen-host interactions and help the identification of serodiagnosis and vaccine targets. This study was undertaken to data mine the genome sequences of *L. interrogans* to identify proteins predicted to be located at cell surface. To characterize two membrane proteins, using *E. coli* as expression system. Binding of these proteins to human components was evaluated. Recognition of these proteins in leptospirosis human sera was performed. Extracts of different species of *Leptospira* were membrane-blotted and probed with antisera raise in mice against each recombinant protein. The interaction of recombinant proteins with human components was evaluated by ELISA. Confirmed leptospirosis human sera were set to interact with recombinant proteins, measuring total IgG antibodies; samples at the onset (MAT-) and convalescent (MAT+) phases of the diseases were employed, and reactivity was compared to commercial normal human serum samples, employed for cut-off calculation. In conclusion, the native proteins are conserved among the pathogenic strains of *Leptospira*. The interactions of the two recombinant proteins with plasminogen, fibrinogen and laminin are dependent on protein concentration, reaching saturation point. The binding of one of the proteins to fibrinogen inhibited the formation of fibrin clot. The plasminogen captured by both recombinant proteins could be converted into plasmin, a mechanism that could help bacterial penetration in the host. Both proteins are capable of inducing immune response in humans, suggesting that these proteins are expressed during infection and may participate in *Leptospira*-host interactions.

## 1152

### THIRTEEN GLOBALLY CONDUCTED PRE-CLINICAL STUDIES ON DNA VACCINES AGAINST LEPTOSPIRA: A SYSTEMATIC REVIEW

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DNA vaccines have gained a novel place in prevention of bacterial infections. This study systematically reviews pre-clinical studies on DNA vaccines against *Leptospira*. All articles in English, regardless of the country of evaluation, were included from the following databases: PubMed, Trip, Google-Scholar, Science-Direct, Cochrane-Library, Google, Open-Grey and

Grey-literature-report. Also, the reference lists of selected articles were screened for suitable studies. PRISMA 2009 guidelines were followed in conducting and reporting of this study results. Quality assessment of the selected studies was done using the SYRCEL's risk of bias tool for animal studies. Thirteen were included in the final analysis. The average quality assessment score was 6.7 (SD  $\pm$  1) out of 10. Thirty-nine percent (n=13) of the study was from Brazil, which was the highest. pTARGET plasmid (31%; n=13) and LipL32 (39%; n=13) were used as the commonest vector and target gene respectively. 31% (n=13) of the studies had *Leptospira interrogans* Copenhageni-Fiocruz-L1-130 as its main strain used in the construction of vaccine. The commonest expression system used was Prokaryotic-E.coli TOP 10 (31%; n=13). Golden Syrian hamster female and BALB/c mice were the commonest animal models used (39% each). Copenhageni Spool, Canicola Hond Utrecht and Pamona (Each 25% - 2/8) were the main strains used in challenging of DNA vaccine. Vector alone treatment (85%) was the commonest method among control groups. The humoral immune response was measured using ELISA in 85% of the studies. Hundred percent potential protective response and zero percent pathological events was seen with interventional DNA vaccines. Enhancement of the efficacy and safety of DNA vaccines against *Leptospira* relies on the finding of optimum vaccination schedule. This could be achieved only with further studies.

## 1153

### MLST ANALYSIS OF *BURKHOLDERIA PSEUDOMALLEI* ISOLATES FROM SRI LANKA

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*Burkholderia pseudomallei* is a Gram-negative bacterium found in soil and water in tropical and subtropical regions worldwide. It causes melioidosis, a severe disease with a broad spectrum of clinical presentations endemic to Southeast Asia and northern Australia. Sri Lanka is situated in an endemic belt and over the last few years we started a project screening undifferentiated fever cases to identify potential melioidosis cases in Sri Lanka. As a result of this screening the number of culture confirmed cases have risen dramatically, with over 50 cases being reported each year in recent years. Genotyping via multi-locus sequence typing (MLST) was carried out to differentiate Sri Lankan isolates from others found worldwide and a large genetic diversity was seen. Sequence types (ST) and isolation data were submitted to the international *B. pseudomallei* database (<http://pubmlst.org/bpseudomallei/>) to aid in understanding the best representation of ST abundance. A total of 108 strains clinical isolates from 2006 until 2015 were genotyped. MLST analysis revealed that they belonged to 46 different STs in which 23 novel STs were found demonstrating great diversity in Sri Lanka. ST1137 is the commonest ST and there were five shared STs (10 isolates), of which three were exclusive to the Southeast Asian region (ST308, ST655 and ST912). Five isolates belonged to ST594 which has been seen among clinical and environmental isolates in both Australia and Thailand. Surprisingly, one of our isolates belonged to ST 132, an exclusive Australian ST which is seen among clinical, animal and environmental specimens. As of 2016, Sri Lanka has the largest representation on the MLST database of all the South Asian countries. We identified 46 STs representing a total of 108 clinical isolates, the commonest of which were STs 1137, 1135, 1132, 1136, 1434 and 1140. These six genotypes accounted for half the isolates and half the deaths. This work outlines our preliminary findings from an externally funded project to identify the strains and study the melioidosis disease burden in Sri Lanka.

## 1154

### EXAMINATION OF LABORATORY DATA FOR SURVEILLANCE OF ANTIMICROBIAL RESISTANCE IN OUTPATIENT URINARY TRACT INFECTIONS IN TAMIL NADU, INDIA — THE RATIONALE FOR CASE-BASED SURVEILLANCE

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The global spread of antimicrobial resistance (AR) is a serious public health threat that requires rigorous surveillance to guide interventions. Laboratory-based approaches, which summarize results of routine clinical specimens, are commonly used for AR surveillance. In India, most published reports describe AR in routine isolates from inpatients in tertiary-care facilities in metropolitan centers; few describe AR in outpatient infections in rural settings. We evaluated a laboratory-based approach to describe AR in isolates from outpatient urinary tract infections (UTIs) in a rural population in Tamil Nadu. Clinicians were interviewed to assess practices for referring outpatients for urine culture. All outpatients who provided a urine specimen for microbiological culture at a district hospital in Tamil Nadu during 2015 were included. Urine specimen results of 218 unique UTI episodes were reviewed. Of 82 (37.6%) culture-positive specimens, 63 (76.8%) had an Enterobacteriaceae isolate; most of these were *Escherichia coli* (52.4%) and *Klebsiella spp.* (20.7%). Of the 63 Enterobacteriaceae isolates assessed, 46 (73.0%) were resistant to third-generation cephalosporins. Twelve Enterobacteriaceae isolates were tested for carbapenem resistance; of these, 10 (85.7%) were resistant. Clinician interviews revealed that urine culture was not routinely performed for all outpatients with UTI symptoms; those who failed empiric UTI treatment were preferentially referred. Resistance to third-generation cephalosporins and carbapenems in tested Enterobacteriaceae isolates from urine specimens was common in rural Tamil Nadu. However, non-uniform microbiological referral practices limit measuring the true extent of AR in outpatient UTIs in this population. Surveillance based on routine clinical specimens might overestimate AR due to systematic bias in patients sampled. Case-based surveillance, which involves sampling and testing all patients with a defined clinical syndrome, would more accurately describe the magnitude of AR infections in a population to direct public health action, including data-driven antibiotic guidance.

## 1155

### UNEXPECTED PREVALENCE AND GEOGRAPHIC SPREAD OF SCRUB TYPHUS IN INDIA

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Scrub typhus (*ST-Orientia tsutsugamushi*) has been recognized as a cause of acute febrile illness (AFI) in India since 1932; until recently, it was considered a rare disease. In 2014, as part of Global Health Security (GHS), we initiated a facility-based AFI surveillance platform. From June 2014-Sept 2016, we enrolled all admitted AFI patients with documented or reported fever <15 days duration from 29 district/sub-district hospitals in 10 states of India. We recorded demographic and clinical data, and tested for bacterial, viral and parasitic diseases, including leptospirosis, malaria, dengue, influenza, Kyasanur Forest Disease, chikungunya, typhoid, brucellosis, and scrub typhus. An optical density of  $\geq 0.5$  was considered probable ST using a serum IgM ELISA. We enrolled 12013 AFI patients; of these, 5942 (49.5%) were positive for any pathogen. Of those with a diagnosis, 577 (9.7%) were ST-positive: Odisha (1/7, 14.3%), Kerala (218/2226, 9.8%), Jharkhand (26/332, 7.8%), Tripura (26/424, 6.1%), Tamil Nadu (42/778, 5.4%), Karnataka (189/4878, 3.9%), Maharashtra (12/379, 3.2%), Assam (39/1458, 2.7%), Gujarat (7/324, 2.2%), Goa (17/1207, 1.4%). ST was the 4th most common pathogen, after influenza (35.5%), dengue (13.7%) and leptospirosis (10.2%). ST-positive patients ranged from 1-65 years (median age 35 years); 62% were male. 10% of patients (58/577) presented with neurological symptoms, and 2% had identified skin eschars. Low platelet counts ( $<150000/\mu\text{l}$ ) were observed in 37% (173) patients, elevated liver enzyme in 69% (227) patients, and raised C-reactive protein (above 6mg/dl) in 81% (119) patients. <1% of patients died. This study documents unexpected prevalence and large geographical spread of scrub typhus across India. As ST is more common than previously estimated, routine testing for ST at district or lower level facilities should be considered. AFI platforms, as are being built under GHS, are critical for the comprehensive detection, characterization, response, and control of known pathogens and may lead to detection of novel pathogens.

## 1156

### PREVALENCE OF BACTERIAL PATHOGENS IN WOUND INFECTIONS AND THEIR ANTIBIOTIC RESISTANCES ALONG THE RIVERS OF THE AMAZON BASIN

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Wound infections caused by antibiotic resistant bacterial pathogens are a growing problem in civilian and military populations. Very little is known about the prevalence and antimicrobial resistant profiles of bacterial pathogens isolated in remote regions. In order to characterize the causes of wound infections and their antibiotic resistances in remote regions, swabs of infected wounds were taken from patients who received medical care on board riverboats serving remote populations in the Amazonian region of Peru. The identification and antimicrobial susceptibility testing of bacteria isolated from the swabs were performed using standardized clinical laboratory procedures. Between November 2015 and September 2016, 52 wound samples from 51 participants  $\geq 18$  years old were taken (65% males, 35% females). In 44 of the 52 samples there was detectable bacterial growth (31 with only one pathogen and 13 with more than one); 1 sample grew yeast and in 7 samples there was no bacterial growth. A total of 60 bacterial strains were isolated. *Staphylococcus aureus* was the most common isolate with 27 isolates (27/60, 45%), followed by 7 isolates of *Aeromonas* spp. (12%), 5 of *Escherichia coli* (8%), 4 isolates of *Streptococcus pyogenes* (7%), 3 isolates of coagulase-negative *Staphylococcus* and 3 of *Pseudomonas stutzeri* (among others). It should be noted that from the 31 samples where only one pathogen was, 68% (21/31) were *S. aureus*. Of all *S. aureus* isolates, 81% (22/27) showed resistance to penicillin, 19% (5/27) were resistant to azithromycin and erythromycin each, 7% to clindamycin and 11% showed inducible

clindamycin resistance, and only one isolate (4%) was methicillin resistant. From the 5 *E. coli* 40% (2/5) were resistant to ciprofloxacin, and only one (20%) was ESBL-producing. *Staphylococcus aureus* was the pathogen most isolated in wound infections in the Amazon basin. The observed resistance profiles suggest that more remote areas, with presumably less antibiotic use, have lower incidences of antibiotic resistance. However, resistance was still detectable in each of these remote regions, exemplifying the far reaching problem of bacterial resistance.

## 1157

### THE CHANGING EPIDEMIOLOGY OF LEPTOSPIROSIS IN MAINLAND CHINA AND ITS IMPACT ON ANNUAL DISEASE BURDEN ESTIMATES

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Leptospirosis morbidity and mortality in China has significantly decreased since the 2000s. Evidence on the epidemiological changes occurring in the last decade and its implication on the burden are essential for formulating effective intervention strategies. We used time-series individual-level leptospirosis data from the Chinese National Disease Surveillance Reporting System (1st January 2005-31st December 2015) to analyze the epidemiological trend and estimate the burden over space and time and demographical groups. A total of 7,763 leptospirosis cases were reported during 2005-2015. Of which, 2,403 cases were laboratory confirmed. During 2005-2010, the incidence was gradually decreased ( $p\text{-trend}<0.001$ ), while from 2011-2015, it was relatively stable ( $p\text{-trend}>0.05$ ). Within the last 5-years, leptospirosis has emerged in the temperate region in northern China. We estimated approximately 10,313 Disability Adjusted Life Years (DALY) were lost due to leptospirosis during 2005-2015, comprising a total of 1,803 years of life with disability (YLD) and 8,509 years of life lost (YLL). The annual burden of leptospirosis was significantly reduced ( $p\text{-trend}<0.001$ ) with the greatest reduction occurred in the endemic provinces in southern China. Males were the most affected group (7,149 DALYs) compared to females (3,723 DALYs). The highest disease burden estimate was attributed to school-age children (10-19 years) living in less-developed provinces of China due to premature deaths. This age group has contributed to more than 30% of the total DALYs lost in the country. The dramatic reduction in the burden of leptospirosis in China observed in the past decade has been accompanied by an epidemiological change in the population most at risk and the geographical areas affected. Our study highlights that these changes reflect massive socio-demographic and ecological changes that China has undergone which their role deserves further investigation. Leptospirosis remains a public health threat in China. Hence, we recommend a strengthened targeted surveillance and control measures particularly in the identified endemic region in China.

## 1158

### DETECTION OF SCRUB TYPHUS IN A SUBSTANTIAL PROPORTION OF ACUTE ENCEPHALITIS SYNDROME PATIENTS IN INDIA: THE CASE FOR ROUTINE TESTING AND EVALUATION

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In India, Japanese encephalitis virus (JE) is the most commonly recognized cause of acute encephalitis syndrome (AES). Recent reports suggest scrub typhus (ST; *Orientia tsutsugamushi* infection) may also contribute to AES burden, though ST testing is not routinely performed in AES patients. We conducted prospective facility-based AES surveillance, and systematically assessed all JE-negative patients for ST. Between January 2014–December 2016, serum and cerebrospinal fluid (CSF) were collected from each patient admitted with AES [fever (>38°C) and ≥1 of altered mental status or seizures] to selected district hospitals in Uttar Pradesh (UP), West Bengal (WB), and Assam. JE-negative patients were tested for seven pathogens according to a standard laboratory algorithm. ST testing was done by Inbios IgM ELISA using locally validated optical density cut-offs, which has shown strong correlation with gold standard assays (IFA). A case of probable ST was defined as a positive serum ST IgM in a patient who tested negative for all other pathogens. Standard clinical data were collected. Among 6770 AES patients enrolled, 5601 (83%) were JE-negative. Of these, 4314 (77%) were tested for ST; 1040 (24%) were positive. The proportion of AES patients with ST was 36% (745/2052) in UP, 26% (91/347) in WB, and 11% (204/1915) in Assam. The majority (69%) of ST cases were detected from July 1–October 31, when the majority (54%) of AES cases was reported. Of 305 ST patients with recorded CSF cytology, 232 (76%) had white blood cell count >10/μL. Of 141 ST patients with recorded data, 20 (14%) reported rash; none had an identified eschar. Of 563 ST patients with recorded outcomes, 34 (6%) died. ST was detected in a substantial proportion of JE-negative AES patients in high AES burden areas of India, though there was wide geographic variability in ST prevalence between UP, WB, and Assam. The overlapping seasonality of ST and JE transmission, the absence of eschar, and frequent detection of CSF pleocytosis in ST AES cases underscores the challenges of distinguishing ST from other AES causes by clinical signs alone, and highlights the need to routinely test AES patients for ST in India.

## 1159

### IN SILICO PREDICTION OF OUTER MEMBRANE PROTEINS FROM *BARTONELLA BACILLIFORMIS* AS CANDIDATE VACCINE

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Carrión's disease caused by *Bartonella bacilliformis* is a major public health problem in Peru. Currently, control of this disease is mainly based on case management and vector control. However, the availability of a vaccine against this disease would be very useful for its prevention. Outer membrane proteins are more promising vaccine candidates against this infection. The KC583 *B. bacilliformis* proteome was analyzed in

order to predict outer membrane proteins. The presence of helix-like transmembrane domains was determined using TMHMM 2.0 server. In addition, all *B. bacilliformis* proteins were analyzed with the pSORT software. Additionally, the presence of peptide signal was analyzed using SignalP, the candidates were compared with PEDANT, Pfam and COG databases. 112 proteins were predicted as outer membrane proteins: 3 autotransporters, 25 outer membrane proteins (5 of these was annotated as lipoproteins), 10 membrane-associated and 74 hypothetical proteins. Our results contribute to identifying outer membrane proteins that are promising vaccine candidates against Carrion's diseases.

## 1160

### THE EPIDEMIOLOGY OF SEVERE MALARIA IN RURAL UGANDA: A FRESH LOOK AT OLD PARADIGMS

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Severe *Plasmodium falciparum* malaria is a leading cause of morbidity and mortality across sub-Saharan Africa. After more than a decade of investment in highly effective interventions, however, few studies have re-examined the epidemiology of severe malaria. We conducted a prospective, observational cohort study in order to describe the epidemiology of severe malaria among children and adults presenting to a rural health center in Uganda. Individuals presenting with a history of fever were screened with a malaria RDT. Those with a positive RDT result had blood collected for microscopy and measurement of hemoglobin and serum chemistries. Severe malaria was defined in accordance with the WHO guidelines for research and epidemiological studies. Ninety-eight of 1,462 (6.7%) of participants with confirmed parasitemia satisfied criteria for severe malaria. The incidence of severe malaria peaked between 2-3 years of age (IRR 17.1, 95% CI 8.4-34.9, p<0.001) and then declined steadily until age 10. However, we also found a second peak among patients ≥50 years of age (IRR 7.78, 95% CI 2.44-24.9, p=0.001). Severe anemia and cerebral malaria were infrequent complications of severe disease, detected in only 5.3% and 3.1% of cases respectively. Instead, lactic acidosis (19.4%) and hyperparasitemia (19.4%) were the most frequently encountered manifestations. Few participants with acidosis demonstrated acidotic breathing and the performance of clinical examination for identifying acidosis was quite low (Sn 15.4%, Sp 76.9%). In conclusion, we found that severe anemia and cerebral malaria were rarely observed among patients with severe malaria. Instead, acidosis was the most common manifestation of severe disease, although few patients demonstrated visible signs of acidotic breathing. Severe malaria was most common in young children, but an elevated risk persisted up to the age of 10, as well as among those >50 years of age. Together, these findings suggest that a reliance on traditional bedside criteria and targeting resources to young children may miss a significant proportion of cases.

## 1161

### THE 2009 WORLD HEALTH ORGANIZATION DENGUE CLASSIFICATION OVER-ESTIMATES DENGUE DISEASE SEVERITY IN SRI LANKA

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Dengue is the most important arboviral disease in the world with a diverse clinical presentation. Accurate diagnosis is important for a favorable outcome. The revised 2009 World Health Organization (WHO) dengue classification was developed to improve sensitivity of diagnosis; the

performance of these criteria has not been widely assessed in Sri Lanka. A cross-sectional study was conducted at the largest tertiary care hospital in Southern Province, Sri Lanka in June 2013- Oct 2014. Children and adults with acute fever were enrolled. Epidemiological/ clinical data and blood samples were obtained at enrollment and 2-4 wks later. Dengue IgM capture ELISA was performed on convalescent sera. Acute sera NS1 antigen testing and paired IgG testing were performed in patients with IgM positivity. Of 366 enrolled patients, 248 (67.8%) were male and 243 (66.4%) were adults. A total of 67 (19.5%) had dengue, with 43.3% primary, 53.7% secondary, and 3.0% unclassified. Patients with dengue were older (26.7 years vs 22.1 years,  $p=0.004$ ) and more likely to report headache (83.6% versus 53.3%,  $p<0.001$ ), joint pains (71.2% versus 42.4%,  $p<0.001$ ), and muscle pains (74.6% versus 44.7%,  $p<0.001$ ). The severity of dengue was low, with no patients having effusions and 5 (7.5%) having signs of hemorrhage. No patients with dengue received intensive care management or died. The sensitivity and specificity of clinical diagnosis were 58.2% and 90.9%, respectively, on admission. Of patients with dengue, the 2009 WHO criteria classified 14 (20.9%) as dengue without warning signs, 42 (62.7%) as dengue with warning signs, and 0 as severe dengue. The sensitivity and specificity of the WHO 2009 criteria for diagnosis were 83.6% (95% CI 74.6- 92.6) and 64.5% (95% CI 58.8-70.2), respectively. The 2009 WHO criteria had high diagnostic sensitivity in Sri Lanka, but severity was greatly over-estimated. In dengue-endemic settings, universal application of the 2009 WHO criteria may result in unnecessary admissions and further strain of overburdened healthcare systems. Algorithms for appropriate triaging of patients to inpatient versus outpatient management are urgently needed.

## 1162

### SURVEILLANCE OF ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS DURING VACCINE TRIAL: EXPERIENCE OF CVD MALI

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Vaccine preventable diseases remain the major cause of mortality in developing world. During the past decade, the number of vaccine trial performed in developing countries is increasing importantly. The need to enroll healthy participants in vaccine trials to assess efficacy is not well understood by the community and adverse events are not accepted by the participants. CVD Mali performed several vaccine trials and has developed a strong strategy to monitor adverse events following immunization among participants. During vaccine trial and study procedures all the medical staff are trained and certified in ICH GCP. After vaccination, immediate AEs and SAEs are assessed for 30 to 60 minutes and the following days according to the study specific protocol. Participants are divided between field workers and community relays who will conduct home visits and remind participants about the clinic planned visits. The main pediatrics hospital, referral health centers and community health centers within the study area are informed about the trial. Participants also have the study ID card with phone contacts of keys study investigators and field supervisors in case of emergency. All AEs and SAEs are managed adequately by the study team. With this multicomponent strategy, site has been able to monitor AEs and SAEs. The number of completed visits has increase while the total lost to follow up go and missed events toward zero. Participants understand that AEs and SAEs are not necessarily linked to the study product and that their well-being is our goal. Community engagement is very strong and leaders are involved in crisis management. Community engagement and good communication between field workers and participants are required for AEs and SAEs follow up and may reduce the risk of lost to follow up in clinical trials.

## 1163

### REASON FOR REFUSAL TO ENROLL SICK BABIES INTO CVD-MALI HOSPITAL BASED SURVEILLANCE STUDY OF INVASIVE BACTERIAL DISEASES FROM 2012 TO 2016

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Invasive bacterial diseases surveillance is essential in monitoring the impact of introduction of new vaccines and the trend of other pathogens. Since 2002, Center for Vaccine Development (CVD-Mali) is implementing a hospital based surveillance in the main pediatric hospital in Bamako, targeting children admitted or seen out as patient. We are describing here the consent process of parents. CVD-Mali Intern identifies eligible inpatient and outpatient, and after brief explanation send the child parent/guardian to the permanent office. Eligible inpatient is defined as children aged 0-15 year old admitted for fever  $\geq 39^{\circ}\text{C}$  and or suspicion of invasive bacterial infection (SIBI). Then the investigator introduce the consent process and provide parent/guardian with the consent form if literate for reading or listening the audio tape of the consent in local language for illiterate and an impartial witness must be present. Parent/guardian and witness will be given enough time and clarification will be provided. Assent is required for children aged from 13 years and above. Study procedure start after getting all required signatures on consent form. From January 2012 to December 2016, we approached legal representatives of 15245 eligible children and proposed participation of their babies in the surveillance. 509 (3.3%) refused to let their infants enrolled in the studies. Different reasons for refusal were: - Don't have money to stay at the hospital and pay for the care; - Don't have someone who can stay at the hospital next to the child; - Prefer immediate care rather than waiting for any kind of investigation; - Prefer to postpone study participant after assessment of initial treatment; - Think that taking blood sample may worsen the child fragile status; - Parent who is present cannot decide alone and propose to wait for someone else to decide child participation in the study. CVD-Mali hospital based surveillance is highly accepted by parents in general. However, refusal cases should not be neglected because of the reasons behind which are linked to poor socio-economics conditions.

## 1164

### ANTIMICROBIAL USE IN UNDER-FIVE CHILDREN WITH DIARRHEAL ILLNESS IN RURAL BANGLADESH

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Unnecessary use of antimicrobials has the potential to increase the development of drug resistance. Due to the self-limiting course of most diarrheal illnesses, use of antimicrobials should be rational; however, antibiotic misuse and overuse use has been widely reported globally, and controlling this irrational use is a major public health challenge. From January 1, 2010 to December 31, 2012, a total of 3,570 under-5 children with diarrhea were enrolled in a tertiary level hospital in Mirzapur, a rural sub-district in Bangladesh, and constituted the study population. Diarrheal stools were collected and tested for enteric pathogens using culture and antigen detection methods. Data entry and statistical analyses were performed using SPSS, Chicago, IL version 20. Associations between antimicrobial use at home and at the hospital, socio-demographic characteristics and diarrheal etiology were evaluated using chi-square test. Finally, logistic regression analysis was performed with the enter method probability of exclusion at  $p=0.10$  to identify the factors significantly

associated with a dependent variable to document the most common underlying indications leading to the prescription of an antimicrobial in diarrheal illness of under-5 children. The rate of antimicrobial use at home was 39% among children aged 0-24 months as opposed to 84% among hospitalized children of a similar age. In a multivariate analysis, distance of facility >5 miles from residence, zinc and ORS use at home, vomiting, greater than 10 stools within 24 hours, greater than 3 days of diarrhea, and presence of rotavirus diarrhea were characteristics of children more likely to receive antimicrobials at home. Children with greater than 10 stools within 24 hours, greater than 3 days of diarrhea, use of antibiotics prior to hospital attendance, fever ( $\geq 37.8^{\circ}\text{C}$ ), rectal straining and *Shigella* infection were more likely to receive antimicrobials during hospitalization or out-patient visits. Antimicrobial use of diarrheal illness in under-5 children requires considerable attention. Education is needed for rational use of antimicrobials at home and in healthcare facilities.

## 1165

### REAL-WORLD ASSESSMENT OF THE WHO GUIDELINES FOR HEPATITIS B IN RESOURCE-LIMITED SETTINGS: A PROSPECTIVE COHORT STUDY IN UGANDA

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Hepatitis B is a major cause of mortality worldwide, despite effective therapy. The cost and availability of investigations, particularly HBV DNA PCR is a major barrier to treatment. We aimed to validate WHO guidelines for identifying individuals requiring hepatitis B treatment in resource-limited settings, which include recommendations for making treatment decisions without the use of HBV DNA PCR. We compared treatment allocation with and without the use of HBV DNA in a cohort of predominantly hospital outpatients in Uganda, a low-middle income country. HBsAg positive, HIV negative, adults were recruited into a prospective cohort in Gulu Province, where HBsAg prevalence is high. Preliminary allocations into treatment and observation groups were made based on liver ultrasound and routine haematological and biochemical blood tests, and taking into account participant age. HBV DNA viral load PCR was performed for each participant and HBeAg testing for selected individuals and final treatment decisions made. Treatment decisions made with and without HBV DNA were compared. Full assessment was completed for 100 participants, treatment was indicated in 20 cases. Combining liver ultrasound, APRI and ALT identified patients for treatment with a positive predictive value of 88.2% and a negative predictive value of 94%, compared with assessment using HBV DNA viral load. Liver ultrasound identified 12/20 individuals requiring treatment. Addition of HBeAg testing as a marker for high viral replication resulted in modest improvements in allocation accuracy. Our data shows that where HBV DNA viral load PCR is unavailable, patients with hepatitis B can be assessed by liver ultrasound and routine laboratory tests alone. These findings will enable physicians in resource-limited settings to initiate treatment more readily. This has implications for the WHO target to reduce viral hepatitis deaths worldwide by 65% before 2030.

## 1166

### NON-OBSTETRIC DEATHS IN WOMEN OF REPRODUCTIVE AGE (12-49 YEARS) IN 12 RURAL COMMUNITIES FROM MAPUTO AND GAZA PROVINCE, SOUTHERN MOZAMBIQUE

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In Mozambique, data regarding death in women of reproductive age are scarce, particularly in rural areas, where the health system has limited coverage. The most recent comprehensive data comes from the post-census mortality survey from 2007-2008. The aim of this study is to estimate the mortality rate and identify non-obstetric causes of death among women of reproductive age. This is a descriptive study to assess mortality rates and causes of death in women 12-49 years using data from a census conducted in 2014 in 12 rural communities located in Gaza and Maputo Provinces, southern Mozambique. This census included household registration to identify women of reproductive age, death registration and verbal autopsy. Women were separated into three age groups: 12-19, 20-34 and 35-49 years, the cause of death was determined using InterVA4. All obstetric related cases of death were excluded because they were published elsewhere. A total of 80,483 women of reproductive age were identified for this study. Of the 246 (3.0 per 1000 women of reproductive age) deaths reported in the previous 12 months, 216 (87.8%) were non-maternal, 23 (9.35%) were maternal and 7 (2.8%) without verbal autopsy information. Communicable diseases, non-communicable disease and road traffic accidents/trauma were responsible for 68.9%, 14.7% and 8.7% of deaths, respectively. The top three causes of death were HIV/AIDS (41.6%), tuberculosis (14.1%), and road traffic accidents or trauma (7.1%). However, age-related differences in top-three causes were observed (malaria (7.7%) at age 20-34 and acute respiratory infection including pneumonia (4.7%) at age 35-49). This study shows that HIV/AIDS remains the most important cause of death in these rural communities. The study findings also reveal the contribution of non-communicable disease, road traffic accidents and trauma which is typical in urban areas suggesting a transition of diseases burden. Strategic public health interventions targeting poor and rural population including HIV care is crucial to save lives in rural Mozambique.

## 1167

### IMPACT OF INTEGRATING A PRE-REFERRAL TREATMENT OF SEVERE MALARIA WITH RECTAL ARTESUNATE AT THE COMMUNITY LEVEL: A NON-INFERIORITY TRIAL IN THE DEMOCRATIC REPUBLIC OF CONGO

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The Democratic Republic of Congo (DRC) adopted the strategy of using at the community level, a dose of rectal artesunate as a pre-referral treatment of severe malaria amongst under five children (CU5) who cannot reach quickly a health care facility and take oral medication. However, its feasibility and acceptability were unknown. To assess the impact of integrating the pre-referral rectal artesunate on the adherence to the referral advice provided by the community health workers (CHWs) and the CHWs and nurses' capacities to identify correctly the danger signs of malaria. A non-inferiority (NI) community trial with a pre- and post-intervention design and a mixed approach was conducted in 51 community care sites (CCS) in 4 provinces (Kasaï-Oriental, Kasaï-Central, Lomami, Lualaba) from August 2014 through June 2015. Surveys targeted at pre-test 387 mothers of CU5, 63 CHWs and 45 nurses; at post-test, 346 mothers, 41 CHWs and 37 nurses. Proportions at 95% confidence intervals were calculated for key indicators. A 15% threshold was considered for NI analyses due to the expected decrease of the adherence to the referral advice after the introduction of the strategy. Rectal route was often used (60.7%) and medicines given rectally were considered more effective (63.6%) and easily administrate (69.7%). Acceptability of the pre-referral strategy was relatively high: 79.4% (CI95: 75.4-83.3) among mothers, 90.3% (CI95: 82.3-96.8) among CHWs, and 97.8%

(CI95: 93.3-100) among nurses. In addition, 41.5% of CHWs and 32.4% of nurses could identify correctly the five danger signs of severe malaria at post-test compare to none at pre-test ( $p < 0.05$ ). The adherence to the referral advice at post-test [84.3% (CI95: 80.6-88.1)] was non-inferior to the pre-test adherence [94.1% (CI95: 91.7-96.4)]. Integration of the pre-referral strategy of severe malaria in the CCS in DRC is feasible and acceptable. It had positive impact on CHWs and nurses' capacities to identify correctly the dangers signs of malaria and on the adherence to the referral advice. However, more information, education and communication are needed for parents of CU5 and trainings for CHWs and nurses.

## 1168

### EVALUATING TRADEOFFS BETWEEN ORAL FLUID AND BLOOD SAMPLES FOR BIOMARKER DETECTION IN RESEARCH AND SURVEILLANCE PROGRAMS: A SIMULATION MODEL

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Biological markers are integrated into population health surveys to obtain more accurate measures of disease outcomes and risk factors. Biomarkers can be measured using point-of-care-diagnostics or samples can be collected for subsequent analyses in the lab (e.g. enzyme immunoassays). The type of sample collected determines the type of assay that can be performed and the test sensitivity. Some sample types may be more easily collected in field settings, particularly during household surveys (e.g. oral fluid is considered more acceptable than blood). A common assumption is that the tradeoff in increased acceptability, and therefore test coverage, compensates for a loss in test sensitivity. In this analysis, a simulated population was constructed to determine the tradeoffs between assay sensitivity and acceptability. The first analysis determined what proportion of those with an outcome would be correctly identified by an assay of varying sensitivity and test coverage. The second analysis determined what degree of bias would be introduced into the prevalence estimate measured using an assay with varying sensitivity, specificity, and test coverage, as well as the degree of bias introduced when coverage is differential by the outcome of interest. Preliminary results showed for every increase in test coverage, the increase in identified individuals was proportional to the sensitivity of the assay. Thus, an assay with a 10% lower sensitivity would have to increase coverage by at least 10% to identify the same proportion of individuals. Under the assumption of less than perfect specificity, assays with higher sensitivity slightly overestimated the prevalence of the outcome while assays with lower sensitivities consistently underestimated the prevalence at all test coverage levels. These results suggest that there is a significant tradeoff in the ability to detect an outcome of interest with a lower sensitivity test that may have moderately higher acceptability levels. When the test is used to estimate prevalence, increased acceptability of a test with lower sensitivity can result in inaccurate and biased estimates.

## 1169

### DELIVERY MECHANISM OF INTERMITTENT PREVENTIVE THERAPY (SULFADOXINE PYRIMETHAMINE) AMONGST PREGNANT WOMEN IN FEDERAL MEDICAL CENTRE KEFFI, NASSARAWA STATE

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Malaria is highly endemic in Nigeria and poses a major health challenge with attendant risk of morbidity and mortality contributing to loss of productivity and economic development. The most vulnerable groups are children below 5 years of age and pregnant women, particularly women in their first and second pregnancy. IPT is recommended in pregnancy to prevent malaria in pregnancy. The following are objectives for the research project:

To establish the mode of administration, frequency of administration and total dosage of IPT given to pregnant women and also to identify the challenges involved with administration and compliance.

Structured interviewer administered questionnaires will be used for data collection from pregnant women to find out their preferred mode of administration of IPT, frequency of administration and also the side effects of the therapy on the mother.

It is hoped that the findings of the proposed research will inform decisions on how to counsel pregnant women to use IPT.

## 1170

### HOW CAN WE KEEP IMMIGRANT TRAVELERS HEALTHY? HEALTH CHALLENGES EXPERIENCED BY CANADIAN SOUTH ASIAN TRAVELERS VISITING FRIENDS AND RELATIVES

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Immigrant travelers who visit friends and relatives (VFR) experience high rates of travel-related morbidity, which has been attributed in part to low uptake of pre-travel health advice. While several barriers to accessing advice have been identified, better characterization of these barriers is needed to inform strategies to overcome them. Consequently, we sought to understand how South Asian VFR travelers perceived and responded to travel-related health risks. We recruited participants and clients from community service organizations for four group interviews between November 2015 and January 2016. Participants were residents of Peel region in Ontario, Canada, who reported visiting friends and relatives in India or Pakistan within the past five years. Interviews were audio-recorded, transcribed, and analyzed thematically. Baxter et al's 'typologies of concern' was used as a framework to understand how participants conceptualized risk. We interviewed 32 travelers who described facing numerous health challenges, from contaminated water and food to pollution to their own personal health, among others. Participants expressed a moderate level of concern for most known risks, which triggered responses to mitigate or avoid the risk where possible or convenient to do so, consistent with Baxter's 'Wait and See' typology. While no participants visited a travel medicine clinic prior to departure, they were knowledgeable of key travel health risks and responded pragmatically with convenient and affordable strategies or behaviours (e.g. drinking bottled or boiled tap water). Responses to risks were context and value dependent. We found that planning a trip in rushed circumstances, a desire to preserve authentic experiences, familial duty to hosts, and financial constraints were competing concerns to travelers responding to health risks. As VFR travelers become knowledgeable of travel health risks, health promotion efforts should emphasize the added value of medical intervention (e.g. immunization and destination-specific health advice), and explore how to best support VFR travelers in light of unique competing concerns they face.

## 1171

### SAFETY AND IMMUNOGENICITY OF AGS-V, A MOSQUITO SALIVA PEPTIDE VACCINE: A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED PHASE 1 TRIAL

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Mosquito-borne diseases continue to cause significant morbidity and mortality worldwide despite on-going control efforts. A new approach to disease prevention focuses on the arthropod salivary-mediated transmission of pathogens. Parasites and viruses carried within mosquito saliva appear to initiate or enhance severity of host infection by taking advantage of saliva-human host interactions. This leads to alteration of the cutaneous environment and modulation of the host's innate and adaptive immune responses, thereby providing a rationale for creating vaccines against mosquito salivary proteins rather than the pathogens contained within the saliva. AGS-v is a vaccine composed of four salivary peptides that are common across a number of arthropods. In this ongoing first-in-human study, we are enrolling and randomizing 45 healthy adult participants to receive the AGS-v vaccine with and without adjuvant (Montanide ISA 51) versus placebo. Vaccinations occur on Day 0 and Day 21 followed by a clean mosquito feeding on Day 42. Primary objectives are: 1) to assess safety via incidence of adverse events and 2) to evaluate humoral and cellular immunity by respectively measuring total AGS-v specific immunoglobulins and Th1-associated cytokine release after incubation of peripheral blood mononuclear cells (PBMCs) with AGS-v antigens. Secondary objectives are post-mosquito feeding measures of AGS-v specific immunoglobulins and Th1-related cytokine release, mosquito survival and fecundity, as well as the effects of saliva-coated Zika virus on cytokine production by PBMCs from immunized individuals. This trial is in process with the plan to complete all vaccinations and mosquito feedings by August 2017. Of note, the data blind will still be in place at the time of the conference.

## 1172

### SAFETY AND FUNCTIONAL IMMUNOGENICITY OF PLASMODIUM FALCIPARUM TRANSMISSION BLOCKING VACCINES PFS230D1M-EPA/ALHYDROGEL AND PFS25M-EPA/ALHYDROGEL ALONE AND IN COMBINATION IN MALIAN ADULTS

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Malaria still is a public health problem. New approaches, such as transmission blocking vaccines (TBV) that prevent mosquito infections, are needed for malaria elimination. We recently reported that a TBV targeting sexual stage antigen Pfs25 induced functional antibodies in US volunteers when conjugated to the carrier protein ExoProtein A (EPA), and are now studying TBV responses in malaria-experienced volunteers in Mali. Before the malaria transmission season, 225 healthy volunteers aged 18-50 years old were enrolled in a dose-escalating, double blind, comparator-controlled study to assess the safety, tolerability, immunogenicity (by ELISA), and functional activity (by SMFA and DSF) of two transmission blocking vaccines (TBV) against *Plasmodium falciparum* malaria (Pfs230D1M-EPA/Alhydrogel® and Pfs25M-EPA/Alhydrogel®) in Bancoumana, Mali. We first assessed the safety of the vaccines administered alone or in combination in 25 participants. The larger cohort then enrolled volunteers to receive either Pfs25M and normal saline (NS) (n=50), Pfs230D1M and NS (n=50), Pfs25M + Pfs230D1M co-administered (n=50), or comparator and NS (n=50) at 0, 1, 6 months in 2015 with a booster vaccination at 18 months given in 2016. Vaccinations have been well tolerated, with most reported AEs being mild or moderate in severity. No serious adverse events related to vaccination were reported. Based on their ability to induce functional immune responses (SMFA, DSF), Pfs25M alone was inferior to Pfs230D1M alone or Pfs230D1M + Pfs25M, while the combination of Pfs230D1M + Pfs25M did not improve on Pfs230D1M alone. These findings will inform future TBV development, such as the trial to test Pfs230D1M-EPA alone with the adjuvant AS01 that started in Mali this year.

## 1173

### BIOMARKERS OF ENDOTHELIAL AND IMMUNE DYSFUNCTION PREDICT MORTALITY IN FEBRILE OUTPATIENT TANZANIAN ADULTS

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Febrile syndromes are among the most common causes of illness globally, accounting for over 1 billion episodes annually. However, most infections are self-limited and few progress to life-threatening infections (LTIs). We currently lack tools to rapidly identify patients who have, or will progress to LTIs and this is a major barrier to rational triage and management of patients at both outpatient and inpatient levels. We hypothesized that measuring biomarkers of pathways implicated in the pathobiology of serious infections would permit the early recognition of LTIs due to multiple etiologies. In this study, we examined consecutive adults (>18 years of age) presenting with fever (>38.0C) to outpatient departments in Dar es Salaam, Tanzania. We quantified circulating levels of markers of endothelial (Ang-2, sFlt-1, sVCAM-1) and immune (sTREM-1, IL-6, IL-8, CHI3L1, sTNFR1, PCT, CRP) dysfunction at clinical presentation, using Luminex® multiplex platform or ELISA, to determine if they predicted disease severity and outcome in "all cause" fever syndromes (i.e. independent of etiology). Of the 519 febrile adults enrolled, 9 died within 48h of presentation and 32 by day 28. Single biomarkers variably predicted 48h mortality: CRP (AUROC 0.55), PCT (AUROC 0.73) versus sTREM-1 (AUROC 0.91) and Ang-2 (AUROC 0.90) which had the best predictive accuracy. By day 28, sTREM-1 remained a good predictor of mortality (AUROC 0.87). Combining a clinical score, qSOFA (quick Sepsis Related Organ Failure Assessment), with even a single biomarker significantly improved predictive accuracy at both day 2 (AUROC 0.94) and day 28 (AUROC 0.91) mortality. These findings validate sTREM-1 and Ang-2 as informative markers to identify individuals at risk of infection-related mortality. Since sTREM-1 and Ang-2 displayed superior performance

to qSOFA, point-of-care rapid diagnostic test versions of these markers could have clinical utility in triage and risk stratification of febrile patients, especially in low resource settings.

## 1174

### OUTER MEMBRANE PROTEINS LSA46 AND LSA77 ARE POTENTIAL VACCINE CANDIDATES AGAINST LEPTOSPIROSIS PROTEINS LSA46 AND LSA77 ARE POTENTIAL VACCINE CANDIDATES AGAINST LEPTOSPIROSIS

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Leptospirosis is a major public health problem caused by pathogenic spirochetes of the *Leptospira* genus. The available vaccine formulations induced short term-immunity and protect only against the serovars included in the preparation. Development of a universal, cost-effective vaccine is long being pursued. The aim of this study is to evaluate the immunoprotective potential of Lsa46 and Lsa77 proteins, individually or combined, with Alum adjuvant. The amplified gene sequences were cloned into the vector pAE for expression of recombinant proteins. The recombinant proteins Lsa46 and Lsa77 were purified by affinity chromatography under denaturing conditions. Hamsters were immunized with 50 µg of Lsa46 and Lsa77 or 25 µg of each protein mixed in 10% Alhydrogel, and antibodies against Lsa46 and Lsa77 evaluated. After immunization, hamsters were challenged with an intraperitoneal inoculum of virulent leptospires. The recombinant proteins were purified by affinity chromatography and confirmed by Western blottings probed with polyclonal antibodies raised in hamsters against the proteins. Lsa46 and Lsa77 were recognized by confirmed leptospirosis human serum samples. Furthermore, mouse anti-Lsa46 and anti-Lsa77 sera recognized the corresponding leptospiral Lsa46 and Lsa77 proteins in virulent Fiocruz L1-130 and pathogenic attenuated M20 whole cell lysates. The data showed that recombinant proteins are capable to stimulate antibody immune response in hamsters. Immunoprotection evaluation of recombinant proteins followed by challenge with virulent leptospires showed that both proteins conferred partial protection. However, only animals immunized with Lsa77 exhibited reduction in renal colonization when compared with Lsa46. Immunization with combined Lsa46+Lsa77 resulted in 90% protection after challenge. Although the death observed with PBS-control group were inconsistent, leptospiral burden among the survivors were lower than the control group. In conclusion, the results indicate that Lsa46 and Lsa77 are potential vaccine candidates against *Leptospira*.

## 1175

### SAFETY AND FUNCTIONAL IMMUNOGENICITY OF PFS25M-EPA/AS01 AND PFS230D1M-EPA/AS01 TRANSMISSION BLOCKING VACCINES AGAINST *PLASMODIUM FALCIPARUM* IN MALIAN ADULTS

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Transmission blocking vaccines (TBV) prevent mosquito infections and could be critical tools for malaria elimination in Sub-Saharan Africa. NIAID scientists have enhanced TBV immunogenicity by conjugating recombinant forms of sexual stage antigens such as Pfs25 and Pfs230 to carrier proteins including ExoProtein A (EPA), and by formulating vaccines in more potent adjuvants. A phase 1 dose-escalating, double-blind, randomized, comparator-controlled trial (NCT02942277) of the safety, tolerability, and immunogenicity of the conjugated vaccines Pfs25M-EPA and Pfs230D1M-EPA formulated with AS01 adjuvant developed and contributed by GSK, is currently being conducted in healthy adult volunteers in Mali. The study started in January 2017 with the unblinded pilot safety cohort in Sotuba, a peri-urban area of Bamako, where 65 healthy adult volunteers aged 18-50 years old were enrolled and have thus far received two doses of the scheduled three vaccine series at 0, 1, and 6 months. Among the 45 participants receiving TBV in AS01 adjuvant, 15 received a low vaccine dose (16µg Pfs25, n=5; or 13µg Pfs230, n=5; or 16µg Pfs25 + 13µg Pfs230, n=5), while 30 received a high vaccine dose (47µg Pfs25, n=10; or 40µg Pfs230, n=10; or 47µg Pfs25 + 40µg Pfs230, n=10). 20 participants received the comparator vaccine Engerix-B. After initial review of the safety cohort, the main double-blind phase of the study started on March 30th, 2017 in Bancoumana and Donéguébougou, two rural villages outside of Bamako, Mali, where to date 196 out of the planned 240 healthy adult volunteers have received first vaccinations with either 40 µg Pfs230 or Engerix-B. To date, vaccinations have been well-tolerated with few related vaccine adverse events (AEs); most reported AEs have been local injection site reactivity, particularly mild injection site pain. Safety data will be presented from both the unblinded pilot safety cohort and blinded field cohort. Immunogenicity including functional serum activity post vaccination from the unblinded pilot safety cohort will be presented.

## 1176

### COMMON SCREENING FINDINGS AND EXCLUSION CRITERIA FOR ADULTS WILLING TO ENROLL IN A MALARIA TRANSMISSION BLOCKING VACCINE STUDY IN SOTUBA, MALI

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For malaria vaccine testing, volunteers must be enrolled from a suitable site and population. Screening is a critical step to identify and enroll appropriate participants into early phase studies. We are conducting a malaria transmission blocking vaccine study among Malian adults in Sotuba, a peri-urban area of Bamako. Once informed consent was obtained, each individual underwent clinical and laboratory assessments. The screening aimed to assess inclusion and exclusion criteria that preclude the enrolment of adult subjects into a dose-escalating, open label, randomized, pilot phase 1 study. In January 2017, we screened 175 volunteers and enrolled 65 volunteers into the malaria vaccine study of Pfs25M-EPA/AS01 and Pfs230D1M-EPA/AS01. Of 175 screened, 114 (65.1%) were eligible for enrollment. Of the 61 (34.9 %) subjects not eligible for enrollment, the main reason was volunteer withdrawal of consent (23/61; 37.7%) before or at the time of enrollment. Of the remaining 38 subjects, 31 (81.6%) failed screening secondary to laboratory abnormalities. The most frequent reason for screen failure was

the hepatitis B surface antigen positivity (9.1%; 16/175), followed by high blood pressure (3.4%; 6/175). Hepatitis C (3.4%; 6/175) and HIV (1.7%; 3/175) frequency was low in this population. In this area, less than 35% of volunteers failed to be enrolled, with voluntary consent withdrawal being the primary reason followed by abnormal laboratory results and high blood pressure. Ratio of screened to enrolled (1.5:1) in Sotuba is lower than other vaccine trial sites in Mali in rural areas.

## 1177

### BURDEN OF COMMON ILLNESSES AND THE PROTECTIVE EFFECT OF BREASTFEEDING IN EARLY CHILDHOOD IN MAL-ED, AN EIGHT-SITE COHORT STUDY

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Children in low-income countries experience multiple episodes of illness in early childhood, and the relationships among these illnesses is not well understood. In the MAL-ED cohort study, 1,731 children were enrolled at or near birth and followed for two years in eight sites (in Bangladesh, Pakistan, India, Nepal, Brazil, Peru, South Africa, and Tanzania). Field workers visited households twice a week and inquired about symptoms the child experienced and their diet. Logistic regression was used to determine if history of illness (in the last 30 days) was associated with incidence of diarrhea or ALRI, accounting for exclusive breastfeeding. Exclusive breastfeeding in the first three months of life was protective against diarrhea. Children with history of diarrhea, fever, or ALRI who were also exclusively breastfed were half as likely to experience a new episode of diarrhea in the first three months of life (diarrhea: OR 0.44, 95% CI 0.31, 0.62; fever: OR 0.61, 95% CI 0.48, 0.77; ALRI: OR 0.50, 95% CI 0.29, 0.86). Fever was associated with greater odds of incident diarrhea between 3-5 months (OR 1.44, 95% CI 1.29, 1.61), and in children > 6 months, diarrhea and fever were both associated with higher odds of incident diarrhea (diarrhea: OR 1.15, 95% CI 1.07, 1.22; fever: OR 1.40, 95% CI 1.33, 1.48). Fever increased the chance of ALRI at all ages (0-2: OR 2.69, 95% CI 1.99, 3.62; 3-5: OR 2.70, 95% CI 1.81, 4.03; >6: OR 3.04, 95% CI 2.74, 3.38), and breastfeeding practices did not change that relationship. Exclusive breastfeeding in the first three months of life was protective against new episodes of diarrhea, which underscores the importance of exclusive breastfeeding in early childhood. Previous illness was associated with greater risk of new illness, indicating that public health programs would benefit from targeting those high morbidity children and households.

## 1178

### CHILDHOOD NEURODISABILITY: CHALLENGES FACED BY CHILDREN AND THEIR FAMILIES IN RURAL NEPAL

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In Nepal, 1.94% of the population is reported to have disability (Nepal census data, 2011). The first National, representative household survey carried out in Nepal in 2014 - 2015 identified a lower score in most indicators of level of living, in households with a person with disability in both urban and rural Nepal. Nepal poses unique challenges due to poverty, a predominantly rural population, the challenging terrain and

strongly held cultural beliefs. Little is known about the prevalence and impact of childhood neuro-disability on families in Nepal. There is lack of data available from Nepal on this topic. We undertook qualitative research among the caregivers of 24 children from 23 families (18 boys, 6 girls; mean age 8.3y) in rural Nepal between 2013 and 2014. Focus group discussions and face to face interviews guided by structured questionnaire were employed. Thematic analysis was carried out to identify the key issues. The children had conditions ranging from cerebral palsy to genetic neuro-degenerative conditions. The key issues identified were difficulty in obtaining appropriate and timely medical advice, financial challenges, practical challenges with schooling, cultural beliefs and taboos that influence the child and the family, and the psychological impact of the illness on the children and caregivers. There is often a significant delay in confirming the diagnosis, and in many cases the diagnosis is not yet clear. Limited facilities for medical treatment and therapy makes it challenging to access these regularly. Among the group, 18 caregivers reported significant anxiety and fear about their child's future. Three children lived in single parent households. Alcohol addiction, domestic abuse and abandonment by a parent was reported in 4 families. Most of our families reported that children were treated differently by the community (19/23), but 3 caregivers reported that their children were positively supported and loved by the community due to their disability. Our data identified gaps in clinical service, educational support and psychological support of children with neuro-disability and their families in rural Nepal.

## 1179

### A NOVEL PUTATIVE LIPOPROTEIN OF *LEPTOSPIRA INTERROGANS* THAT INTERACTS WITH LAMININ, PLASMINOGEN AND COMPLEMENT COMPONENTS

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Leptospirosis is a zoonosis globally disseminated caused by pathogenic spirochetes of the genus *Leptospira*. Rodents are the main reservoirs of the disease and constantly eliminate live leptospires in their urine, contaminating soil and water. Preventive measures to control leptospirosis are difficult to implement. Thus, understanding leptospiral pathogenic mechanisms is critical for the efficient development of vaccines and diagnostic tests. Our group has identified leptospiral surface proteins with the ability to bind extracellular matrix and plasma components, which could mediate adhesion and facilitate invasion through the hosts. This work aims to characterize the role of a probable lipoprotein encoded by the gene LIC13259 of *L. interrogans* serovar Copenhageni in pathogenesis. The gene LIC13259 was cloned into the expression vector pAE. The plasmid pAE-LIC13259 was employed to transform *E. coli* strains for protein expression studies. After purification of the recombinant proteins, mice were immunized for polyclonal antibody production. The ability of recombinant protein to interact with the extracellular matrix proteins, complement and human plasma components was evaluated. The coding sequence LIC13259 was cloned and expressed in their soluble form successfully. The recombinant protein was purified by nickel affinity chromatography and appeared as a single band after SDS-PAGE analysis. Mice immunized with the recombinant protein LIC13259 stimulated effectively antibody immune response. LIC13259 exhibited adhesion properties and was able to bind laminin. Studies with human plasma components demonstrated that LIC13259 interacts with plasminogen and in the presence of an activator could generate plasmin. Furthermore, LIC13259 was able to interact with vitronectin, C7, C8 and C9 complement components in a dose-dependent manner and could recruit them from normal human serum. Our data suggest that LIC13259 is a multifunctional protein that might be involved in invasion and complement immune evasion processes within the hosts.

### ASSESSMENT OF *LEPTOSPIRA INTERROGANS* PROTEINS LIC11711 AND LIC12587 AND THEIR INTERACTIONS WITH EXTRACELLULAR MATRIX AND PLASMA COMPONENTS

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Leptospirosis is a worldwide zoonosis of endemic occurrence in tropical countries. In Brazil, *Leptospira interrogans* serovar Copenhageni is prevalent in urban centres, affecting several species of animals including humans. Therefore, the understanding of leptospiral interactions with host components and the immune system is important to elucidate pathogenicity mechanisms and to develop strategies to eliminate the microorganism. In this work, we aimed to express and to characterize two putative membrane proteins of *L. interrogans* serovar Copenhageni and assess their interactions with human components. Recombinant LIC11711 and LIC12587 proteins were expressed in *E. coli* DE3 Star pLysS strain and then purified by metal affinity chromatography. For native proteins localization assay, intact *L. interrogans* strain M-20 and saprophytic *L. biflexa* cells were immobilized and detected with homologous antiserum and secondary anti-mouse/HRP by ELISA; cytosolic protein LipL31 was used as negative control. The interaction of recombinant proteins with human extracellular matrix and plasma components was evaluated by ELISA. Recombinant proteins were obtained from the soluble fraction after cell lysis and purified by metal affinity chromatography. Protein purity was observed by SDS-PAGE. Both proteins were detected in intact *L. interrogans* but are absent in the saprophyte *L. biflexa* cells. LipL31, a cytosolic protein, was obtained only in basal level, suggesting that both proteins are probably located on the leptospiral surface. Binding interactions with host components laminin and plasminogen were dose-dependent, but didn't reach saturation for both recombinant proteins. In addition, when bound to PLG, the component was converted into its enzymatically active form, plasmin, in the presence of the plasminogen activator uPa. The proteins encoded by the genes LIC11711 and LIC12587 are probably located at bacterial surface and could mediate host-pathogen interactions.

### 1181

### PLASMODIUM FALCIPARUM CONTROLLED HUMAN MALARIA INFECTION IN MALARIA EXPOSED VOLUNTEERS: CAN IT INFORM MALARIA VACCINE TRIALS IN THE FIELD

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A clinical trial was conducted in Mali, West Africa to assess the safety, immunogenicity and protective efficacy of a 3-dose regimen of Sanaria® PfSPZ Vaccine (1.8x10<sup>6</sup> PfSPZ) administered by direct venous inoculation (DVI) against homologous controlled human malaria infection (CHMI) by DVI. 29/30 (96.7%) subjects enrolled to receive PfSPZ Vaccine during the dry season completed all 3-doses. ~6 weeks later, 15 infectivity controls and 29 vaccinees underwent CHMI with 3.2x10<sup>3</sup> PfSPZ Challenge. All 44 subjects received a full treatment course of artesunate/amodiaquine

(ASAQ) approximately 7 weeks prior to CHMI. Primary protective efficacy endpoint was detection of 1st positive blood smear (BS). All subjects were treated with artemether/lumefantrine when BS positive or at day 27 post CHMI. All 44 subjects completed CHMI follow-up. Reported AEs post CHMI were few, with vaccinees reporting 5 AEs (4/29 subjects; 13.8%) and infectivity controls 8 AEs (7/15 subjects; 46.7%). All reported AEs were mild to moderate. Only one AE, granulocyte decrease, was deemed related to CHMI. Of subjects undergoing CHMI, 0/29 vaccinees and 1/15 infectivity controls became BS positive. By qPCR, 0/29 vaccinees and 8/15 (53.3%) infectivity controls were positive. Most qPCR positive infectivity controls were positive only for a single timepoint (5/8; 62.5%). By qPCR vaccine efficacy was significant (p<0.001) by time-to-infection analysis and 100% (p<0.001, 95% CI 73-100%) by proportional analysis. In comparison, participants who underwent the same vaccination series and were assessed for protective efficacy against natural malaria infection during 24 weeks had a per-protocol vaccine efficacy of 51% (p=0.004, 95% CI 20-70) by time-to-infection analysis and 24% (p=0.031, 95% CI 2-41) by proportional analysis. Protective efficacy against homologous CHMI at 6 weeks exceeded vaccine efficacy against natural occurring infection measured over 24 weeks; given the incomplete infectivity achieved with 3.2x10<sup>3</sup> PfSPZ in the controls, higher doses of PfSPZ Challenge via DVI, or CHMI with heterologous parasite strains, should be assessed to increase the stringency.

### 1182

### CASE REPORT OF SIGNIFICANTLY ABNORMAL LIVER ENZYMES IN HEALTHY VOLUNTEERS ENROLLED IN A MALARIA VACCINE CLINICAL TRIAL: IMPACT OF EMPIRIC ANTIMALARIAL DRUG TREATMENT ON VACCINE SAFETY EVALUATIONS

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A double blind, randomized clinical trial was conducted in Mali, West Africa to assess the safety, immunogenicity and protective efficacy against natural malaria exposure of a three-dose regimen of Sanaria® PfSPZ Vaccine (1.8x10<sup>6</sup>) administered via direct venous inoculation (DVI). All study volunteers (n=175) enrolled received at least one, and in some cases three, 3-day treatment regimens with artesunate/amodiaquine (ASAQ) for *Plasmodium falciparum* (Pf) clearance prior to immunization. During the execution of the vaccine trial we noted multiple unanticipated significantly elevated transaminases (Grade 3 to 4) in four participants (2 PfSPZ Vaccine, 2 placebo) at varying stages in the study (14 to 65 days post ASAQ). All four subjects were asymptomatic at presentation with no associated agranulocytosis. All laboratory abnormalities resolved without sequelae (duration 169 to 214 days). Testing for potential of other etiologies, through imaging, expanded laboratory testing, and serology, identified no other possible contributing causes, except a traditional medicine provided to the first subject presenting with liver enzymes derangements. Serum antibodies to AQ are pending at this time. AQ has previously been linked to severe serum aminotransferase elevations in a small proportion (1%) of recipients, but the impact of AQ may be underestimated if routine follow-up testing is not done, and if, as with all 4 volunteers in this study, the abnormalities remain asymptomatic.

## 1183

**THE MANAGEMENT OF HYDROCELE IN THE HEALTH DISTRICT OF KOLLO****Adamou Bathiri A. Salissou**, Gnanadou G. Issa*Onchocerciasis and lymphatique filariasis programme manager, Niamey, Niger*

In Niger, more than half of the population lives in Areas with risk of lymphatique filariasis infection and the disease affect 1/5 of the people living in the 31 endemic districts. The debilitation effects of the disease like hydrocele aggravate the cycle of poverty, and those suffering are often unable to provide their families with everyday necessities. They are at the bottoms of society, away from school, and community life. In 2016, an active census of hydrocele cases have been conducted in 4 health centers of the district of Kollo. 156 suspected patients were actively diagnosed by community agent. After the confirmation, 71.16% of the presumed hydrocele were confirmed and the rest (28.84%) are hernias. A free hydrocele surgery camp was organized in March 2017 in the district and the technique used is a resection of the vaginal. A total of 187 interventions were carried out on the 127 patients operated. 87.4% of the patients operated came from the villages where the active census were conducted beforehand. 12.6% came by themselves because of the radio announcements. The hydrocele represents 63.3% of the interventions performed, 32.06% were hernias and the rest others. After one week, of postoperative follow-up and after two dressings were renewed, there were 0% complications and the patients returned home safe. After 3 week-end all the patients are cured and the wounds healed.

## 1184

**RECENT SUCCESSFUL CROSS-BORDER ONCHOCERCIASIS ELIMINATION ACTIVITIES - OUR EXPERIENCE****Moses N. Katarwa**<sup>1</sup>, Tom Lakwo<sup>2</sup>, Isam Zroug<sup>3</sup>, Biruck Kebede<sup>4</sup>, Peace Habomugisha<sup>5</sup>, Oumer Shafi<sup>6</sup>, Tony Ukety<sup>7</sup>, Edridah Tukahebwa<sup>8</sup>, Zerihun Tadasse<sup>8</sup>, Nabil Aziz<sup>9</sup>, Kamal Hashim<sup>3</sup>, Ismael Byaruhanga<sup>10</sup>, Leonard Lopay<sup>11</sup>, Uvon Awaca Pitchouna<sup>12</sup>, Makoy Yibi Logara<sup>13</sup>, Lauri Hudson-Davis<sup>1</sup>, Emily Griswold<sup>1</sup>, Frank Richards<sup>1</sup>

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As the shift from control to elimination of onchocerciasis progresses in Ethiopia, Sudan and Uganda, cross-border transmission has become an increasingly apparent obstacle to success. Without a clear understanding of the situation on the other side of the border, part of a transmission zone cannot be verified for interruption of transmission. There are cross-border transmission zones between Ethiopia and Sudan; Ethiopia, Sudan and Republic of South Sudan (RSS); Uganda and Democratic Republic of Congo (DRC); and possibly Uganda and RSS. Since 2014, Ethiopia and Sudan have successfully coordinated activities in the Galabat/Metema cross border focus and ensured that co-endemicity with lymphatic filariasis is also tackled in the area. Health workers from DRC and Uganda have epidemiologically and entomologically assessed three of four transmission zones along their common border. It was not possible to tackle the fourth due to civil strife, but there is determination to do so as soon as possible. Other cross-border activities planned for and keenly awaited are between Uganda and RSS. Most of the necessary discussions and planning has been done, but insecurity and funding in RSS remains a stumbling block. The factors responsible for our success and the challenges we encountered

are critical to informing future cross-border operations for onchocerciasis elimination, and possibly other public health problems. These include: utilization of local knowledge and institutions; building trust; identifying trusted leaders, required resources, and cost containment; standardization of approaches; co-ordination between relevant government departments and partner institutions; addressing lymphatic filariasis co-endemicity; and operational flexibility.

## 1185

**THE SIGNIFICANT SCALE UP AND SUCCESS OF MASS DRUG ADMINISTRATION FOR LYMPHATIC FILARIASIS IN ZAMBIA: ACCELERATING TOWARDS THE ELIMINATION GOAL OF 2020****Deborah R. Edwards**<sup>1</sup>, Elizabeth Chizema<sup>2</sup>, Caroline Phiri<sup>2</sup>, Namasiku Siyumbwa<sup>2</sup>, Tedious Sokesi<sup>3</sup>, Tina Chisenga<sup>2</sup>, Brent Thomas<sup>1</sup>, Sarah Martindale<sup>1</sup>, Hannah Betts<sup>1</sup>, Louise Kelly-Hope<sup>1</sup>, Chummy S. Sikasunge<sup>4</sup>, Mark Taylor<sup>1</sup>

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Lymphatic filariasis (LF) is a mosquito-borne disease that is endemic in 85 districts of Zambia, and targeted for elimination through the implementation of mass drug administration (MDA) of albendazole (ALB) and diethylcarbamazine citrate (DEC) to at-risk populations. Prevalence mapping conducted between 2003-2010 using the rapid immunochromatographic test (ICT) card found an overall rate of 7.4% (range 0-54%). Pre-MDA sentinel site surveys conducted in 2012 and 2014 indicated a significant decline in LF prevalence with most districts reporting <1%, which has been associated with the scale-up of insecticide treated bed nets (ITNs). This suggests that MDA including ALB/DEC may further reduce LF transmission to critical elimination thresholds. This study highlights the success of nation-wide MDA since 2015, including the community mobilization strategy, reported and verified treatment coverage rates, reasons for non-compliance, challenges faced by community drug distributors (CDDs) and number of morbidity cases (i.e. hydrocele, lymphoedema) found in relation to district risk. In 2015, Prevalence mapping community mobilization helped to successfully implement the MDA campaign. Overall, high ALB/DEC rates were reported in 2015 (mean 106%; range 44%-136%), and 2016 (mean 101%; range 58% to 138%), and independently verified high coverage in 10 (79%-93 %) and 5 districts (83-94%), respectively. There was little non-compliance, with 'being away at the time' and/or 'unaware of the MDA activity' cited as main reasons. CDDs noted that problems with transport was the main challenge. Only 372 hydroceles and 255 lymphoedema cases were reported among the 10.2 million target population, with most cases found in previously highly endemic districts. This significant scale up and success of nationwide ALB and DEC distribution - the largest ever reported in sub-Saharan Africa - with high verified coverage, and minimal morbidity case reported together with large scale impact of ITNs, suggests that the Zambia LF Programme could swiftly move to a surveillance phase and accelerate towards the elimination goal of 2020.

## 1186

**THE IMPACT OF SEMI-ANNUAL MASS DRUG ADMINISTRATION FOR MULTI-SPECIES LYMPHATIC FILARIASIS IN INDONESIA: A MODELLING APPROACH****Suzanne Verver**<sup>1</sup>, Periklis Kontoroupi<sup>1</sup>, Taniawati Supali<sup>2</sup>, Peter U. Fischer<sup>3</sup>, Sake J. de Vlas<sup>1</sup>, Gary J. Weil<sup>3</sup>, Wilma A. Stolck<sup>1</sup>

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Many countries have achieved an enormous decrease in LF infection prevalence through implementation of annual mass drug administration (MDA), but a number of countries lag behind. Acceleration strategies are

needed to achieve the 2020 elimination goal worldwide. Mathematical modelling suggested that the required duration of interventions can be halved by switching from annual treatment to 6-monthly treatment. This needs confirmation by empirical data. We aim to refine model-based estimates of the required duration of annual versus semi-annual MDA for achieving LF elimination, by analyzing DOLF project data. These include a community intervention study in Sikka district, Flores, Indonesia that compared trends in brugian and bancroftian filariasis infection in 3 communities after 3 years of annual or biannual MDA with diethylcarbamazine and albendazole. We fitted the LYMFASIM simulation model to antigenaemia prevalence (*W. bancrofti*), antibody prevalence (*B. timori*), and microfilaraemia (mf) prevalence and intensity (both species) at baseline. Thereafter we assessed whether the model-predicted trends are in accordance with the observed data. Lastly we estimated the number of treatment rounds required to bring mf prevalence below 1%. The baseline prevalence in the community with biannual MDA was higher (8.7 and 10.3% for *W. bancrofti* and *B. timori*, respectively) than for the two communities with annual MDA (0 and 0.4% for *W. bancrofti* and 3.9 and 4.9% for *B. timori*). Our modeling approach accurately reproduced the observed trends. Because of the low baseline mf prevalence in the communities with annual MDA, these communities reached the 1% pre-TAS threshold faster than the community with annual MDA. Our modelling suggests that the target would have been achieved later than in the biannual village, if the baseline mf prevalence was equally high. The total required duration of MDA can be about halved by treating biannually instead of yearly, for both LF species, provided that good coverage levels are maintained. This is a useful strategy to accelerate MDA where biannual MDA can be implemented.

## 1187

### A COMPREHENSIVE ASSESSMENT OF PERSISTENT *WUCHERERIA BANCROFTI* IN HOTSPOTS IN GALLE COASTAL EVALUATION UNIT IN SRI LANKA 9 YEARS AFTER STOPPING MASS DRUG ADMINISTRATION

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The Sri Lankan Anti-Filariasis Campaign (AFC) distributed 5 rounds of MDA (DEC plus ALB) according to WHO guidelines to some 10 million people in 8 endemic districts between 2002 and 2006. All districts met WHO criteria for verification of lymphatic filariasis (LF) elimination as a public health problem in 2016. We previously reported results from comprehensive post-MDA surveillance that was conducted in Galle district between 2013 and 2014. These results and a district wide molecular xenomonitoring study suggested that there were many hotspots in coastal EU in Galle district with ongoing transmission. One of these hot spots called Balapitiya PHI (Population: 17,500) had alarmingly high LF parameters: community CFA rate (3%, 1.8-4.8 CI), MF rate (1%, 0.4-2.2% CI); school children CFA rate (1.2%, 0.5-2.8% CI), Bm14 antibody rate (5.7%, 3.7-8.4% CI) and filarial DNA rate in *Culex* (5.2%, 4.2-6.3% CI). Microfilaraemia rates in this area ranged from 0.9% in 2013 and 0.6% in 2016 suggesting that 2 rounds of MDA in 2014 and 2015 may have had some effect. We reexamined 22 of 168 PHM areas in the Galle district coastal EU after 2 rounds of MDA, and 8 of these were drawn from Balapitiya. Approximately 660 *Culex* pools collected from 22 PHMs in Galle were tested for filarial DNA. 179/660 (27%) pools were positive for filarial DNA. Interestingly, a higher percentage of mosquito pools [107/240 pools (45%)] sampled from Balapitiya contained filarial DNA. This hotspot may require additional rounds of MDA with improved coverage or a test and treat program along with integrated vector management. Coastal Galle district and Balapitiya provide excellent opportunities for applied field research to compare different methods for erasing hotspots and for strengthening Transmission Assessment Surveys.

## 1188

### HYPO-ENDEMIC ONCHOCERCIASIS HOTSPOTS: CHARACTERIZING RISK, DEMOGRAPHY, INFRASTRUCTURE AND ENVIRONMENT TO FACILITATE THE SCALE UP OF ALTERNATIVE STRATEGIES FOR ELIMINATION IN CENTRAL AFRICA

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Community directed treatment with ivermectin (CDTi) has been successful in reducing and controlling the global prevalence of onchocerciasis, with elimination now targeted in all endemic countries. However, in Africa, severe adverse events (SAEs) from ivermectin treatment, including death, have been reported in some areas that are hyper-endemic for loiasis. We define 'hypo-endemic onchocerciasis hotspots' as areas of overlapping onchocerciasis hypo-endemicity and loiasis hyper-endemicity. In such hotspots, the potential risks of CDTi may outweigh the benefits to individual patients, and if left untreated, could contribute to on-going transmission and prevent meeting elimination targets for onchocerciasis. Alternative intervention strategies such as anti-*Wolbachia* therapy, vector control and/or the Test and (not) Treat need to be implemented in such hotspots to ensure safety and impact. It is important however to first understand the local risk, demography, infrastructure and environment that characterise the hotspots. This study, therefore, aimed to characterise five hypo-endemic hotspots across central Africa. Firstly, by using onchocerciasis and loiasis prevalence data to define the scale and spatial extent of risk; secondly, by examining key demographic factors including: age, sex, population density and distribution; thirdly, infrastructure factors including: health facilities, roads and nearest large town, and finally, environmental factors, including: rivers, forests and elevation. These key characteristics were summarised, mapped with key information and used to develop a simple algorithm/decision matrix to facilitate country programmes in identifying high-risk areas, target populations, available human resources, access to health infrastructure and logistical requirements to successfully implement alternative interventions. This study considers whether this pragmatic approach can bridge an existing gap between academic research and programme implementation in hypo-endemic hotspots, in countries currently falling behind in the WHO onchocerciasis elimination agenda.

## 1189

### MEASURING THE NUMBER OF REPRODUCTIVE ADULT FEMALES AND DEFINING TRANSMISSION ZONES FOR FILARIAL NEMATODES USING POPULATION GENETIC MEASURES

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For most organisms, there are limits to the distance over which mating can occur between any two individuals, giving rise to population structure in which genetic relatedness is correlated with distance: close together, more related (i.e. more likely to share common ancestors) and further

apart, less related. The result is that measures of genetic relatedness can be used to assign an individual to their population of origin, and that measures of population structure can define the boundaries within which mating between two individuals is likely to occur. For filarial nematodes, two individuals can only mate if they are transmitted to the same host, so measures of genetic relatedness in filarial parasites are an indirect measure of the distances over which transmission occurs within a given population. Consequently, measures of population structure define the boundary of a parasite transmission zone and estimate the probability that an individual parasite is a "resident" of that transmission zone or is an immigrant. Similarly, measures of population structure can be used to estimate the risk of parasite transmission between two locations (for example, across a border between countries/states/districts with differing degrees of success in parasite elimination). We have used a variety of genotyping tools (whole genome sequencing, whole and partial mitochondrial and *Wolbachia* sequencing, and single locus genotyping) to characterise population structure and hence transmission zones for *Onchocerca volvulus* in West Africa and have shown that we can resolve population structure and transmission zones over several spatial and temporal scales. These measures may provide objective tools with which to determine the boundaries of implementation and evaluation units for mass drug administration and cessation of treatment. We have also coincidentally used genotyping data to estimate the number of females contributing to the microfilarial population in the skin of an individual patient, providing a novel measure of parasite infection intensity, and to track the rate at which a parasite population declines under MDA.

## 1190

### DEVELOPING THE FIRST NATIONAL DATABASE AND MAP OF LYMPHATIC FILARIASIS CLINICAL CASES IN MALAWI

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The Lymphatic Filariasis (LF) Elimination Programme in Malawi is on track to meet the Global Programme to Eliminate Lymphatic Filariasis (GPELF) targets of 2020 by interrupting transmission with mass drug administration (MDA) and alleviating suffering by managing morbidity and preventing disability (MMDP). This study aimed to highlight the LF programme's approach to determining the number of people affected by lymphoedema and hydrocoele, and developing the first national database and morbidity map for targeted interventions across the 26 endemic districts. Such information is essential for certification of LF elimination as a public health problem. The first data on morbidity was collected during national MDA campaigns in 2009-2010 when community drug distributors (CDDs) reported the number of lymphoedema and hydrocoele cases at the health-centre-level. Once MDA was stopped in 2014, the three districts with the highest baseline prevalence and reported number of cases during MDA (Chikwawa, Nsanje and Karonga), were selected for intensive patient mapping using the mHealth tool *MeasureSMS-Morbidity* in which health surveillance assistants (HSAs) reported all cases in their communities by SMS. Each district reported approximately 350 lymphoedema and 800 hydrocoele cases, and provided specific village-level patient data on age, sex, condition and severity of condition (mild, moderate, severe) which could be used to further understand spatial-epidemiological patterns. Focal mapping is now planned for six to eight districts to help develop predictive maps in the remaining 23 districts. Each district will be strategically selected, considering its location, environment, baseline prevalence, and morbidity data reported by MDA. This will allow country-wide data and a map to be generated, highlighting and classifying areas of 'low', 'medium' and 'high' predicted prevalence/case numbers, which will then be verified.

This information will allow the Malawi LF Programme to appropriately plan and deliver a basic package of care to those suffering from the disabling and debilitating clinical manifestations of LF across the country.

## 1191

### FAMILIAL AGGREGATION AND HERITABILITY OF LOA LOA INFECTION

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Large scale treatment with ivermectin (IVM) against onchocerciasis needs to be expended to hypo-endemic areas to reach the elimination goal. In these areas, localities where community-directed treatment with IVM (CDTI) cannot be applied because of possible occurrence of *Loa*-related severe adverse events (SAE) have to be identified. At individual level, people at risk of SAE are those harboring >30,000 *Loa* microfilariae per mL of blood. For a given prevalence of microfilaremia, the proportion of people with such high densities varies significantly between communities. We hypothesized that the latter observation is related to the existence of familial clusters of hypermicrofilaremic individuals which would be the consequence of a genetic predisposition to present high *Loa* microfilarial densities. We tested this hypothesis in 10 villages in the Okola Health District of Cameroon. Intrafamilial correlation coefficients and heritability estimates were assessed for both *Loa* microfilaremia and individual microfilarial densities by controlling for age, sex, *Mansonella perstans* microfilaremia and household effects. Pedigree charts were constructed for 1,126 individuals. A significant familial susceptibility to be microfilaremic for *Loa* was found for first-degree relatives ( $p = 0.08$ ,  $P < 0.05$ ; heritability = 0.23). Regarding individual microfilarial densities, a significant familial aggregation was demonstrated ( $p = 0.36$  for first- and 0.27 for second-degree relatives). For first-degree relatives, the highest coefficients were found between mothers and daughters ( $p = 0.57$ ). Overall heritability estimate for intensity was 0.24. These results suggest that the *Loa* microfilaremic status is mainly driven by environmental factors and habits, while a genetic component governs the microfilarial density. These results support the hypothesis that a genetical predisposition to be hypermicrofilaremic exists, leading to the presence of familial clusters of individuals at risk for post-ivermectin SAE. This finding should be taken into account for developing sampling strategies to identify communities where CDTI cannot be applied.

## 1192

### PREDICTIVE VALUE OF OV16 ANTIBODY PREVALENCE IN DIFFERENT AGE GROUPS FOR ELIMINATION OF AFRICAN ONCHOCERCIASIS

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Onchocerciasis is targeted for elimination in Africa by 2025 through mass drug administration (MDA) with ivermectin. Current WHO guidelines for stopping MDA and verifying elimination require that the Ov16 antibody prevalence in 0-9 year old children is brought below 0.1%, but the empirical evidence underlying the choice of age group and threshold is still limited. We assessed the predictive value of different Ov16 antibody prevalence thresholds for elimination of onchocerciasis, for various age groups, a variety of endemic settings and various MDA scenarios. We used the individual-based stochastic ONCHOSIM model to simulate trends in infection and Ov16 antibody prevalence levels during and after MDA. We

simulated 750 scenarios, varying with respect to pre-control endemicity level and MDA characteristics (frequency, coverage and duration of MDA). Each scenario was simulated 10,000 times, and each time we recorded the model-predicted Ov16 prevalence in various age groups (one year after the last MDA round) and the outcome in terms of ongoing transmission vs. elimination (non-zero vs. zero mf prevalence 50 years after the last MDA round). We found that the sensitivity and specificity of Ov16 antibody prevalence for predicting elimination highly depends on the pre-control epidemiological situation, history of MDA, the age group that is sampled, and the chosen Ov16 antibody prevalence threshold. Still, threshold values can be defined such that positive predictive values for elimination are close to 100% regardless of the history of MDA. Importantly, the Ov16 antibody prevalence in school-aged children (age-group 5-14) performs best in predicting elimination. Although exact estimation of thresholds remains difficult due to uncertainties regarding antibody response dynamics, our study indicates that 1) sampling school-age children instead of children under ten increases the accuracy of anti-Ov16 prevalence as a predictor for elimination; 2) the current threshold of 0-1% is too stringent except for the most highly endemic settings; and 3) a differentiated approach to defining thresholds based on pre-control endemicity is pertinent.

### 1193

#### EFFORT TOWARDS ELIMINATION OF LYMPHATIC FILARIASIS IN CAMEROON: RESULTS OF A TRANSMISSION ASSESSMENT SURVEY IN 31 HEALTH DISTRICTS IN NORTHERN REGIONS

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Lymphatic filariasis (LF) is one of the major neglected tropical diseases in Cameroon. The northern regions (Far North and North) were classified by the Ministry of Public Health as LF endemic, based on historical and clinical data, and underwent mass drug administration (MDA) with ivermectin and albendazole in all the health districts (HDs) of the two regions, with financial support from the United States Agency for International Development. Following stopping MDA in 5 HDs that successfully passed their first transmission assessment survey (TAS 1) in 2014 after 6 rounds of effective MDA, a further 31 HDs completed 5 rounds of effective MDA and successfully passed their pre-TAS in 2014 and 2015. TAS 1 in these 31 HDs was subsequently conducted in 2016 using the Alere™ Filariasis Test Strip (FTS). The 31 HDs were grouped in 9 evaluation units (EUs). As the school enrolment rates are below 75% in the northern regions, the TAS 1 was carried out using a community-based cluster sampling according to the World Health Organization guidelines. A total of 13,957 children aged 6-7 years old were enrolled in 267 villages (clusters) and tested using FTS. Overall, 10 positive cases were found in the 9 EUs. No positive case was found in 3 EUs, and 1, 2 and 4 positive cases were found in 4, 1 and 1 EUs, respectively. According to the critical cut-off value for each of the 9 EUs (18 or 20), all EUs passed the TAS 1, suggesting that MDA can be stopped and surveillance organized in these HDs. The results showed that Cameroon has made significant progress in eliminating LF. However, the sample size in one EU was significantly lower than the sample size generated by the Survey Sample Builder because one HD in that EU was not surveyed due to insecurity issue. MDA in this EU may have to continue until further assessment.

#### WHERE ARE WE WITH ONCHOCERCIASIS IN MALI AFTER FORTY YEARS OF IMPLEMENTATION OF CONTROL ACTIVITIES?

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Mapping carried out from 1971 to 1987 showed that 35 health districts (HDs) in Sikasso, Ségou, Mopti, Koulikoro and Kayes regions in Mali were endemic with onchocerciasis (OV). Mali began its OV program in 1974 through vector control with the support of the Onchocerciasis Control Program in West Africa (OCP). From 1988 onwards, community-directed treatment with ivermectin (CDTI) was conducted in hyper-endemic foci (20 HDs) to control morbidity. In 2007, OV treatment was integrated into the mass drug administration (MDA) for lymphatic filariasis elimination in the national integrated neglected tropical disease (NTD) program with effective treatment coverage, funded by the USAID. The aim was to consolidate previous achievements and contribute to OV elimination by 2025. The 1971-1987 baseline data gave a median prevalence of 42% (range: 10-73%), using cutaneous biopsy. During the vector control period with OCP, the median prevalence in 1987 after 12 years of control was 16.5% (range: 3-34%). Since the introduction of CDTI in 1988, surveys by cutaneous biopsy showed a gradual decline in prevalence: median prevalence of 2% (range: 0-7%) in 1998 (after ten years of CDTI) and 0% in 2008 for all the surveyed HDs. Since integrated MDA, the prevalence from surveys by biopsy in 2009-2015 has been 0%, which confirmed the previous prevalence obtained with CDTI. The first entomological surveys in 1976 and 1987 showed a median annual transmission potential (ATP) of 497 (0-2914). After 12 years of vector control the median ATP was 70 (29-1116) in 1987. In the last entomological surveys from 2009 to 2015, the infectivity rate was less than 0.5/1000 blackflies (*Simulium*), achieving the threshold biting rate of <100 per person per year. Currently, treatment for LF has stopped in all districts co-endemic with OV. The National Program plans to conduct serological assessment using OV16 in order to determine if treatment for OV can also be stopped in the 20 districts concerned. The country has created the National Committee for the Certification of Elimination of Onchocerciasis that will determine the way forward on the validation of OV elimination in Mali before 2025.

### 1195

#### ELIMINATION OF LYMPHATIC FILARIASIS AS A PUBLIC HEALTH PROBLEM IN NIGER: PROGRESS AND CHALLENGES TO REACHING THE TARGET BY 2020

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Lymphatic filariasis (LF) is endemic in 31/42 districts (HDs) across all regions of Niger with baseline microfilaria prevalence ranging from 1% to 52%. Niger aims to eliminate LF as a public health problem by 2020. The strategy is by annual mass drug administration (MDA) with ivermectin and albendazole. Niger began MDA in 2007 in 10 HDs, and progressively reached full geographical MDA coverage in 2014. By 2017, 30/31 endemic HDs have conducted 5 or more rounds of effective MDA

with epidemiological coverage of  $\geq 65\%$ . 17 HDs have undergone the first transmission assessment survey (TAS 1): 8 in 2013, 6 in 2014 and 3 in 2016. Of these, 11 HDs passed TAS 1 and stopped MDA (3 in 2013, 5 in 2014 and 3 in 2016); 6 HDs failed the TAS 1 (5 in 2013, 1 in 2014 and 0 in 2016). 7 other HDs will undergo TAS 1 in 2017. Other 6 HDs recently completed pre-TAS (and those that failed TAS 1 recently underwent re-pre-TAS) and will undergo TAS 1 in 2018 if results allow (pending). The remaining 1 HD finished mapping in 2014 and MDA is ongoing, but it is anticipated that it may be able to stop MDA in 2020. The reason for the continued high prevalence in certain locations and the failure of TAS 1 in some HDs may be due to a highly mobile population (nomadic populations and/or migrants for economic reasons for part of the year), which may have led to sub-optimal MDA coverage in certain sectors of the population, even if overall minimum coverage was met. In addition, the grouping of HDs into evaluation units (EUs) may also have been a factor (e.g. in two EUs that failed in 2013, all positive cases were found in only some of the districts in each EU). In order for Niger to reach its elimination targets, the National LF Program proposes twice-yearly MDA in areas with mobile populations to ensure a better chance to find nomadic groups and ensure at least one annual treatment for economic migrants. More intensive social mobilization and advocacy among leaders will be needed to ensure adherence to MDA. In addition, great care will be taken when deciding eligibility of HDs for TAS 1.

## 1196

### EFFICACY AND SAFETY OF MOXIDECTIN PLUS ALBENDAZOLE, MOXIDECTIN PLUS TRIBENDIMIDINE, AND MOXIDECTIN ALONE VERSUS ALBENDAZOLE PLUS OXANTEL PAMOATE AGAINST *TRICHURIS TRICHIURA* AND CONCOMITANT SOIL-TRANSMITTED HELMINTH INFECTIONS: A RANDOMIZED CONTROLLED TRIAL

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Hundreds of millions of people are affected by soil-transmitted helminth (STHs) that are represented by *Trichuris trichiura*, *Ascaris lumbricoides* and hookworms. Infection is most intense and debilitating in children and adolescents. However, their treatment relies on few drugs only. The benzimidazoles are the treatment of choice in preventive chemotherapy, hence most commonly used. However these drugs have a little effect against *Trichuris trichiura*. Moxidectin, in a preliminary study, has shown good activity against this infection. The aim of our study is to assess the efficacy of different combinations of drugs against *T. trichiura* infection and concomitant co-infections with other STHs. In addition, we aim to compare the sensitivity of Kato-Katz and PCR since the sensitivity of Kato-Katz is fairly low, despite being the recommended tool. We conducted a randomized single-blinded trial in *T. trichiura* infected adolescents on Pemba Island, Tanzania. We enrolled 640 adolescents (12-18 years old). At baseline each participant was asked to provide two stool samples, which were examined according to WHO standard procedures (Kato Katz method) and one subsample was shipped to Swiss TPH for PCR procedure. Each participant was randomly assigned to one of the four treatment arms: 1) moxidectin (8 mg), 2) moxidectin (8 mg)/tribendimidine (400 mg), 3) moxidectin (8 mg)/albendazole (400 mg) and 4) albendazole (400 mg)/oxantel pamoate (25 mg/kg). Adverse events were monitored at several time points post-treatment. The participants were asked to provide another two stool samples for follow-up 14-21 days after treatment. Stool samples were again analyzed with Kato-Katz and a sub-sample was shipped to the Swiss TPH for PCR examination. Cure and egg reduction rates of the different treatments will be presented. I will show how the drugs were tolerated by the adolescents, together with the diagnostic

performance of the two methods. Our results are of great importance as we will have elucidated the trichuricidal activity of novel treatments and drug combinations, which might serve as treatment alternative.

## 1197

### EVALUATION OF THE ANTHELMINTHIC ACTIVITY OF COMPOUNDS EXTRACTED FROM *DALEA PARRYI*, *D. POGONATHERA* AND *D. NANA* (PLANTAE, FABACEAE)

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Hookworm infection is a major cause of iron-deficiency anemia, malnutrition, growth delay and cognitive defects in children in endemic countries where about a billion people are affected. Severe iron-deficiency anemia due to hookworm infection during pregnancy might lead to severe consequences for the mother, the unborn fetus and the neonate including impairment of milk production and low birth weight. Control strategies relying on mass distribution of benzimidazole drugs are hampered by frequent re-infections. In addition, reports from the field indicate increasing low efficacy of the current anthelmintics to treat hookworm and other soil-transmitted nematodes. In our quest to identify alternative therapeutic tools for the control of hookworm, we have tested compounds extracted from plants for their anthelmintic activity against the adult stage of the hookworm, *Ancylostoma ceylanicum*. We have reported that two compounds namely tephrosin and deguelin obtained from the plant species *Dalea ornata* can induce 100% mortality of adult hookworm *ex vivo* by 24 hours post-incubation. Consequently, we have rationally collected other plants of *Dalea* genus and evaluated their anthelmintic activity. Data from compounds isolated from *Dalea parryi*, *D. pogonathera* and *D. nana* will be presented. In preparation of our *in vivo* evaluation using our hamster laboratory model of hookworm infection, toxicity of these compounds to mammalian cells is being evaluated by flow cytometry and their effects on cell proliferation by BrdU, a colorimetric assay. Detailed results will be presented.

## 1198

### PREVALENCE OF SOIL TRANSMITTED HELMINTHIASIS AND SCHISTOSOMIASIS AMONG SCHOOL GOING CHILDREN IN SELECTED COUNTIES, KENYA, 2013-2015

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Soil transmitted helminths (STHs) are the most common parasitic infection, infecting an estimated 2 billion people worldwide with over 600million School aged children living in intensive transmission areas. Kenya has been providing prophylactic chemical treatment (PCT) for STHs among school-going children over the past 5 years in 27 counties of which 19 did not have baseline data. Our objectives aimed to determine the prevalence and geographical distribution of STHs and Schistosomiasis (SCH) in Kenya. This was a cross sectional survey conducted using multistage stratified sampling within 19 counties located in Western, Central and Rift-valley region of Kenya. We randomly sampled five schools in each sub-counties and stool samples collected from 50 pupils in each school aged 5-14 years as per world Health Organization (WHO) guidelines. We analyzed stool samples using Kato-Katz technique to identify STH and *Schistosoma mansoni* eggs. We also collected Urine samples from 50 pupils in 5 randomly selected schools in sub-counties in Migori, Marsabit and Homa Bay counties and tested for Schistosomiasis hematobium by using filtration and microscopy. We analyzed data using Ms. Excel, Epi-Info and calculated summary statistics. A total of 18,054 stool samples and 2,950 urine samples were collected. Mean age of pupils were 10.7 years (standard deviation [SD]

2.1). Females constituted 50.6% (n=9,135). Overall STH prevalence in the 19 counties was 5.6%. Prevalence was highest in Narok county (21.2%), Migori 17%, and Homa Bay 12.1%. Overall Schistosomiasis prevalence was 5.6%, with *Schistosoma mansoni* at 4.1% and *Schistosoma hematobium* 1.5% with Migori having the highest prevalence (21.6%). In conclusion, there was still a high prevalence in some counties implementing PCT which would indicate a high transmutability or inadequate treatment. There is need to consider WHO guidelines on sanitation, midterm review of STH and SCH endemicity and effectiveness of intervention is recommended.

## 1199

### INVESTIGATING THE DIFFERENTIAL IMPACT OF SCHOOL AND COMMUNITY-BASED INTEGRATED CONTROL PROGRAMS FOR SOIL-TRANSMITTED HELMINTHS IN TIMOR-LESTE: THE (S)WASH-D FOR WORMS PILOT STUDY

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Deworming programs for STH control are usually targeted to school-age children (SAC), through school-based delivery systems, in line with current World Health Organization guidelines. However, a recent meta-analysis shows greater reductions in STH prevalence in children following community-wide deworming, compared to child-targeted deworming. Furthermore, although water, sanitation and hygiene (WASH) interventions are thought to be important for sustainable STH control, alongside regular distribution of anthelmintic drugs, there has been limited research integrating deworming and WASH. The (S)WASH-D for WORMS pilot study aimed to compare the impact of school- and community-based integrated deworming and WASH programs on STH in SAC in six remote communities in Timor-Leste. STH prevalence and intensity were measured in SAC at baseline and six months after anthelmintic delivery, using quantitative PCR. All communities received a deworming and WASH program at the primary school (targeting SAC), and three communities additionally received a community-wide deworming and WASH program. The impact of the study intervention on STH infection rates was estimated. For hookworm, a greater reduction in prevalence at follow-up was seen in the intervention arm (62.5% reduction, 15.1% to 5.7%) compared to the control arm (33.2% reduction, 14.8% to 9.9%). Results of a mixed effects multivariable regression model, with hookworm infection at follow-up as the outcome, suggest that there is a trend towards reduced odds of infection in the intervention arm. The results of the pilot study demonstrate not only the feasibility and acceptability of both the study procedures and the deworming and WASH program, but more importantly provide proof of principle for our hypothesis that a community-wide approach to STH control will reduce STH infections in children more than an exclusively school-based approach.

## 1200

### HYGIENIC BEHAVIORS AND RISKS FOR ASCARIASIS AMONG COLLEGE STUDENTS IN KABUL AFGHANISTAN

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The most at-risk group for ascariasis is older children and teenagers, but the mechanism by which they are at risk is poorly understood, particularly in developing countries. In this study, the proportion of university students in Kabul, Afghanistan, with ascariasis was estimated, along with the distribution of risk factors for infection. We stratified sampling of students

from Kabul Medical University between those who lived in a group hostel vs those who lived in a private home. Students were asked about risk factors with a questionnaire, and they provided a stool sample to test for presence of ascaris eggs. A total of 15.8% of students had ascariasis. Living in a hostel was a strong risk factor for ascariasis (21.2% vs 10.4% in houses). Prevalence of certain risk factors was higher for students in hostels vs homes, like eating street food (51.2% of those in hostels vs 45.8% of those in homes), and eating unwashed vegetables (17.3% vs 8.1%). In conclusion, living in a group hostel was shown to be a risk factor for ascariasis in this study, potentially because the students engaged in at-risk behaviors, like eating food outside of the home. Interventions looking to reduce ascariasis could focus on educating students in group hostels about healthy eating behaviors.

## 1201

### CHILD DEVELOPMENT CENTER-BASED SURVEILLANCE AND CONTROL OF SOIL-TRANSMITTED HELMINTH INFECTIONS IN PRESCHOOL-AGE CHILDREN IN SELECTED LOCAL GOVERNMENT UNITS IN THE PHILIPPINES

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The study aimed to describe the parasitologic status of preschool-age children (PSAC) through child development center (CDC)-based sentinel surveillance methodology and to develop and evaluate a CDC-based mass drug administration (MDA) for soil-transmitted helminthiasis (STH) in PSAC in selected sites in the Philippines. For the sentinel surveillance, 30 PSAC in CDCs were targeted from each *barangay* (village). Stool samples were collected in CDCs and examined using the Kato-Katz technique in Cavite, Guimaras, Iloilo, Negros Occidental, Davao del Norte, and Iloilo and Lapu-Lapu cities in 2013-2015. A CDC-based MDA scheme in PSAC was implemented in Guimaras, Davao del Norte, and Lapu-Lapu City in July 2016. MDA coverage rates were obtained, while the implementation of the scheme was described using a monitoring form. Good practices and challenges in the implementation were described through interviews. The survey included 1,732 PSAC. The cumulative STH prevalence and moderate-heavy (M-H) intensity prevalence are 24.9% and 10.3%, respectively. Five schistosomiasis cases were found in Lapu-Lapu City and Davao del Norte. The WHO target for M-H intensity STH prevalence of less than 1% was not met, despite the program being implemented for almost a decade. Good practices included the active involvement of local stakeholders, while challenges included the low turnout for stool collection in some barangays. On the other hand, Lapu-Lapu City and Davao del Norte both reported 100% MDA coverage, while Guimaras reported 86.78% MDA coverage. Good practices included capacity building of health workers and child development workers, social media campaign, health promotion and education integrated in the CDCs, and directly observed deworming. Challenges included absentees and insufficient health workers. Biennial CDC-based sentinel surveillance and biannual CDC-based MDA may be considered to improve STH surveillance and control in PSAC.

## 1202

**CHARACTERISTICS AND OUTCOMES OF STRONGYLOIDIASIS IN SOUTHERN THAILAND**

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Epidemiological study on strongyloidiasis in southern Thailand unclear. Even the incidence of helminthic infection in this area is relatively high. A cross-sectional study was conducted to determine the clinical characteristics and outcomes of *Strongyloides stercoralis* infection among inpatients of Songklanagarind Hospital, the tertiary care hospital and referral center in southern Thailand from 2005-2015. Samples of each isolate were examined with microscopy and conventional culture. Then molecular techniques were used to identify the phylogenetic tree of the isolatable strains. Results: Of the 470 inpatients with clinical suspicious of strongyloidiasis with routine laboratory report. Those aged ranged from 21 to 91 years with median of 60 years (interquartile [IQR] range from 45 to 68 years). Four hundred and four patients were male, accounting to 86 %. Most common medical underlying disease was systemic lupus erythematosus (SLE) (50%), solid organ malignancies (36%) and hematologic malignancy (19%), subsequently. Most of patients (70%) were plant gardeners and rice formers. Most common clinical manifestation were fever (90%), respiratory symptoms (70%) including dyspnea, pleuritic chest pain and cough. Routine laboratory showed significant eosinophilia (65%) while anemia and thrombocytopenia was rare. Mortality rate was 59 % and length of hospital stay after infection ranged from 14 to 123 days (IQR; 45 to 90 days). Time to identify the infection was 25 days (IQR; 9-21 days). With logistic regression analysis showed the delayed treatment > was associated with higher mortality. Culture for *S. stercoralis* was positive for 77 isolates from 56 patients. Most common positive-culture sample was sputum (57 samples) accounting to 74 %. Most strains fell into type 4, the common strain in Asian Pacific region, while based on published sequences for species, they cannot be distinguished from the strains were identified in this area. In conclusion, strongyloidiasis is one of the fatal causes of the patients with immunocompromised in southern Thailand and yielded high mortality. Early diagnosis is challenged.

## 1203

**SYSTEMATIC NON-ADHERENCE TO TREATMENT IN HELMINTH MASS DRUG ADMINISTRATION PROGRAMS: INTERACTIONS WITH DISEASE-SPECIFIC TRANSMISSION DYNAMICS**

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In mass drug administration (MDA) aimed at elimination of disease, sufficient treatment coverage is essential for success. Non-adherence to treatment is a barrier to achieving adequate coverage. A critical question concerns the likelihood of non-adherence at one round of treatment predisposing to non-treatment at subsequent rounds. Transmission modelling indicates that the impact of non-adherence varies between helminth diseases. We examine the underlying reasons for this disease-specific heterogeneity through an individual-based stochastic computational model and discuss implications for targeting of MDA in treatment policy.

## 1204

**SOIL-TRANSMITTED HELMINTHIASIS IS UNDER CONTROL IN SEGOU, SIKASSO, KAYES, MOPTI AND KOULIKORO - FIVE REGIONS IN MALI**

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Segou, Sikasso, Kayes, Mopti and Koulikoro regions in Mali are endemic with soil-transmitted helminths (STH). Initial mapping conducted in 2004-5 showed that 28 of a total of 38 health districts (HDs) in the five regions had STH infections where the prevalence ranged 0.3-33.5% and 7 HDs had prevalence over 20%. The prevalence of hookworm was the highest of the three STH species in the 38 HDs: 19 HDs had hookworm prevalence of ≥1%, with the highest prevalence being 33.5%. Taking advantage of the integration of mass drug administration (MDA) against neglected tropical diseases (NTDs) in Mali, which started in 2007, STH treatment was integrated with that of lymphatic filariasis (LF) in all endemic HDs. Thus the entire population over five years old was targeted for treatment with albendazole. In addition, since 1999, deworming of children under five years has been conducted twice per year during vitamin A supplementation campaigns. To evaluate the impact of mass treatment, STH prevalence was assessed in 2014-16 in 84 sentinel sites in 38 HDs during schistosomiasis sentinel site survey in schools. A total of 5053 children aged 7 to 14 years were evaluated. The Kato-Katz method was used to diagnose STH infections using stool samples. STH infection was found in the sampled school children in only 4 of the 38 HDs surveyed (hookworm in three HDs and *Trichuris* in one), with prevalence ranging from 0.82% to 1.63%, and no heavy infection was found. According to the World Health Organization criteria, the current results showed that STH is successfully under control in all 38 HDs. The treatment of the entire population through deworming and the LF MDA campaign has been conducted in the country's all 65 HDs, and this may have successfully controlled STH throughout Mali. The NTD program plans to conduct further assessments to confirm the control of STH in Mali and will integrate STH assessments with LF transmission assessment surveys. With the LF MDA being gradually stopped in Mali, discussion is under way to integrate STH deworming with schistosomiasis MDA targeting mainly school age children in HDs where the baseline STH prevalence was over 20%.

## 1205

**ASSESSING BETWEEN-VILLAGE HETEROGENEITY OF HOOKWORM TRANSMISSION IN A LOW-INTENSITY SETTING**

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Since the London Declaration on NTDs in 2012, the extent to which mathematical modelling has been used to inform public health policy within NTD research has increased markedly. Frequently, however, only cross-sectional data from specific areas within a country of interest are available, which are often collected for other goals rather than informing mathematical models. Data that are required to inform models are scarce as they are difficult and expensive to collect, especially true for high-quality longitudinal data. As such, an assumption may be required that results obtained from the models are applicable to not only the rest of country, but potentially to other countries as well. However, it is well known that prevalence differences are present both between and within

countries, and therefore differences in disease dynamics may also exist. In this study, we investigate whether heterogeneities in transmission dynamics of hookworm exist between villages and their impact on model predictions and policy recommendations. We apply a highly detailed, longitudinal dataset consisting of 45 villages collected from a study into soil-transmitted helminth control in Tamil Nadu, India. Although the mean prevalence across the 45 villages was 19%, the village-level prevalence ranged from 2% to 45%. The high degree of variation in hookworm prevalence, combined with the individual-level, longitudinal nature of the data, result in a dataset of great use in investigating our desired aims. Transmission parameters will be fitted to the age-prevalence profiles for each village separately using maximum-likelihood estimation. These parameters will then each be used within the mathematical models to investigate the impact of village-specific parameter estimates on policy recommendations.

## 1206

### MULTIPLEX POLYMERASE CHAIN REACTION FOR DETECTION OF HOOKWORMS AND *STRONGYLOIDES STERCORALIS*

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Diagnosis of gastrointestinal parasites has traditionally relied on stool microscopy, which has low diagnostic sensitivity; although relatively simple and low cost in terms of equipment, the main species of hookworms that infect humans, *Necator Americanus* and *Ancylostoma duodenale*, cannot be differentiated through microscopy of eggs. Moreover, *Strongyloides stercoralis* is the most difficult to diagnose, due to the intermittent nature of larval shedding in the stool as well as the relatively low numbers of larvae found in the stool. As an alternative technique, we developed a Multiplex PCR assay to identify and detect mixed infection with *Necator Americanus*, *Ancylostoma duodenale* and *Strongyloides stercoralis* in human's fecal samples. The multiplex PCR reaction system was established by optimizing the reaction conditions. Results showed that the three target fragments corresponding to the three species used in this work were specifically amplified. The detection limit was ten eggs for hookworms and one larval for *Strongyloides stercoralis*. To measure sensitivity and specificity three groups of fecal samples previously examined by five microscopy test were evaluated. Group 1: 5 patients without parasites; group 2: 10 patients with infection with other parasites (*Ascaris lumbricoides*, *Trichuris trichiura*, *Giardia intestinalis*, and *Hymenolepis nana*) and group 3: 40 patients with single and mixed infections with hookworms and *Strongyloides stercoralis*. No amplification occurred in any of the samples of groups 1 and 2. All the samples from group 3 were positive with single and mixed infection by multiplex PCR, and we could distinguish the species of hookworms. In conclusion, the established multiplex PCR assay is a convenient, rapid, and species-specific identification method for molecular detection and epidemiological investigation in regions of multiples soil-transmitted helminths.

## 1207

### OPERATIONAL CHALLENGES ON THE IMPLEMENTATION OF MASS TEST AND TREAT APPROACH FOR THE ACTIVE SCREENING AND TREATMENT OF HELMINTHS AND PROTOZOA IN PRE-SCHOOL CHILDREN FROM BONGO, ANGOLA

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Neglected Tropical Diseases impose a massive burden to health systems, especially in sub-Saharan Africa. The Health Research Center of Angola (CISA), have been investigating integrated interventional strategies willing to control helminths and protozoa in under 5 children, using mass test-and-treat approach. This study aim at documenting the main operational challenges on the implementation of this strategy. Data was collected from under 5 years old children between July and September 2016. It included the collection of stool samples for the diagnosis of intestinal helminth and protozoa (by ParasiTrap and Kato-katz), and the collection of urine for the diagnosis of urogenital schistosomiasis (by filtration technique). Praziquantel and albendazole were administered to infected children. A total of 657 children's were evaluated. From those, 83% delivered urine samples, collected mainly in the supplied container (64%) and in the chamber-pot (30%). On the other hand, a total of 78% stool samples were delivered, where 99.6% were successfully processed by ParasiTrap and 94.7% by Kato-katz. The stool samples were mainly collected from the chamber-pot (51%) and from the floor (27%). In total, 247 children were infected with at least one of the studied parasites, 31% needed Praziquantel, 54% needed Albendazole and 15% needed both drugs. From all infected children, only 222 individual treatment sheet (the main proof of treatment) were signed by the caretakers and 202 were signed by the technician. The main limitations observed here were failure in delivering samples, inadequate sample volume, collection of samples in/from inappropriate containers/locations, impossibility of performing katokatz in diarrheal samples, lack of compliance to treatment appointments or visits, non-supervision of all intakes (in the cases of multiple dosages/drugs) and inadequate filling of treatment records. It should be noticed that the adoption of mass drug administration, as a possible way to overcome these limitations, would entail the unnecessary treatment of uninfected children and limit the number of parasites species included on research studies.

## 1208

### ANTIBODY RESPONSES TO *PLASMODIUM FALCIPARUM* ANTIGENS IN HUMAN IMMUNODEFICIENCY VIRUS-INFECTED ADULTS IN BONDO SUB COUNTY HOSPITAL, WESTERN KENYA

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Malaria and human immunodeficiency virus (HIV) infections are co-endemic in sub-Saharan Africa. HIV infection has been associated with severe and frequent malaria episodes. The effect of HIV on malaria immunity is poorly understood but studies have shown altered malaria-specific antibody responses and B-cell phenotypes. Major alterations in CD4+ T cell populations, disruptions in lymphoid tissue and chronic inflammation may alter malaria-specific B cell responses beyond total antibody levels. However, the effect of HIV infection on malaria-specific

antibody isotypes and subclasses is unknown, including the influence of viral load (VL), CD4+ cells and CRP levels on these malaria-specific antibody responses. We enrolled 190 adult participants (52 HIV-uninfected and 138 infected) at Bondo sub-county hospital in western Kenya at the time of HIV testing. We measured antibody isotypes (IgM and total IgG) and subclasses (IgG1-4) in plasma against AMA-1 and GLURP-R0 antigens using ELISA. Quantification of CRP, CD4+ cells and VL was obtained using ELISA, FACSCount system and Abbott m2000 analyzer respectively. We compared malaria-specific antibodies in HIV-infected and uninfected subjects and also associate these measurements with CD4+ cells, VLs and CRP. IgM, IgG1 and IgG3 levels against AMA-1 and GLURP-R0 were higher in HIV-infected individuals ( $P<0.01$ ). Higher CD4+ cell counts were positively and negatively associated with total IgG and IgG1 responses to AMA-1 respectively ( $P<0.05$ ), VL was negatively associated with total IgG to AMA-1 and positively associated with both IgM to AMA-1 and IgG1 to AMA-1 and GLURP-R0 ( $P<0.05$ ) while IgM and IgG1 levels to AMA-1 and GLURP-R0 respectively were positively correlated with CRP levels of HIV-infected individuals ( $P<0.05$ ). In conclusion, in HIV-infected adults, antibodies associated with malaria protection in prior studies, specifically IgM, IgG1, and IgG3, were significantly increased, and often increased with increased viral load. Since HIV increases risk of clinical malaria, future studies should assess antibody affinity and function in these individuals.

## 1210

### LONGITUDINAL ASSESSMENT OF CD4 RECOVERY AFTER ART INITIATION IN ART-NAÏVE HIV-INFECTED ADULTS IN FOUR AFRICAN COUNTRIES

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With mortality rates decreasing from increasing use of combination antiretroviral therapy (cART), HIV infected adults are surviving longer. Characterizing the impact and efficacy of cART use in older adults can inform care strategies for this population in resource-limited settings where access to cART is now rapidly expanding. We examined changes in CD4 count after cART initiation, stratified by age, in a unique longitudinal African Cohort Study (AFRICOS) across 11 PEPFAR-supported facilities in four African countries; Tanzania, Uganda, Kenya and Nigeria. ART-naïve adults who started ART while in the cohort and underwent evaluations six and twelve months after ART initiation were included in the analyses among two age categories: <50 and >50 years based on the WHO definition for older age. We assessed the association between age group and population-averaged CD4 count using linear regression with generalized estimating equations and an independent working correlation to account for repeated observations in the same individual. Variables for time since ART initiation and the interaction between age group and time were included in the model to evaluate longitudinal changes in CD4 by age stratum. Between Jan 2013 and Jan 2017, 72 HIV-infected participants, eligible for inclusion in these analyses were enrolled. Participants had a median age of 38.6 years (IQR 30.3-46.1) and 48 (67%) were females. 61 (85%) participants were <50 years old and 11 (15%) were >50. At the time of ART initiation, participants who were >50 had a lower mean CD4 count than did participants <50 years old (199 vs 310 cells/mm<sup>3</sup>,  $p<0.001$ ). This disparity between the older and younger age groups persisted after 12 months of ART (256 vs. 361 cells/mm<sup>3</sup>,  $p<0.001$ ), representing a mean increase of 59 cells/mm<sup>3</sup> in the older group compared to 51 cells/mm<sup>3</sup> in the younger group. Despite the

fact that patients >50 years old in this cohort demonstrated similar CD4 responses to ART initiation as were observed among <50 years patients, and lower baseline CD4 counts and immune senescence that is inherent in aging, current case finding strategies are probably inadequate for older individuals.

## 1211

### EVALUATION OF MALARIA STATUS IN INDIVIDUALS WITH AND WITHOUT HIV INFECTION

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The public health crisis of malaria-HIV co-infection is rapidly expanding in sub-Saharan Africa. Lowland western Kenya in particular is holoendemic for malaria transmission and also has high rates of HIV/AIDS infection. Previous studies have shown that individuals infected with HIV experience more frequent and more severe episodes of clinical malaria and the risk increases with advancing HIV disease. However, the impact of HIV infection on asymptomatic malaria, which represents the majority of malaria infections in adults in high transmission areas, remains unclear. We hypothesize that there are high levels of asymptomatic malaria prevalence and malaria parasitemia in HIV-infected compared to HIV-uninfected persons. To address this question, we used sensitive molecular techniques to determine the point prevalence of malaria infection of malaria parasites in HIV-infected and HIV-uninfected participants. Using 700 samples collected in a HIV/malaria co-infection study in a malaria endemic region in western Kenya, the presence of any species of malaria was first determined using an improved assay based on genus-conserved sequences of the *Plasmodium* 18S ribosomal gene. All infections with  $\geq 1$  parasite per 50ul sample were detectable by our assay. Second, if positive for malaria, we utilized a panel of highly species-specific qPCR assays to determine the presence and quantity of mixed species infections including *P.falciparum*, *P.ovale* and *P.malariae*. We report the point prevalence and magnitude of asexual malaria parasitemia as well as the prevalence of infection with multiple species in HIV-infected versus HIV-uninfected persons. Preliminary results show that our quantitative PCR assays are highly sensitive and can be used on blood samples collected on filter paper from malaria endemic areas where high prevalence of sub-microscopic parasitaemia have been reported. The relevance of this study is that HIV-malaria co-infected individuals may be more efficient reservoirs of malaria and therefore there is need for higher public health priority of persons at risk for both diseases.

## 1212

### PREVALENCE OF MICROSCOPIC AND SUBMICROSCOPIC MALARIA INFECTION AMONG PATIENTS LIVING WITH HIV INFECTION AND HIV NEGATIVE INDIVIDUALS IN GABON

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Malaria infection is frequent in endemic among asymptomatic individuals as well as submicroscopic infection who constitute the parasite reservoir. In Gabon, submicroscopic malaria infection has been frequently detected among febrile patients, but its prevalence has not been investigated in people living with HIV (PLHIV) most represented by adults where malaria susceptibility increasing this last year. The present study aims to determine the frequency of microscopic and submicroscopic infection among PLHIV compared to the seronegative patients in different areas of Gabon. A survey was conducted in rural (Koulamoutou and Oyem)

and urban (Lireville) areas of Gabon from March 2015 to June 2016. The microscopic malaria diagnosis was performed by blood smears. The gene encoding the 18S subunit of riosomal RNA was amplified to detect of submicroscopic malaria infection. In total, 339 samples were analysed, among them, 61,1% (n=207) were PLHIV. The prevalence of malaria infection was 31,8% (108/339). The parasite were detected in 27,7% (94/339) by microscopic and 5,7% (14/245) by submicroscopic. The largest proportion of malaria infected samples by microscopic was found among HIV seronegative patients compared to the PLHIV (45,5% vs 15,4%) (p <0.01). Contraste to submicroscopic malaria infection, the prevalence was higer among PLHIV compared to the seronegative patients (6,9% vs 2,7%). This profil of prevalence was comparable according to the fever, the asymptomatic PLHIV were the carried of submicroscopic infection (9,6%; n=12/125). Among PLHIV, frequency of malaria vary according to the use of cotrimoxazole (CTX). This frequency was low among patients with microscopic infection compared to the submicroscopic infections individuals (4,6% vs 12,1%). The present study highlight that submicroscopic and asymptomatic malaria infection not uncommon among HIV infected patients compared to the seronegative patients. The protective effect of CTX were observed. However, submicroscopic infection were frequente among PLHIV with taking CTX.

## 1213

### CHARACTERIZATION AND IDENTIFICATION OF CYP2B6 POLYMORPHISMS IN A CONGOLESE HIV-1 POSITIVE COHORT NAIVE TO TREATMENT

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Antiretroviral therapy has greatly improved the prognosis of AIDS. However, polymorphisms in genes involved in the enzymatic metabolism of drugs seem to be partly blamed for being responsible for the variability in responses to antiretroviral drugs including toxicity. Thus, genotyping of CYP2B6 G516T has been suggested to identify slow metabolizers for precision medicine. In Republic of Congo, highly active antiretroviral therapy (HAART) for HIV / AIDS consists of two nucleoside reverse transcriptase inhibitors (NRTIs) combined with a non-nucleoside reverse transcriptase inhibitor (NNRTI), Efavirenz or Nevapirine (EFV or Nvp), or with a protease inhibitor (PI). Unfortunately, no data are available on the variation of CYP2B6 in the Congolese population. ART (Antiretroviral Therapy) is initiated without a genetic test of the CYP2B6 G516T. Therefore, the aim of this study was to determine 1) the prevalence of the polymorphisms of the CYP2B6 gene at position 516 in Congolese population and 2) to predict the proportion of GG genotype (rapid metabolizer), GT genotype (intermediate metabolizer) and TT Genotype (slow metabolizer), and finally to show the importance of genetic testing in personalized medicine in the country. We collected blood samples from thirty-six HIV positive patients consulting the anti tuberculosis national Center in Brazzaville. Human DNA was extracted and CYP2B6 G516T genotyping was done using Polymerase Chain reaction-restriction fragment length polymorphisms (PCR-RFLP). Our results showed a distribution of 9/36 for the GG genotype (extensive metabolizer), 12/36 for the GT genotype (intermediate metabolizer) and 15/36 for the TT genotype (slow metabolizer). This study shows in a small sample size, the high presence of this mutation in the Congolese population and it would be of interest to conduct a larger study and to explore the possibility to incorporate this test in the national treatment algorithm to improve health care of HIV positive patients.

## 1214

### ART ADHERENCE

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For diseases, such as human immunodeficiency virus (HIV), rural communities must often grapple with adopting a Western biomedical approach to treatment or use traditional healing methods rooted in generations of religious and cultural practice. Despite advocacy for a biomedical approach to treating HIV with antiretroviral therapy (ART) in South Africa, traditional healing preferences remain prominent. It is unknown what factors influence these preferences in rural Limpopo province and whether a better understanding of these health seeking behaviors could be utilized to improved ART uptake and adherence while maintaining a culturally important connection to traditional practices. A structured literature review was performed to identify rates of ART uptake and adherence based on levels of economic disadvantage. We then sought to identify at least five districts in Limpopo province representing the major ethnic groups living in communal settings, and whose belief systems are at least partly African traditional. Next, purposive and snowball sampling methods were designed to draw a sample of 30 participants evenly sampled from each district. Semi-structured interviews will be used to collect data. Data will be analyzed through grounded theory method. Structured literature review found that non-adherence to ART was more prevalent in communities which are economically disadvantaged, and was particularly common in areas characterized by communal subsistence farming lacking formal education and employment. Stigma and discrimination also resulted in patients on ART taking their medications in concealment to cover their HIV positive status or defaulting treatment rather than risk stigmatization. Participant enrollment for qualitative study has yet to begin. Economically disadvantaged populations in rural Limpopo are at major risk for lack of ART uptake or non-adherence but complimentary engagement of the traditional healing community may improve HIV outcomes. Ongoing qualitative study will elucidate the most promising community-based mechanisms.

## 1215

### RATES OF TUBERCULOSIS DIAGNOSIS AMONG AN HIV-POSITIVE COHORT IN 4 AFRICAN COUNTRIES

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Tuberculosis (TB) disproportionately affects people living with HIV (PLWH) but is underdiagnosed due to atypical signs and symptoms and the paucibacillary nature of the disease among HIV-positive people. Diagnosing TB is a challenge to health systems. For this analysis, we describe the rates of TB diagnosis among PLWH in an African cohort study. The African Cohort Study is an ongoing 15 year prospective observational HIV focused cohort that enrolls adults at 11 PEPFAR-supported health facilities in Uganda, Kenya, Tanzania, and Nigeria, all high burden HIV/TB countries. HIV and co-infection clinical parameters and laboratory assessments are obtained at enrollment and every 6 months. TB diagnosis is either bacteriologically confirmed (via GeneXpert® MTB/RIF or acid-fast bacilli smear) or clinically diagnosed on the basis of symptoms and history. We evaluated rates of TB diagnosis among PLWH in our cohort and described associated clinical and demographic characteristics. Between January 2013 and December 2016, 2,488 HIV-infected participants were enrolled. During this period, 111 (4.5%) subjects were diagnosed with TB; 64

(58%) of those cases were bacteriologically confirmed and 47 (42%) were clinically diagnosed. TB incidence was 2.4 cases/100 person years; with 1.4 cases/100 person years and 1 case/100 person years diagnosed among the bacteriologically confirmed and clinically diagnosed, respectively. Although incidence of active TB among PLWH is not well known, a systematic review of intensified TB case finding in HIV clinics in Sub-Saharan Africa found TB rates of 8.2% [Kranzer et al. Lancet 2010]. Among PLWH in South Africa, TB incidence was 5% among patients with comparable CD4 counts to our cohort (mean CD4 465.8 cells/mm<sup>3</sup>) [Lawn et al. JID 2011]. Therefore, our cohort's incidence of 2.4% may be under-diagnosing TB. Continued aggressive screening and diagnosis for TB is warranted.

## 1216

### TRENDS IN THE PREVALENCE OF HIV/AIDS IN THE STATE OF MISSISSIPPI: A FIVE YEAR REVIEW

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Acquired immune deficiency syndrome (AIDS) is a disease of utmost concern all over the world. The etiology is the human immunodeficiency virus (HIV). It is one of the most serious sexually transmitted diseases (STDs). But it can also be transmitted by contact with infected blood from mother to child during pregnancy, childbirth, or breast feeding. There is no cure for it but there are available antiviral drugs that can be used to mitigate its severity. The signs and symptoms vary depending on the stage of infection. Mississippi with a population of 2.9 million, was said to have the 7th highest rate of HIV infection in the U.S. in 2011. The purpose of this study is to assess the trend in the prevalence of HIV/AIDS within the last five years (2011-2015). The study is based on the statistical analysis of the prevalence reports in literature and the Mississippi State Department of Health. The literature review showed that the prevalence of HIV/AIDS in the State of Mississippi in the five years under review appears to have been continuously increasing rather than decreasing. However, there is no significant difference on year to year basis from 2011 to 2015 ( $P > 0.05$ ). HIV infection by sex showed a preponderance of males infected as against females ( $P < 0.05$ ) for each of the five years reviewed. The study showed the highest prevalence in 2011 and the lowest, in 2012 and increased yearly from 2013-2015. But the increase was not very significant ( $P > 0.05$ ). African Americans had the highest number of diagnosed cases of all the races within the review period, even though they make up only 38% of the State's population. They represent 78% of people in Mississippi with HIV/AIDS. These results are very revealing. The trend show how the prevalence of HIV/AIDS is highest in African Americans and appear to be increasing and much less in the caucasians and appear to be decreasing. It is recommended that more efforts be made to reduce the prevalence of HIV/AIDS among African Americans in the State of Mississippi.

## 1217

### PROSPECTIVE VALUE OF QUANTIFERON TB GOLD FOR ACTIVE TUBERCULOSIS IN ART NAIVE HIV POSITIVE INDIVIDUALS IN THE AFRICAN COHORT STUDY

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Tuberculosis (TB) causes globally significant morbidity and mortality. Among people with HIV, the diagnosis of TB is difficult as the detection of acid-fast bacilli in sputum via smear, culture or PCR (TB Xpert) often fails. We evaluated whether immune response to TB, measured by Quantiferon TB Gold (QFT) could improve detection. The African Cohort Study (AFRICOS) prospectively enrolls adults at 11 PEPFAR-supported facilities in Uganda, Kenya, Tanzania, and Nigeria. HIV management history and laboratory assessments were obtained at entry and every 6 months. Clinical diagnosis of TB was determined by national guidelines. Baseline laboratory evaluation involved screening for TB using TB Xpert for all individuals and QFT for ART naïve participants. Between January 2013 and August 2016, 2263 HIV infected participants were enrolled, with a mean follow-up of 1.05 years (range 0 to 3.5 yrs). The mean age was 39.7 yrs and 58.9% were women. At enrollment, 1494 (66%) of participants were taking antiretroviral treatment (ART); 777 (34%) were ART-naïve. 233 (10.3%) of those on ART reported a previous episode of TB, in contrast to 20 (2.6%) of the ART-naïve. At enrollment, 15 of 2263 (0.7%) participants were newly diagnosed with active TB, 10 of them with a positive Xpert and 5 with clinical criteria. During follow-up (2381 person-yrs), a total of 44 cases of TB were clinically diagnosed (1.8 cases per 100 py), 25 among the ART naïve group (3.6 per 100py), compared to 19 (1.1 per 100 py) among the ART experienced. Of these 44 cases, all had a TB Xpert completed, of which 23 had a confirmed positive result. Of the 777 ART-naïve participants, QFT results are available for 549 of which 176 (32.1%) were positive. Out of the subgroup with a positive QFT result 7.8% had a positive Xpert compared with 1.69% of the QFT negative group. Our results suggest an increased risk for developing TB in ART-naïve individuals in a prospective cohort study. Individuals with a positive QFT among ART-naïves are at increased risk for developing active TB. For TB suspects for whom the microbiological proof of TB through Xpert or culture is difficult, testing with QFT can improve the management of patients.

## 1218

### PATTERNS OF HIV STATUS DISCLOSURE TO HOUSEHOLD MEMBERS IN AN AFRICAN COHORT

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Persons living with HIV (PLWH) who do not disclose their status to members of their household may not seek out HIV care and treatment due to fear of stigma or discrimination. These analyses describe patterns of HIV status disclosure, including to household members, and evaluate associations between disclosure, stigma and outcomes such as ART use, and virologic suppression. The African Cohort Study (AFRICOS) prospectively enrolls participants at clinics in Kenya, Nigeria, Tanzania and Uganda. At enrolment of PLWH, a structured questionnaire is administered, a review of ARV medications performed, and blood samples collected. For these analyses, PLWH living with  $\geq 1$  person were categorized based on disclosure to household members. Categories of persons aware of each participant's HIV status were tallied based on self-report. Characteristics were compared between the two disclosure groups. Multivariable Poisson regression models with robust variance estimators were used to calculate risk ratios (RRs) and 95% confidence intervals (CIs) for associations between disclosure to household members and (1) ART use and (2) virologic suppression (HIV RNA  $< 50$  copies/mL). As of 31 December 2016, 2490 PLWH were enrolled and 2296 of them lived

with  $\geq 1$  person. Of these, 1813 (79%) had disclosed their HIV status to a child, 1748 (76%) to a household member, and 1346 (59%) to a spouse or partner. As compared to PLWH who had not disclosed, those who had disclosed to a household member were older (median 39.2 [interquartile range 32.5-46.4] vs 37.6 [30.8-45.6] years,  $p=0.007$ ), more likely to be male (45% vs 24%,  $p<0.001$ ), more likely to be married (72.4% vs 29.9%,  $p<0.001$ ), and more likely to be prescribed ART (70% vs 57%,  $p<0.001$ ). Most participants in both groups denied any experience of HIV-related stigma (88% vs 89%,  $p=0.32$ ). Adjusting for other factors, disclosure to household members was associated with increased likelihood of both ART use (RR 1.09 [95% CI 1.06-1.12]) and virologic suppression (RR 1.29 [95% CI 1.16-1.44]). Efforts to encourage disclosure—particularly among young, unmarried men—may improve ART adherence and clinical outcomes in PLWH.

## 1219

### POPULATION LEVEL ANALYSES TO EXAMINE COMORBID HIV/AIDS INFECTION IN SUB-SAHARAN AFRICA AND TRANSMISSION OF DRUG RESISTANT MALARIA PARASITES

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Modeling in previous studies showed that co-infection with HIV and malaria has the potential to amplify transmission of both diseases through immunosuppression and higher disease burden. However, few studies emphasize the impact of asymptomatic malaria on transmission dynamics. Antifolate prophylaxis for HIV-associated opportunistic infections can increase gametocyte burden, and also may increase parasite drug resistance. Accordingly, we seek to establish the prevalence of drug resistant genotypes within individual patients and at the population level as a prelude to investigate transmissibility of resistance alleles using paired patient and vector samples interrogated by targeted deep sequencing. For these studies, genomic DNA was extracted from dried blood spots obtained from patients in western Kenya. Because parasite DNA is present against a high background of human DNA from leukocytes in these samples, gene-specific targets are initially amplified using a hemi-nested PCR approach. A subsequent PCR further amplifies the targets while adding universal adapters to both 5' and 3' ends of the amplicons. Finally, PCR using primers complementary to the universal adapters adds molecular barcodes and Illumina sequencing adapters. This method allows a greater number of unique identifiers to be added to each library using fewer primers and enables pooling of libraries into a single lane for sequencing. Data are demultiplexed by dbcAmplicons and quality filtered using the SeekDeep analysis pipeline. Data from our validation study suggest that alleles conferring moderate to high resistance in the dihydropteroate synthetase (DHPS) and dihydrofolate reductase (DHFR) genes are approaching fixation in a region of high malaria transmission in Western Kenya. In the context of holoendemic malaria and HIV transmission, fixation of resistant genotypes significantly limits utility for sulfadoxine-pyrimethamine for intermittent preventative therapy. By quantifying and evaluating the transmissibility of drug resistant genotypes, we hope to inform better strategies for the control and treatment of HIV and malaria.

## 1220

### CO-INFECTION MALARIA-HELMINTHIASIS IN PREGNANT WOMEN AT THE GENERAL HOSPITAL OF KIMPESE, DEMOCRATIC REPUBLIC OF CONGO

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Parasitic infections are a major cause of morbidity and mortality in pregnant women. Soil Transmitted Helminthiasis, Schistosomiasis and malaria have a negative impact on the woman and the child who is in gestation, especially by the anemia that they can cause during pregnancy. The objective of this study is to determine the prevalence of co infection malaria-helminthiasis during pregnancy. This is a preliminary cross-sectional descriptive study of 128 pregnant women attending antenatal clinics at IME / Kimpese hospital from January to May 2016. Biological examinations allowed diagnosis of malaria and intestinal helminthiasis and Dose hemoglobin. The frequency of malaria during pregnancy was 32%. In relation to malaria prevention, 64% of women received ITP and 89% were under LLIN. Helminth infection was found in 57% of pregnant women. Malaria co-infection with helminth infections was observed in 24% of pregnant women with 23% *Ascaris lumbricoides*, 16% *Schistosoma mansoni* and 8% hookworm. *A. lumbricoides*, *Schistosoma* spp and hookworm infection co-infected 3% of the pregnant women. However, 65% of the pregnant women had anemia. Malaria-helminth infections co-infected nearly one-quarter of the women receiving pre-natal consultations at the Kimpese HGR. More than 60% of these women had hemoglobin levels below 11g%.

## 1221

### UNUSUAL MORPHOLOGIES AND REPRODUCTION OF CRYPTOCOCCUS NEOFORMANS

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Two cases of immunocompromised patients with cryptococcal meningitis were recorded in the year 2008. The aim of this report is to present unusual morphologies of *Cryptococcus neoformans*. The samples of cerebrospinal fluid obtained from a 7 year-old child, National Institute of Child Health, Lima, Peru, and an adult aged 47, National Hospital Carrión, Callao, Peru, were processed using the modified Chinese stain technique. Both samples showed unusual morphologies and reproduction of *Cryptococcus neoformans*: cells with pseudohyphae, hyphae, successive budding and germ tubes, presented here in two- and three-dimensional images. *Cryptococcus neoformans* was confirmed using culture and biochemical methods. In conclusion, unusual morphologies and reproduction of *Cryptococcus neoformans* have been found in cerebrospinal fluid of immunocompromised patients.

## 1222

**RETROSPECTIVE HOSPITAL REVIEW OF THE INCIDENCE HIV AND SYPHILIS IN HAITI FROM 2008-2016**

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Haiti is among the poorest countries in the Western Hemisphere and has the highest number of people living with Human Immunodeficiency Virus (HIV) in the Caribbean region. Damaged infrastructure following multiple natural disasters has led to an increase risk of HIV and syphilis transmission, due to limited access to health services. Retrospective clinical data focusing on HIV and syphilis was extracted from five African Methodist Episcopal Service and Developing Agency (AME-SADA) network clinics located in the Ouest department of Haiti, capturing data from 2008-2016. Monthly incidence and annual trends were then examined. All data was double entered to ensure accuracy. Between 2008 and 2012, 3.9% (263/6580) of those tested for HIV were found to be positive, while 3.02% (150/4966) number of patients were found to be infected with syphilis. Between 2013 and 2016, the HIV prevalence decreased to 3.4% (523/15331) and syphilis cases increased to 6.2% (996/15973). In 2016 alone, the prevalence of HIV was 3.67% (190/5169) while syphilis was 14.3% (189/1319). However, the 2016 HIV prevalence is relatively high compared to previous years, which may indicate surging transmission and requires monitoring. Studies in Haiti have shown that syphilis infection or having any other sexually transmitted infections (STI's) increases the risk of facilitating HIV transmission and acquisition. In addition, HIV/syphilis infection during pregnancy can lead to adverse birth outcomes, congenital syphilis and increases the risk of mother to child transmission of HIV. Ongoing efforts and funding are critical to reducing the ongoing epidemic of HIV and other STIs in Haiti.

## 1223

**CHARACTERIZATION OF THE POTENCIAL DIAGNOSTIC OF POLYANTIGENS FOR DETECTING *TRYPANOSOMA CRUZI* IN THE CHRONIC PHASE OF CHAGAS DISEASE**

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Chronic Chagas disease (CCD) diagnosis is based on serological methods employing crude, semipurified or recombinant antigens, which eventually may result in low sensitivity or cross-reactivity. An alternative method for solving this problem is the use of chimeric molecules composed by conserved and repetitive epitopes of distinct parasite structures. Our objective was to evaluate the use of chimeras in immunoassays for the diagnosis of CCD. The chimeras IBMP-8.1, IBMP-8.2, IBMP-8.3 and IBMP-8.4 were purified by chromatography and their purity assessed by SDS-PAGE. Circular dichroism (CD) and dynamic light scattering (DLS) were used to assess the hydrodynamic radius of the chimeras, to evaluate their stability and to select the buffering system that offers the lowest state of molecular aggregation. Serological tests for detection of anti-*Trypanosoma cruzi* were performed by ELISA and liquid bead microarray using a panel of 857 positive and 689 negative serum samples for CCD. Cross-reactivity was assessed by using 1079 serum samples from patients with unrelated diseases. The purification was efficient since SDS-PAGE indicated the absence of degradation of chimeric proteins. Analyses of CD and DLS showed that the four chimeras had low aggregation tendency in carbonate buffer, pH 9.6. Serological testing showed high values of sensitivity (Sen) and specificity (Spe) for IBMP-8.4 (Sen-99.3%; Spe-100%).

The performance of other molecules was satisfactory, being the Sen and Spe values above 94%. Cross-reaction was observed in 0.46%, 0.85%, 0.46% and 0.37% of the samples tested against IBMP-8.1, IBMP-8.2, IBMP-8.3 and IBMP-8.4, respectively. The results indicate that the chimeras can be safely used in diagnostic assays for CCD.

## 1224

**USE OF CHITOSAN MICROPARTICLES TO CAPTURE AND CONCENTRATE *TRYPANOSOMA CRUZI* DNA IN URINE OF EXPERIMENTALLY INFECTED GUINEA PIGS**

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Chagas disease is a neglected tropical disease, caused by the protozoan *Trypanosoma cruzi*, which is a major public health problem in Latin America and a potentially serious emerging globally. Detection of *T. cruzi* DNA in urine has been demonstrated before but has low sensitivity due because of the dilution effect of molecules in urine, DNA degradation and presence of PCR inhibitors. In recent years, more practical and accurate diagnostic tools have been developed using non-invasive samples for rapid detection of cases using microparticle and nanoparticle platforms. The chitosan, a cationic biopolymer, obtained from the deacetylation of chitin can interact with anionic molecules included DNA. Here we synthesized microparticles of chitosan by cross-linking with 1% glutaraldehyde to concentrate small fragments of *T. cruzi* DNA in urine and detection of Transrenal DNA (Tr-DNA) of experimentally infected guinea pigs (*Cavia porcellus*). DNA detection was performed using real time PCR target in parasite satellite (166 bp) and kinetoplast DNA (118 bp) sequences. Our results showed that the detection limit of DNA in urine by qPCR was 0.1 parasites/ml for both target sequences. The specificity was 100 % and the sensitivity was 25 % using satellite DNA target and increase to 68 % using kinetoplast DNA target. In conclusion, chitosan microparticles could be used to capture and concentrate *T. cruzi* DNA in urine samples and can increase the sensitivity of qPCR. Detection of *T. cruzi* Tr-DNA could be used for diagnosis of Chagas disease and for the evaluation of treatment efficacy.

## 1225

**ANTI-LEISHMANIAL ACTIVITIES OF SYNTHETIC ENDOPEROXIDES, N-89 AND N-251**

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Leishmaniasis is a major problem worldwide which causes significant morbidity and mortality in cases of visceral leishmaniasis. New chemotherapeutics are urgently needed due to severe side effects and drug resistance plaguing current drugs. The new antimalarial synthetic organic compounds based on endoperoxide structure (N-89 and N-251) were found to possess strong antimalarial and antischistosomal properties, which suggested a broad spectrum of anti-parasitic activities. We therefore investigated the antileishmanial activities of both N-89 and N-251. N-89 exhibited inhibition activity against promastigote forms of 4 *Leishmania*

parasites *in vitro*; *L. major* (PM 2), *L. donovani* (Dd8 and BD38) *L. infantum* (EP173). N-251 also showed inhibition activity against all *Leishmania* spp promastigotes screened *in vitro* except *L. major* (Friedlin). In addition, N-251 induced parasite morphological change resulting in round-shaped *L. donovani* promastigotes mostly lacking nuclei and ability to divide. This was further confirmed when N-251 treatment resulted in parasites with fragmented DNA, a classical feature of apoptosis. Furthermore, 80/20 ratio combination of N-251/miltefosine enhanced activity with reduced toxicity resulting in improved selectivity against *L. donovani* promastigotes. Further *in vivo* studies showed N-251 to significantly reduce parasite burden in both pre-infection and post-infection orally treated BALB/c mice. Results therefore confirm the antileishmanial activities of N-89 and N-251 and also suggest the apoptotic effect of N-251. Further studies to investigate efficacy of N251/miltefosine combination therapy *in vivo* and elucidation of mechanisms of action of N-251 *in vitro* are ongoing.

## 1226

### CO-ENCAPSULATED HOST- AND PARASITE-DIRECTED THERAPIES TO TREAT VISCERAL LEISHMANIASIS

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Visceral leishmaniasis is the second deadliest parasitic disease worldwide, causing an estimated 200,000 to 400,000 new infections annually. Despite its far-reaching effects, there exist very few treatment options, all of which are associated with adverse side effects. Moreover, emerging resistant strains of *Leishmania donovani* limit options even further. It is estimated that nearly 60% of newly-diagnosed visceral leishmaniasis cases are caused by resistant strains of *L. donovani*. One strategy to circumvent resistance mechanisms is to deliver host-directed therapeutics in combination with parasite-directed therapeutics. Previous work in our lab has shown that resiquimod, an agonist for the intracellular toll-like receptor 7/8, can effectively clear *L. donovani* parasite loads, which reside within phagocytic cells. Furthermore, there is evidence for synergistic interactions between suboptimal doses of the parasite-directed amphotericin B and the host-directed resiquimod. Using an acid-sensitive biopolymer (Acetalated dextran (Ac-DEX)) microparticle (MP), we have co-encapsulated resiquimod and amphotericin B at a synergistic ratio capable of passively targeting macrophages and other phagocytic cells. The Ac-DEX MPs allows greater spatiotemporal control of therapeutic delivery, potentially requiring less drug and subsequently resulting in fewer off-target effects than traditional therapies. Furthermore, encapsulation in Ac-DEX MPs mitigates the need for cold-chain storage, rendering this treatment more accessible worldwide than current formulations, which often require administration at a healthcare facility. Ultimately, treatment with both host- and parasite-directed therapeutics could reduce the resistance of emergent strains while minimizing adverse side effects.

## 1227

### THE STRONG HEARTS PILOT: RESULTS OF A PRIMARY-CARE SCREENING PROGRAM FOR *TRYPANOSOMA CRUZI* IN EAST BOSTON, MASSACHUSETTS

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The United States is home to approximately 300,000 people who are infected with *Trypanosoma cruzi*, though less than 1% of those infected have been treated with benznidazole or nifurtimox. We designed and are implementing a screening program for Chagas disease in East Boston, Massachusetts. The aim of this study is to describe the results of a pilot to

integrate Chagas disease screening and referral for treatment into primary care for high-risk populations. This screening pilot is being implemented in the adult, pediatric and obstetrics departments of a large community health center. More than one third of people in this community are from a Chagas-endemic country. Prior to screening, at least one continuing education session about Chagas disease was offered to participating providers. The screening strategy recommends that all patients <50 years old who had lived in Mexico, Central or South America for at least 6 months be offered a one-time screening test. Country of origin was recorded in the electronic medical record with each test ordered. The health center laboratory stored a sample of serum for each patient screened to facilitate rapid confirmatory testing. The pilot established a referral process with a local infectious disease department to ensure close follow-up for infected patients. Community outreach to educate at-risk communities was simultaneously implemented. In the 3 months prior to the pilot, 7 screening tests were ordered across all departments. During the first 2 weeks of the pilot, providers from Adult Medicine ordered 60 screening tests and providers from other departments ordered an additional 2 screening tests. Of these 62 screening tests, only 10 were resulted to date (9 negatives and 1 positive) and 52 were still pending. The patients screened included 28 females (45%) and 34 males (55%). The leading countries of origin for these patients were El Salvador with 23 patients and Colombia with 14 patients. In conclusion, screening for Chagas disease is feasible and accepted by both providers and patients in primary care settings that serve large communities of high-risk patients.

## 1228

### HIGH RESOLUTION MELTING ANALYSIS TARGETING HSP70 AS A FAST AND EFFICIENT METHOD FOR THE DISCRIMINATION OF *LEISHMANIA* SPECIES

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Protozoan parasites of the genus *Leishmania* cause a large spectrum of clinical manifestations known as Leishmaniasis. These diseases are increasingly important public health problems in many countries both within and outside endemic regions. Thus, an accurate differential diagnosis is extremely relevant for understanding epidemiological profiles and for the administration of the best therapeutic protocol. Exploring the High Resolution Melting (HRM) dissociation profiles of two amplicons using real time polymerase chain reaction (real-time PCR) targeting heat-shock protein 70 coding gene (*hsp70*) revealed differences that allowed the discrimination of genomic DNA samples of eight *Leishmania* species found in the Americas, including *Leishmania (Leishmania) infantum chagasi*, *L. (L.) amazonensis*, *L. (L.) mexicana*, *L. (Viannia) lainsoni*, *L. (V.) braziliensis*, *L. (V.) guyanensis*, *L. (V.) naiffi* and *L. (V.) shawi*, and three species found in Eurasia and Africa, including *L. (L.) tropica*, *L. (L.) donovani* and *L. (L.) major*. In addition, we tested DNA samples obtained from standard promastigote culture, naturally infected phlebotomines, experimentally infected mice and clinical human samples to validate the proposed protocol. In conclusion, HRM analysis of *hsp70* amplicons is a fast and robust strategy that allowed for the detection and discrimination of all *Leishmania* species responsible for the Leishmaniasis in Brazil and Eurasia/Africa with high sensitivity and accuracy. This method could detect less than one parasite per reaction, even in the presence of host DNA.

## 1229

### TISSUE IMPRESSION SMEAR AS A SUPPLEMENTARY DIAGNOSTIC TEST FOR HISTOPATHOLOGY IN CUTANEOUS LEISHMANIASIS

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Diagnosis of cutaneous leishmaniasis is a new challenge faced by the dermatologists in Sri Lanka. Majority of patients are diagnosed clinically, due to lack of facilities in areas where the disease is highly endemic, and light microscopy of slit skin smears (SSS) is used to supplement the clinical diagnosis whenever possible. Histopathology of skin biopsy is used as a second line investigation if the SSS is negative or when the lesion is atypical. This study compares the tissue impression smears (TIS) with histopathology for diagnosis of CL in Sri Lanka. A total of 111 patients with clinically suspected CL were included and a single skin punch biopsy specimen was obtained from the active border under local anaesthesia. A TIS was prepared by gently rolling the biopsy on a glass slide. The biopsy specimen was then fixed in 10% neutral buffered formalin, processed routinely for histopathology and examined under a conference microscope for Leishman-Donovan bodies. Tissue impression smears were air dried, fixed in methanol, stained with Giemsa and examined under a light microscope for amastigotes. These two methods were compared by applying the z-test for difference between two proportions to discordant pairs. The study group consisted of 87 (78.4%) males and 24 (21.6%) females with a mean lesion duration of 6.2 months (SD=7.2, range: 1-48). Majority of lesions were either papules or nodules (66, 59.5%), and 32 (28.8%) presented as ulcerated lesions. Lesions were mainly found on the upper limb (63, 56.8%), followed by lower leg (22, 19.8%). Tissue impression smear was positive in 78 (70.3%) patients and histopathology was positive in 63 (56.8%) patients. Both TIS and histopathology were positive in 58 (52.2%) patients. In 20 (18%) patients amastigotes were seen only by TIS and in 5 (4.5%) patients amastigotes were seen only by histopathology. Amastigotes were more likely to be detected in TIS than histopathology ( $p = 0.001$ ). Considering the high positivity and ease of performance of TIS we recommend it as a supplementary diagnostic test in instances where a skin biopsy is performed on skin lesions suggestive of leishmaniasis.

## 1230

### IDENTIFICATION OF ANTI-*TRYPANOSOMA CRUZI* LEAD COMPOUNDS WITH PUTATIVE IMMUNOMODULATORY ACTIVITY

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Chagas disease (CD) is an important public health problem not only in Latin America where it is endemic but it is increasingly spreading in other areas such as Europe, North America, Japan and Australia. Around 7 million people are affected worldwide and approximately 7,000 deaths occur annually. The current drugs used for the treatment of Chagas disease are not effective during the chronic phase and cause numerous toxic side effects. In this context, the balance of the host immune response seems to be a key element for therapeutic success, where a pro-inflammatory microenvironment modulated by IL-10 has shown to be relevant to potentiate anti-*Trypanosoma cruzi* drug activity. The present work aimed to identify the potential immunomodulatory activity of the new therapeutic candidates anti-*T. cruzi*, K777, Pyronaridine (PYR) and Furazolidone (FUR) in peripheral blood mononuclear cells (PBMC) from noninfected subjects (NI) and patients with chronic CD. Our results showed low toxicity of the compounds with IC<sub>50</sub>= 13.1 μM (K777); 5.9 μM (PYR) and greater than 20 μM (FUR) for PBMC. In addition, K777 showed no impact on the exposure index (EI=culture in the presence of the drug/culture with medium only) of phytohemagglutinin-stimulated PBMC, while PYR and FUR induced increased EI of monocytes and T lymphocytes at late stages of apoptosis in NI subjects. The analysis of the cytokine signatures in culture supernatants demonstrated a mixed pro-inflammatory profile, with expressive elevation of IL17, in all treatments employed from CD patients. Moreover, K777 induced a pro-inflammatory response (TNF-α+CD14+/IFN-γ+CD4+) modulated by IL-10 (+IL-10+CD4+/IL-10+CD8+), while PYR and FUR triggered a more inflammatory response by increasing TNF-α+CD14+ and reducing IL-10+ from CD4+/CD8+ T cells. Signature analysis of intracytoplasmic cytokines corroborated with the proinflammatory/modulated (K777) and pro-inflammatory (PYR/FUR) profiles found for these compounds. In conclusion, K777 lead compound may induce beneficial changes in the immunological profile of patients with chronic CD and may contribute to a more effective therapy against the disease.

## 1231

### CHAGAS DISEASE IN THE GRAN CHACO ECOREGION: FROM SURVEILLANCE AND CONTROL TO DIAGNOSIS AND TREATMENT

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Approximately 1.6 million people in Argentina are infected with *Trypanosoma cruzi* (4% of the population) and 7.3 million are exposed to Chagas disease (CD). Less than 1% of them have access to diagnosis and etiological treatment and an estimated 1,300 children are infected annually due to congenital transmission. Mundo Sano (MS) develops surveillance and control (S&C) actions in Santiago del Estero (Gran Chaco Ecoregion) since 2002 as well as operational research on *Triatoma infestans*, main vector of CD in the region. In 2005, MS incorporated the component of Sanitary Improvement of Rural Housing to its S&C program as an innovative strategy to reduce vector transmission of CD. This program has currently covered 11 rural communities, covering 1587 inhabitants from 384 households. Moreover, since 2015, MS has also included the diagnosis and treatment of CD in rural areas in order to improve the situation of underdiagnosis and to aid in the access to etiological treatment. In this study, the data from one of these rural communities is presented. Pozo Herrera (General Taboada Department, Santiago del Estero - Argentina), a settlement of the southeast of the Gran Chaco Ecoregion, is inhabited by 120 people that are distributed in 39 households. This settlement was added to the program of vector S&C in 2010. S&C consists of intra and peridomestic inspection (animal pens, deposits, chicken coops) and its subsequent chemical control with 5% beta-cypermethrin at least once a year, as well as the improvement of housing. This improvement includes the waterproofing of roofs, whitening of walls, ceiling improvement, paint, animal pens and the construction

of water wells and latrines. After six years of implementation, there was a marked reduction in the household infestation rate of *T. infestans* from 31% to 2.7%. In this manner, what started as a purely S&C project has evolved into an integral project that has enabled Pozo Herrera to enter into the diagnosis and treatment phase of the program, allowing for the etiological treatment of CD with Benznidazol (ABARAX® - Elea) following national guidelines.

## 1232

### CIRCULATING MIRNAS PROFILE AS POTENTIAL SIGNATURE OF BENZNIDAZOLE TREATMENT TOXICITY IN CHAGAS PATIENTS

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Chagas disease is an infectious disease caused by the protozoan parasite *Trypanosoma cruzi* (*T. cruzi*). It is endemic in Latin America where it currently affects around seven million people. Even after 100 years of its discovery by Carlos Chagas, there is still a lack of effective treatment for the chronic phase. In addition, the only two drugs available for its treatment, Benznidazole and Nifurtimox, are associated to a great amount of toxicity and no gold-standard biomarkers to assess treatment effectiveness have been described. Therefore, in light of the latest advances showing microRNAs (miRNAs), post-transcriptional regulators of gene expression, as potential biomarkers for drug treatment, we evaluated the effect of benznidazole treatment in Chagas' patients plasma microRNA expression. A group of 41 Chagas patients were enrolled in this study. Blood samples for *T. cruzi* qPCR were collected before, at 60, 180, and 360 days after Benznidazole treatment. Treatment was considered successful when no qPCR positive results were obtained after treatment. A MicroRNA profiling (754 miRNAs) was performed comparing before and 1 year after treatment samples. The comparison between post and pre-treatment samples resulted in 11 differentially expressed miRNAs: 9 upregulated (hsa-miR-181a, hsa-miR-130b, hsa-miR-660, hsa-miR-320, hsa-miR-29a, hsa-miR-29c, hsa-miR-93, hsa-miR-142-3p, hsa-miR-886-3p) and 3 downregulated (hsa-miR-340, hsa-miR-335, hsa-miR-197). Pathway analysis resulted in "Hepatic Fibrosis/ Hepatic stellate cell activation" as one of the most enriched pathways indicating that these benznidazole-induced miRNAs could be regulating targets related to liver damage.

## 1233

### THE POTENTIAL IMPACT OF VISCERAL LEISHMANIASIS VACCINES: EXPLORATIONS WITH DIFFERENT DETERMINISTIC AGE-STRUCTURED TRANSMISSION MODELS

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Current interventions to control visceral leishmaniasis (VL) focus on diagnosis and treatment of cases and on vector control. At this point there is no human vaccine available. However, vaccine development has been ongoing for decades and the results of various studies strongly support the possibility for immunoprophylaxis of VL in the future, suggesting vaccines to be of great additional benefit for individuals at risk. This study explores the potential health impact of different hypothetical vaccines at population level using two age-structured mathematical models that capture the VL transmission dynamics between humans and sandflies. The models differ regarding the main reservoir of infection; in symptomatic and asymptomatic individuals. We incorporate different types of vaccines that

1) lower the risk of infection, 2) lower the risk of developing clinical VL, 3) lower the risk of developing PKDL and 4) a vaccine after which susceptible individuals become immune. Other assumptions take into account different durations of the vaccines' effect and various population coverage levels. The vaccine protecting individuals from developing infection reaches the largest decrease in VL incidence, with the model with the main reservoir of infection in asymptomatic individuals. The vaccine that decreases the development of PKDL has the least impact, only causing a minor decrease in incidence after at least 10 years of an active vaccination programme. The same is true for the model with the main reservoir of infection in symptomatic individuals, however showing a slower decline in VL incidence. The vaccine target product profiles (TPP) are also identified for specific VL targets including the minimum required vaccine efficacy, duration of effect, coverage levels and which age groups best to include in a vaccine program would one become available. In conclusion, these new insights aid in guiding vaccine development and support policy makers with the development of potential future vaccination strategies for the elimination and control of VL.

## 1234

### POTENTIATION OF BENZNIDAZOLE EFFECT BY COADMINISTRATION OF REPURPOSED DRUGS ACTING IN THE INVASION OF HOST CELLS BY *TRYPANOSOMA CRUZI*

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Benznidazole (BZN) treatment of chronically infected Chagas disease patients shows limited or no effectiveness, and the development of more effective drug regimens is one of the major challenges in the field. Host mechanisms involved in *Trypanosoma cruzi* cell invasion and replication offer novel therapeutic targets that have been little explored. We identified two drugs, used for other diseases, that inhibit host cell functions crucial for *T. cruzi* invasion. We hypothesized that treatment with these two drugs could synergize with BZN against *T. cruzi* infection of host cells *in vitro*. Different concentrations of BZN alone, each of the two drugs alone, or their combination were tested against the BZN-resistant Colombian strain of *T. cruzi*. We measured parasite release in the supernatant of cultures of human non-phagocytic HEK293T and phagocytic THP1 cell lines. The concentrations for half-maximal anti-*T. cruzi* activity (IC50) for BZN alone on HEK293T cells and THP-1 cells were 31 µM and 29 µM respectively. Combination of the two identified drugs with BZN reduced the BZN IC50 by 4 to 8-fold as compared to BZN alone. In addition, we analyzed the effect of treatment on the number of intracellular amastigotes after 48h and observed that the combination of the two drugs with a suboptimal concentration of BZN (25 µM) reduced intracellular parasites in THP1 cells by 4 to 10-fold as compared with BZN (25 µM) alone. The drugs had no direct effect on the viability of axenic epimastigotes and trypomastigotes. Results indicate that the combination of repurposed drugs that inhibit host cell functions crucial for *T. cruzi* invasion strongly synergized with BZN in the control of *in vitro* infection by the BZN-resistant Colombian strain of *T. cruzi*. The use of repurposed drugs acting on the host, to synergize with and increase the effect of Benznidazole even at lower - and probably less toxic - doses may have important implications for the treatment of benznidazole-resistant Chagas disease and chronic phase of infection, and animal experiments are under way.

## 1235

### BLOOD CLOT BASED QPCR FOR THE DIAGNOSIS OF CHAGAS DISEASE

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Chagas disease remains an important public health problem especially in endemic countries. The qPCR has become the most important tool for the diagnosis and monitor *Trypanosoma cruzi* load in peripheral blood samples. We have shown previously for qPCR that DNA extracted from clot yields greater sensitivity than DNA extracted from either buffy coat or whole blood samples. However, phenol-chloroform DNA extraction from clot samples is troublesome and toxic, DNA yield is highly variable and DNA stability is poor. The objective of the present study was to develop a better and more reliable DNA extraction method for clot samples. Whole blood – guanidine-EDTA (GEB) and clot samples were obtained from mothers in a previous study of congenital transmission of Chagas disease. We analyzed 100 match GEB and clot samples; 58 were from subjects positive to Chagas disease by serology. A new methodology was developed for blood clot samples based on a combination of the fast-prep technique and the standard method for GEB extraction. Detection of *T. cruzi* was performed by qPCR targeting the nuclear satellite sequences. Out of the 58 samples from Chagas positive individuals by serology, sixteen were positive by qPCR, when either DNA from GEB or clot was used while four samples were positive only in the clot samples. None of the 43 GEB or clot samples from serology negative subjects were positive by qPCR. The new methodology for DNA extraction from blood clot samples increases DNA yield and stability, thus largely improving the molecular diagnosis of Chagas disease.

## 1236

### DIAGNOSIS OF CHAGAS DISEASE BY QPCR IN DIFFERENT SAMPLES FROM NEWBORNS

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Chagas disease, caused by *Trypanosoma cruzi*, affects 8 million people around the world. Congenital transmission has become an important public health concern. Early diagnosis in new born babies is important to implement an effective treatment, since treatment is best tolerated in young babies. The qPCR is an important diagnostic tool specially in new born babies, but sensitivity on the target sequence and on the sample used as source of DNA. In the present study, we compare the use of three different samples as source of DNA: clot, guanidine-EDTA whole blood (GEB) and tissue obtained from the umbilical cord. Archived matched samples (n=100) obtained in a previous study of congenital Chagas transmission were analyzed by qPCR targeting satellite sequences of the *T. cruzi* genome. GEB samples were DNA extracted using previously standardized methodology. DNA from blood clot and cord tissue were extracted using a new developed methodology based on a combination of the fast-prep technique and the standard method for GEB extraction. Three samples were positive when DNA extracted from either of the three sources was tested by qPCR. Two were positive only when DNA from GEB and cord tissue were employed and one sample was positive only when DNA from cord tissue was used, thus sensitivity for GEB, clot and cord tissue was 3; 6 and 7% respectively. We have developed a new DNA extraction technique for DNA extraction from umbilical cord tissue in addition we have shown that tissue from umbilical cord is an important DNA source for the diagnosis of Chagas disease.

## 1237

### DIAGNOSING LEISHMANIASIS BY TARGETING THE ARGININE PERMEASE (AAP3) CODING SEQUENCE

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The leishmaniasis are caused by around 20 different species of the protozoan parasite *Leishmania*. The clinical outcome of the disease and the effectiveness of the treatment protocols is partly dependent on the species, therefore species identification is paramount. Correct identification of *Leishmania* species generate important data for epidemiological and ecological studies. This study was undertaken to develop a rapid, specific and sensitive diagnostic assay for differentiation of *Leishmania* species targeting the *aap3* coding sequence. We designed four pairs of primers flanking polymorphic sites on the *aap3* coding sequence for amplification in real-time PCR and subsequent HRM analysis. Amplicon 1 was used to group the parasites into three main groups: (I) *L. (L.) donovani*, *L. (L.) infantum*, *L. (L.) tropica*, *L. (L.) major*; and (II) *L. (L.) mexicana*, *L. (L.) amazonensis*; and (III) *L. (V.) braziliensis*, *L. (V.) guyanensis*, *L. (V.) lainsoni*, *L. (V.) naiffi*, *L. (V.) shawi*, *L. (V.) panamensis*. For further distinction, three amplicons were designed to differentiate the species within the groups. The assay was able to distinguish all the species tested. Specificity was further investigated comparing the dissociation profiles of different strains of the same species. Further, the amplification profile for other trypanosomatids was distinct from the ones observed for *Leishmania* species. No amplification was detected for mouse, rat or human DNA. In conclusion, *aap3*-HRM analysis is a fast strategy for detection and discrimination of *Leishmania* species. Distinct profiles were found for phylogenetically close organisms and host DNA was negative. The limit of detection was about 0.5 parasites per reaction after a pre-amplification step. Our results indicate that the AAP3 coding sequence could also be a good target for species differentiation by sequencing.

## 1238

### SURROGATE MARKERS OF CURE FOR CHAGAS DISEASE IN CHILDREN TREATED WITH BENZNIDAZOLE DISEASE IN CHILDREN TREATED WITH BENZNIDAZOLE

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Chagas disease (CD), caused by *Trypanosoma cruzi* is the most neglected tropical disease in Latin America. There are no reliable tests or validated biomarker(s) to ensure parasitological cure in chronically infected patients. In adults, seronegative conversion can take decades. However, seronegative conversion in children occurs in few months to a few years, which made children serum samples ideal for CD biomarkers validation. Thirty CD children mainly infected by vertical transmission and diagnosed *T. cruzi* positive by microscopy and PCR (time point S0) were treated with Benznidazole (BZ) 5-8 mg/kg/day for 60 days (time point S1). Treatment efficacy was assessed by serological tests at the end of BZ treatment and follow up until seronegative conversion with at least two serological tests (time point S2). Twenty children from the same range of age were used as healthy controls (HC). Immunoblot and a proteomic-based-assay were used to assess cure in these children using fragments of Apolipoprotein A-1 (ApoA1) and Fibronectin (FBN), biomarkers identified previously in our laboratory. Immunoblot evaluation of ApoA1 and FBN fragments shown gradual reduction or absent of fragments at S1, compared to fragments at diagnosis (S0), and their clearance at seroconversion (S2). Similar results were observed using mass spectrometry. In addition, using intact proteins analysis, a 28109 Da protein was detected in HC serum samples but not

in CD. MS/MS analysis confirmed the protein corresponds to ApoA1 full length. These new data suggest fragments of ApoA1 and FBN are valid biomarkers and demonstrate their better predictive capacity than serology. These biomarkers might be useful to predict cure in clinical settings as well as treatment efficacy in clinical trials of new drugs and drug regimen.

## 1239

### REGIONAL DIFFERENCES OF INFLUENZA LIKE-ILLNESS SYNDROME IN CHILDREN UNDER 5 YEARS, DEMOGRAPHIC AND HEALTH SURVEY (DHS) PERU 2010 - 2014

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Acute respiratory diseases commonly present a seasonal pattern. However, due to its geographic diversity, it is thought that in Peru there may be seasonal differences at the regional level. We analyzed data from 2010 to 2014 of the Demographic and Health Survey (DHS) Peru. Our outcome variable was the variation of the average prevalence of influenza-like illness (ILI) in children under five years between March-December in the five years, defined by the report of the mother over the presence of cough and fever in the last two weeks. Following WHO guidelines, we defined an epidemic peak as 2 DS above the median. The co-variable of interest was the geographic region categorized in coast, highlands and amazon. We report complete data of 45,445 children. The average prevalence of ILI in the five years differed between the coast, highlands, and amazon (15.5%, 16.2% and 20.1% respectively,  $p < 0.001$ ). In March, the average prevalence of ILI was: coast (11.8%), highlands (12.2%) and amazon (16.1%). The median of the frequency of ILI in coast was 15.6%, highlands 16.4% and amazon 19.7%. Epidemic peaks for coast and highlands were presented between April-June (18.1%) and May-September (18.5%) and for amazon two peaks between May-July (26.1%) and October-November (20.1%). The prevalence in the coast decreased in July (13.4%) and had a slight increase in September (16.3%), while in the sierra and amazon decreased in November (15.9%) and December (16.1%). We observed regional differences in the seasonality of the influenza like-illness syndrome, with an earlier onset in the coast compared with the other regions, which could be due to the beginning of classes and greater population of children in schools. On the other hand, the high frequency of ILI in our amazon may be due to a high circulation of other respiratory viruses and bacteria, which have been reported for tropical areas. These findings will serve to design specific preventive actions by region.

## 1240

### IMMUNODETECTION OF PYRAZINE-2-CARBOXYLIC ACID

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Mycobacterium tuberculosis (Mtb) is globally associated to high morbimortality. Drug resistance is threatening the efforts to control this disease. Pyrazinamide (PZA) is a pro-drug integrated in both first and second line treatment schemes due to its unique ability to kill Mtb in its latent stage. Resistance to pyrazinamide is a predictor of poor prognosis, and a cause of treatment abandon and lengthening. Despite genetic polymorphisms in the *pncA* gene, explaining and predicting to a great extent PZA resistance, molecular analysis of clinical isolates is not feasible at great scale since its high costs and complexity, plus not every mutation causes enzyme malfunctioning and PZA resistance. Phenotypic analyses attempt to quantify the metabolite of PZA: pyrazinoic acid (POA). A resistant strain would not convert PZA in to POA at a critical required rate, whereas a susceptible strain will do, and would expel POA in the extracellular environment at a certain rate, allowing its quantification. The

Wayne Test, serves as a simple but not highly sensitive colorimetric test for PZA resistance. We have developed an indirect competitive ELISA test using rabbit serum against POA. POA was covalently linked to Keyhole Limpet Hemocyanine, and inoculated in rabbits. In this test, Bovine Serum Albumin linked to POA is fixed in a Maxisorp Microplate at 40 ng/well and incubated with rabbit antiserum diluted to 1:50000 and a solution of POA. POA positive samples (typical of the supernatant of a MODS liquid culture of a sputum sample of a PZA susceptible TB patient), will block the polyclonal antibodies from binding the BSA linked to POA, causing a decreased colorimetric signal in contrast to negative controls (supernatant of sputum culture of negative controls). The optical density ratios are proportional to the POA concentration in a negative sigmoidal curve fashion. We standardized this assay with serially diluted POA concentrations and found the limit of detection (LOD) at 80ug/mL, which is more sensitive than the Wayne test (500ug/mL). We expect to improve LOD by further diluting competitor agents and using chemiluminescence, which is usually 10x more sensitive.

## 1241

### EFFECT OF CARBOXY TERMINAL MUTATIONS OF RIBOSOMAL PROTEIN S1 OF MYCOBACTERIUM TUBERCULOSIS ON INTERACTION WITH PYRAZINOIC ACID

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Pyrazinamide (PZA) is a first-line drug used in the treatment of tuberculosis. The importance of the study of this drug lies in that it acts mainly on microorganisms in a state of dormancy and yet its mechanism of action is little known. The ribosomal protein S1 (*rpsA*) has been reported as the target of pyrazinoic acid (POA), the active form of pyrazinamide. Thus, in this study we evaluate how a mutation recorded at the terminal carboxyl terminus of *rpsA* can confer resistance to the pyrazinamide strain despite not being at the site of interaction between the *rpsA* and the POA, but rather in a Highly flexible and distant region. Our first hypothesis proposes a loss of affinity of the *rpsA* for the POA due to steric hindrance since the mutation would generate that this highly flexible end more frequently visit the site of interaction and avoid the interaction between both. To evaluate our hypothesis we performed an in silico study, where it was evaluated by docking peptides generated from the carboxyl terminal end and its interaction with the binding site between *rpsA*-POA. Three recombinant proteins with truncated carboxyl terminal end were produced at sites proposed by the in silico study and also contained the mutation conferring resistance of the strain to the drug. The affinity for isothermal titration microcalorimetry was evaluated and our results show that the mechanism by which the mutation recorded at the carboxy terminal end of *rpsA*, which in turn changes the affinity of the *rpsA*-POA, is not generated by a steric hindrance, And that this mutation would be generating other interactions that would change their affinity. To assess what could be the interactions of the carboxy-terminal end and the *rpsA*-POA interaction site, site-directed mutagenesis was generated by *rpsA* recombinant proteins and affinity was determined. To assess what could be the interactions of the carboxyl-terminal end and the site of interaction *rpsA*-POA will be generated by site-directed mutagenesis directed recombinant proteins of *rpsA* with which it is intended to raise the explanation of how a mutation generates the change of affinity between *rpsA* and POA.

## 1242

**HIGH TUBERCULOSIS AND MULTIDRUG RESISTANT TUBERCULOSIS RATES IN A PERUVIAN COHORT**

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Tuberculosis and multidrug resistant *Mycobacterium tuberculosis* (TB & MDRTB) remain significant public health threats worldwide. Peru ranks the second highest in estimated number of new TB cases per year and first in MDR and extremely drug resistant (XDR) TB cases in the Americas; however TB rates in Peruvian military populations are unknown. Here we report results from a cohort under the Peruvian National TB Program (PNTBP) at the Peruvian Naval Medical Center serving active duty military, family members and Peruvian Navy civilians. All symptomatic patients at the military health units in Lima, Peru, received medical exams, smear microscopy and X-rays in accordance with PNTBP guidelines. TB positive patients were registered into the program (serving 16,490 active duty military), a first-line TB drug was started, and culture and sensitivity tests were performed according to PNTBP. Centralized Peruvian microbiology laboratories performed Ogawa and proportion tests to detect TB and MDR/XDRTB. In 2014, primary TB cases were detected in 79 patients; of these 55 (incidence: 33.4/10,000 persons/year) were active duty military, 5% were MDRTB, 78% were male, 85% were pulmonary TB, and 1 was HIV positive. All of these patients completed treatment and were cured. Conversely, in 2015, 93 primary TB cases were detected; 63 (incidence: 38.2/10,000 persons/year) were active duty military, 8% were MDRTB, 83% were male, 80% were pulmonary TB, 4 (4%) were HIV positive, and 4 (n=4) succumbed to the infection. However, no statistically significant differences between 2014 and 2015 were observed for these parameters. Of interest, a teenager in the study presented with XDRTB. High prevalence of TB and MDR/XDRTB has been found in active duty military personnel in this Peruvian Navy cohort; further analysis is required to elucidate risk factors. Control and preventive measures should be implemented to mitigate TB and MDR/XDRTB in military settings.

## 1243

**METHODS OF A STUDY EVALUATING THE IMPACT OF LUNG ULTRASOUND (LUS) ON MANAGEMENT OF PNEUMONIA IN LOW-RESOURCE SETTINGS**

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Pneumonia is the leading infectious cause of death in children worldwide. To address this mortality, it is critical to explore modalities to improve outcomes, such as improved diagnostics. In low-resource settings (LRS), pneumonia is diagnosed using World Health Organization (WHO) Integrated Management of Childhood Illness (IMCI) guidelines that rely on assessing variable and subjective clinical signs like respiratory rate and chest indrawing. Lung ultrasound (LUS) is a promising pneumonia diagnostic technology. There is evidence that indicates that LUS may be as sensitive and specific as chest radiography (CXR). Additional advantages of LUS include its portability, ease of use, lower cost, and absence of ionizing radiation. We will conduct a pilot study among 240 children aged 2 through 23 months presenting to sub-district hospitals in Mozambique and Pakistan (200 cases and 40 controls). Eligible cases will present with cough <14 days and/or difficult breathing and chest indrawing. Eligible controls will present with cough <14 days and/or difficult breathing with no chest indrawing, fast breathing or fever. Children will receive local standard of care including WHO IMCI assessment as well as CXR and LUS performed at enrollment. Physician panels will interpret the CXR and

LUS to ensure consistency and accuracy of interpretation. Respiratory specimens for viral and bacterial testing will be collected from children at enrollment, along with blood for disease screening. Enrolled children will be followed for 30 days post-enrollment; LUS will be performed on enrollment, and on days 2, 6, and 14. The primary outcome will be LUS findings at enrollment with secondary outcomes including patient outcomes, repeat LUS findings, viral and bacterial test results, and patient status after 14 and 30 days of follow-up. Qualitative data will also be collected to assess feasibility, usability and acceptability among healthcare providers and caregivers. By generating this evidence to accelerate LUS as a point-of-care device, a paradigm shift and advance over present-day approaches to the detection and diagnosis of pneumonia can be achieved in LRS.

## 1244

**MATERNAL VITAMIN D SUPPLEMENTATION DURING PREGNANCY AND LACTATION TO PREVENT ACUTE RESPIRATORY INFECTIONS IN INFANCY IN DHAKA, BANGLADESH (MDARI TRIAL): A PROSPECTIVE COHORT STUDY NESTED WITHIN A RANDOMIZED CONTROLLED TRIAL DURING PREGNANCY AND LACTATION TO PREVENT RESPIRATORY INFECTIONS IN INFANCY IN BANGLADESH (MDARI TRIAL)**

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Infancy is a high-risk period for severe acute respiratory infections (ARI). Severe ARIs are often preceded by viral upper respiratory tract infections. Vitamin D status is a candidate modifiable early-life determinant of the host antiviral immune response that may influence the risk of ARI-associated morbidity in high-risk populations. The MDARI trial is a prospective cohort study nested within the Maternal Vitamin D for Infant Growth (MDIG) study in Dhaka, Bangladesh in which 1300 pregnant women were randomized to one of 5 groups of weekly vitamin D supplementation: placebo, 4200 IU, 16800 IU, or 28000 IU from 2nd trimester to delivery plus placebo from 0-6 months postpartum; or, 28000 IU prenatal and until 6-months postpartum. Infants consented into the MDARI sub-study underwent active home-based weekly surveillance during the first 6 months of life that included inquiry about ARI symptoms and a standardized clinical assessment. Additional surveillance visits were conducted when caregivers reported ARI symptoms. Infants meeting clinical ARI criteria had a mid-turbinate nasal swab performed and tested by reverse transcription polymerase chain reaction for 8 viruses (influenza A/B, parainfluenza 1/2/3, RSV, adenovirus, and human metapneumovirus (hMPV)). The primary outcome is incidence of microbiologically confirmed ARI. Secondary outcomes include incidence of ARI associated with specific pathogens (influenza A or B, RSV), clinical ARI, and hospitalized ARI. 1214 (94%) of MDIG participants consented to enrollment in the MDARI study. There were 8333 instances of possible ARI reported of which 3860 (46%) met the study definition of ARI and 3810 (99%) nasal swabs were performed. 4.4% of samples tested positive for RSV, 1.7% for influenza A, 1.0% for influenza B, 4.9% for hMPV, 3.8% for adenovirus, 7.1% for parainfluenza virus, and 1.1% tested positive for more than one viral pathogen. The dose-ranging effect of vitamin D supplementation during pregnancy and lactation on infant ARI in Bangladesh will be summarized and the significance of the findings for maternal vitamin D supplementation guidelines will be discussed.

## THE ASSOCIATION OF COUGH FREQUENCY WITH THE MICROBIOLOGICAL DYNAMICS OF TUBERCULOSIS IN PATIENTS WITH ACTIVE PULMONARY TUBERCULOSIS

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Prolonged cough is a signal feature of tuberculosis infection, and an assumed determinate of tuberculosis transmission. Among HIV-negative patients receiving appropriate therapy, it has been demonstrated that cough rates drop rapidly in the first 14 days of treatment, and that cough frequencies are associated with bacillary load as determined through the microscopic-observation drug-susceptibility (MODS) assay. To further evaluate the association between patient cough and microbiological results in patients with active pulmonary tuberculosis, we conducted a prospective cohort study of recently diagnosed tuberculosis patients in Lima, Peru, at Hospital Dos de Mayo, and Hospital Cayetano Heredia. Cough frequency was determined based on 4 hour recordings using a vibration-sensor based device with a semi-automated cough detection algorithm, and drug resistance was determined using the MODS assay. Paired sputum samples and cough recordings were taken at six time points: at days 0 (or pre-treatment), 3, 7, 14, 30, and 60. Our final cohort included 59 HIV-negative patients with drug-sensitive tuberculosis on appropriate therapy, 7 HIV-positive patients with drug-sensitive tuberculosis, 6 HIV-negative patients on first-line therapy whose disease was subsequently identified as mono- or multidrug resistant, and 1 HIV-positive patients with drug-resistant tuberculosis. Preliminary results confirm that cough detection based on a 4-hour vibration-sensing device and a semi-automated algorithm produced similar estimates of cough frequency compared to a 24-hour, audio-based device. Cough frequency decreased with treatment, patients with a positive MODS result coughed approximately twice as much as patients without cough. In conclusion, cough is associated with microbiological positivity in HIV positive and MDR patients as well as patients with drug-sensitive TB.

## WORLD PNEUMONIA DAY 2011-2016: TWITTER CONTENTS AND RETWEETS

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Pneumonia remains a major killer of children. In 2015, 703,900 children <5 years died of lower respiratory infection globally. World Pneumonia Day (Nov 12) is an annual event advocating for pneumonia awareness. Social media (e.g. Twitter) has become part of the health communication strategy adopted by the campaign. In this study, 28,181 original tweets with hashtag #pneumonia were retrieved on Nov 21, 2016. From this Twitter corpus, 6 sub-corpora of tweets, one month before and one month after the World Pneumonia Day from 2011 to 2016 were extracted (n=998, 1409, 1036, 1369, 1221, and 688 respectively, N=6721). The underlying topics of the 6 sub-corpora were identified via unsupervised machine learning (Latent Dirichlet Allocation). Topics were then manually coded if they belonged to the themes of Raising Awareness, Promoting

Intervention, and Call to Action. Given the overdispersion of retweet counts and inflated number of zeros, we used multivariable hurdle regression models to assess whether themes of Twitter contents were associated with number of retweets, controlling for hashtag count, URL presence, and the age of the tweet. We found that tweets that raised awareness, promoted interventions and called the audience to action were more likely to have retweets (adjOR=2.11, 95%CI 1.89, 2.35; 1.37, 95%CI 1.21, 1.55; 1.99, 95%CI 1.40, 2.85) and if retweeted, to have a higher retweet count by 188%, 122% and 78% respectively (adjPR=2.88, 95%CI 2.36, 2.51; 2.22, 95%CI 1.76, 2.79; 1.78, 95%CI, 1.03, 3.14). Tweets with URL links were also more likely to have retweets (adjOR=1.27, 95%CI 1.13, 1.42) and a 65% higher retweet count (adjPR=1.65, 95%CI 1.35, 2.02). We also described the Twitter topics on pneumonia and their changes over the years. In 2015 and 2016, price of vaccines occurred in the list of top 10 topics of highest number of original tweets and retweets. Our study suggests that among #pneumonia tweets, tweets raising awareness of pneumonia, promoting interventions, and calling their audience to action were more engaged by Twitter users and were likely to be retweeted than others (e.g. sharing personal experience with pneumonia).

## ASSOCIATION BETWEEN SELF-REPORTED SYMPTOMS WITH OBJECTIVE COUGH AND DYNAMIC MYCOBACTERIAL MICROBIOLOGY IN PATIENTS WITH ACTIVE PULMONARY TUBERCULOSIS

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Subjective cough measures have shown utility in assessing quality of life among patients with chronic cough and may also have applicability in patients with Tuberculosis (TB). With existing difficulties in quantitatively prognosticating TB patients, patient-reported of symptoms might also be used to aid in clinical management. Our study evaluated the association between patient-reported cough and objective cough frequency and the association of patient reported symptoms to sputum bacillary load determined by the microscopic-observation drug-susceptibility (MODS) assay. We conducted a prospective cohort study in Lima, Peru at Hospital Cayetano Heredia and Hospital Dos de Mayo on patients recently diagnosed with active pulmonary tuberculosis. The Leicester cough questionnaire and a questionnaire about other TB related symptoms were administered at six times points: at days 0 (or pre-treatment), 3, 7, 14, 30, and 60, with paired sputum samples and cough recordings at each point. 73 patients were enrolled in our study. Preliminary analysis of 47 patients revealed that patient-reported fatigue and difficulty breathing dropped most slowly with treatment, while fever dropped most quickly. The mean Leicester cough score was found to decrease over time from 18.1/40 at day 0 to 9.6/40 at day 60. Correlation between objective cough rate, in epochs or fits of coughing per hour, with Leicester Cough scale was 0.50. There was a notable relationship between reported improvements in symptoms in the first 3 days of treatment, and MODS positivity (OR: 0.84, 95% CI: 0.78 – 0.91, p < 0.001). Increasing self-reported cough frequency was also significantly related to less days to MODS positivity (each question option p < 0.05). In conjunction with other methods of quantifying TB symptoms, a self-reported symptom questionnaire could be a valuable tool for determining prognosis for patients with TB, especially in low resource settings.

## 1248

**ASIA SURVEILLANCE FOR ACUTE NOVEL RESPIRATORY INFECTIONS**

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Novel respiratory virus generation often occurs first among livestock, then infects man before adapting and causing epidemics. Viral groups prone to this include influenza viruses, coronaviruses, adenoviruses, and enteroviruses. In this work we are seeking to identify novel respiratory viruses causing morbidity in humans or emerging in domestic animals which have potential to infect humans. We ultimately seek to detect and characterize these viruses before they fully cross-over to humans and cause epidemics. To do this we have developed a multi-phase surveillance program beginning with severe acute respiratory infection (SARI) caused by these viral groups at four hospitals in Vietnam. The aim of this first phase is to develop a sustainable sentinel surveillance system that strengthens the ability to detect emerging infections. Four sentinel military and civilian hospitals will enroll 400-450 subjects annually. Duke-NUS and NMRC-A have trained local partners in employing molecular techniques using algorithms developed at Duke for evidence in nasopharyngeal samples of human adenovirus, influenza viruses, enterovirus, and coronavirus infections. When an algorithm indicates a patient may be suffering with a recognized human virus, the specimen will be shared with Duke-NUS for further culture and sequence-based study. Additionally, an aliquot of the specimens which screen negative for known pathogens will also be screened for veterinary viruses. Data analysis will include: Counts of SARI detection by month and by site, proportional etiology of likely viral etiology types, and risk factors for severe disease (hospitalization or death). In the preliminary results from the first two months of surveillance, 19 patients have been enrolled and 37% have had an etiology detected (5=influenza A, 1= influenza B, 1=enterovirus). Culture and sequence-based analyses are pending. We plan to expand the surveillance in 2017 to include domestic livestock surveillance (swabs and bioaerosol sampling) in Vietnam, and to begin similar novel respiratory pathogen surveillance at the human-animal nexus in Malaysia.

## 1249

**NON-TREATMENT OF FAST BREATHING PNEUMONIA - THE RETAPP TRIAL**

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“World Health Organization (WHO) defined fast breathing pneumonia” among children 2-59 months of age in low resource settings mandates outpatient antibiotic therapy with high dose amoxicillin. WHO itself recognizes limitations of this approach-non-specificity of the clinical diagnosis, large proportion due to viral infections and giving antibiotics where they are not needed, resultant disruption in microbiome, consequent poor growth along with the increased burden on scarce health resources. To fill the evidence gap for giving amoxicillin in fast breathing pneumonia a double blind randomized placebo controlled non-inferiority trial using parallel assignment is conducted in low income squatter settlements of Karachi, Pakistan. Children 2-59 months old with fast breathing, without any WHO-defined danger signs and seeking care at primary health care center are randomized to receive either three days of placebo or amoxicillin. Primary outcome is difference in cumulative treatment failure between two groups (new clinical sign based on preset definitions indicating illness progression or mortality) on day 0, 1, 2 or 3

of therapy. From September 2014-November 2016, 62,442 children were triaged, 34934 (55.9%) presented with cough or difficulty in breathing. 5550 (15.8%) met the inclusion criteria i.e. having cough for less than two weeks with tachypnoea. Based on eligibility, 2755 were enrolled and received high dose amoxicillin or placebo. With per protocol rate of 96%, overall treatment failure is 3.5% with 1 death. Causes of treatment failure are hospitalization (2%), onset of chest indrawing (2%) and danger signs in 1% among others. Relapse are 66 (2.5%), 33 due to chest indrawing pneumonia. X-ray positivity among treatment failures/relapses is 17%. There were 2 serious adverse events (severe diarrhea). Thus overall rates of treatment failure in fast breathing pneumonia are low. Trial results will serve to support or refute use of antibiotics in this illness. Findings are generalizable to settings with low HIV and malaria prevalence and Hib/Pneumococcal vaccines in their national immunization plan.

## 1250

**ACQUISITION OF PROPER TREATMENT FOR EXTENSIVELY DRUG RESISTANT TUBERCULOSIS PATIENTS IN MALI: WHERE IS THE ISSUE?**

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Extensively drug-resistant tuberculosis (XDR TB), defined as resistance to rifampin and isoniazid plus any fluoroquinolone and at least one injectable second-line drug, has been detected in Mali. This study presents the first three XDR TB patients identified in Mali and describes the barriers to treatment access currently faced in Mali's healthcare system. This alarming development of XDR-TB cases has not been entirely unexpected since resistant infections are the consequence of poor patient management and failure to follow drug treatment regimens established by international authorities. At a referral hospital in Bamako, Mali, two patients presented with XDR TB in August 2016 followed by a third patient in March 2017. Diagnostic testing for the first two patients was completed by the Mali WHO Supra national laboratory (ITM/Antwerp), and testing for the third patient was completed by the Mali University Clinical Research Center (UCRC/USTTB, Bamako). Genotypic findings were suggestive of nosocomial transmission from the first patient to both the second and third patients, which resulted in mixed TB infections. Despite receiving a laboratory-confirmed diagnosis of XDR TB, none of the patients have been given the appropriate treatment, Bedaquiline, to-date. This treatment is not readily available in Mali, and the most significant barrier to treatment access is obtaining the drugs from health authorities, including the Malian government, Global Fund, WHO, and TB Union. Without access to adequate treatment, the identification of XDR TB in Mali will result in little advancement against the global spread of XDR TB. In conclusion, the experience from Mali highlights the need for the developing world to not only have access to diagnostics, but also to effective treatments and clinical guidance. Otherwise, preventable XDR TB infections will continue to develop and spread unchecked.

### TUBERCULOSIS RECURRENCE IN POSTPARTUM

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Active tuberculosis is most common during a woman's reproductive years, the risk in early postpartum women are twice as nonpregnant women. In Jakarta, Indonesia, a 26 year-old woman presented to the Emergency Department with a complaint of worsened dyspnoea 3 days after caesarean section delivery. She was having dyspnoea, coughing with productive white coloured sputum, afebrile with night sweats and weight loss in the past 2 months before delivery. She went to the private hospital, she got cough medicine. She has a history of active TB 6 years ago, and already completed 9 months TB treatment (category-1) with negative acid fast bacilli (AFB) in the end of the course. On examination, she was afebrile, pale but not tachypnic or tachycardic. She was normotensive and the pulse oximetric reading was 96% on room air. Chest auscultation revealed crackles in both lung. Laboratory finding on admission revealed hypochromic microcytic anaemia and neutrophil leucocytosis. Mycobacterium tuberculosis bacillus was detected by sputum AFB staining and positive GeneXpert test without resistance to rifampicin. Chest X-ray on admission showed destroyed of the left lung with pleural effusion, also shows fibrosis with ground glass opacity at the middle lobe of the right lung and fibrosis with calcification at the upper lobe of the right lung. Computed tomographic pulmonary showed atelectasis of the left lung, with retraction of left mediastinal structure, right lung hyperinflation with herniation, fibrosis with ground glass opacity, tree in bud appearance and bronchiectasis. She was initially treated with intravenous ceftriaxone and oral azithromycin for community acquired pneumonia. Anti-tuberculosis treatment under regimen category-2 (rifampicin, isoniazid, pyrazinamide, ethambutol, streptomycin) was started once tuberculosis infection was confirmed together with methylprednisolone (for 3 days). She was discharged after hospitalized for 10 days. The status of TB for the newborn baby are remain unknown. This case reports a challenge to diagnose and treat active TB in postpartum, and show the need of multidisciplinary approaches to deal with it.

### 1252

### DETERMINATION OF MOLECULAR MECHANISMS BEHIND PARASITE EGRESS IN *CRYPTOSPORIDIUM PARVUM* INFECTION

**Samantha Nava**

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Recent studies have identified *Cryptosporidium* as a major contributor to childhood disease in endemic areas. Complications such as stunting, impairments in cognitive development, and malnutrition are caused by persistent and recurring infections. Persistent infection is contributed by the unique life cycle of the parasite, where asexual replication is continuous until intervention by the host immune response. Current clinical interventions are not ideal where only few have demonstrated anti-Cryptosporidial activity and are often associated with relapses in infection. A vaccine to protect or reduce severity of infection would be ideal to protect young children who are most susceptible to complications associated with disease. Merozoite egress is a crucial event in the life cycle of *Cryptosporidium* before the establishment of a persistent infection in susceptible hosts. By targeting the protein factors that are crucial for parasite egress, we could potentially develop a strain of parasite that would have the ability to infect host cells and reproduce asexually, but not complete the lifecycle. Lifecycle arrest will prevent parasites from exiting the cell. We predict this effect will prolong antigen presentation by intestinal epithelial cells, providing the host immune response time

to develop an immune response. This project proposes to look at these factors in *Cryptosporidium parvum* and analyze their role in infection. We hypothesized that proteins related with motility and proteolysis expressed at 24 hours post-infection must have a key role during *Cryptosporidium* egress. Here we will test the hypothesis that CDPK's, Subtilases, or cGMP's are essential molecules for this process. We predict that if these molecules are involved in this mechanism, then the interruption of its function will result in decreased merozoites released after asexual replication.

### 1253

### VACCINE DEVELOPMENT AGAINST *CRYPTOSPORIDIUM PARVUM* INFECTION USING THE INTERFERON GAMMA RECEPTOR KNOCK-OUT MOUSE MODEL

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*Cryptosporidium spp.* infection is a major cause of moderate-to-severe diarrhea in children in sub-Saharan Africa and South Asia and is responsible for more than 200,000 deaths yearly in infants in these regions. There is an urgent need to find new treatments and vaccines for this disease. Advantages of the interferon gamma receptor knock-out (IFNgammaR-KO) mouse model were reported by our laboratory when testing oleylphosphocholine, a compound that prevents lethal *C. parvum* infection. This mouse model also facilitates the evaluation of candidate vaccines against *C. parvum* infection *in vivo*, as demonstrated by experiments on the protective effect of a vaccine candidate, the gp45 *C. parvum* surface protein. IgG, IgG1, IgG2c and several cytokines (for example, IFNgamma and IL-5) were measured by ELISA to evaluate immune responses to vaccination. Intestinal parasite burden following challenge was determined by flow cytometry. Recombinant gp45 combined with the TLR9 agonist CpG-B ODN-2006, a TH1-inducer adjuvant, induced a mixed TH1/TH2 immune response represented by high anti-gp45 IgG titers with a high IgG2c/IgG1 ratio. However, gp45 alone elicited a TH2 immune response illustrated by high IgG1 and IL-5 titers and better protected mice (72% reduction in parasite burden). Therefore, a new combination with aluminium hydroxide, a TH2-inducer adjuvant, is being tested in IFNgammaR-KO mice to determine its ability to elicit a protective immune response and to reduce intestinal parasite burden. The IFNgammaR-KO mouse model is valuable for research on *C. parvum* infection because it is particularly susceptible to infection and mimics clinical signs of cryptosporidiosis in infants. Using this model, we demonstrated the potential of a new vaccine to reduce *C. parvum* parasite burden, a step toward the development of new control measures for infants in developing countries.

### 1254

### RESPONSE OF HOST CELLS TO INJECTION WITH EFFECTOR PROTEINS BY *TOXOPLASMA GONDII*

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*Toxoplasma gondii* and its cousin *Plasmodium spp.* are obligate intracellular parasites that pose a global burden on human health. Prior to invasion, both parasites rapidly inject host cells with proteins from a specialized secretory organelle called the rhoptry. Rhoptry protein secretion is necessary for parasite invasion, survival, and virulence; however, the host response to this event is poorly understood, as traditional methods fail to distinguish it from the host response to additional parasite effector secretion events subsequent to invasion. To characterize the host response specifically to rhoptry secretion, we are circumventing traditional approaches by performing transcriptomic analyses of a naturally occurring and reproducible population of host cells that *Toxoplasma* parasites inject with rhoptry proteins, but then subsequently fail to invade ("uninfected-injected" cells as previously described by Koshy et al., PLoS Path. 8(7):

e1002825 (2012)). This work captures a picture of the earliest stage of a host cell's interaction with *Toxoplasma gondii*, and is expected to reveal host targets of the rhopty proteins.

## 1255

### MATHEMATICAL ANALYSIS FOR A MODEL TO CONTROL CHAGAS DISEASE: FIGHTING AN INFECTION WITH AN INFECTION

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Chagas disease is a vector-borne disease that is endemic across the Americas. We review a single strain infection model for *Trypanosoma cruzi*, the parasite that causes Chagas disease. Then we construct a two strain infection model for *Trypanosoma cruzi* and *T. rangeli* host-vector dynamics. This creates a basis for understanding the dynamics of the competing infections. From here, we can analyze the initial conditions necessary for *T. rangeli* to outcompete *T. cruzi* in a given host-vector population. Recent research has found that infection with the non-pathogenic parasite *T. rangeli* provides protection against infection from the pathogenic parasite *T. cruzi*. No research has yet been done on creating a competition between *T. cruzi* and *T. rangeli* to displace *T. cruzi*, rather than vaccinate or treat animals for the infection. Our two strain model allows us to explore this dynamic. We propose an introduction of *T. rangeli* into host populations endemic with *T. cruzi*. This briefly creates an infection wave of *T. rangeli*, but the single introduction of the *T. rangeli* is insufficient to create a break in the *T. cruzi* infection wave. Multiple introductions of *T. rangeli* under ideal conditions can significantly reduce *T. cruzi* infection, but this effect stops if reintroductions of *T. rangeli* are stopped. Introduction of *T. rangeli* into at risk populations can reduce the invasion rate of *T. cruzi*.

## 1256

### EVALUATION OF THREE COMMERCIAL DIAGNOSTIC TESTS FOR CRYPTOSPORIDIUM INFECTIONS IN HUMANS

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Diarrhea is the second greatest killer of small children worldwide, responsible for 800.000 deaths of under 4-year-olds every year. This is more than AIDS, measles and malaria combined. Even when not fatal, diarrhea often leads to malnutrition, and malnourished children are more prone to develop diarrhea, a vicious cycle difficult to interrupt. Most lethal diarrhea in small children (irrespective of HIV status) is caused by rotavirus, which is soon to be controlled by vaccination programs. This is closely followed by the eukaryote *Cryptosporidium*, which is one of the commonest, and at the same time most poorly understood, water-borne parasites of humans. To aid diagnosis and to support control programmes adequate diagnostic tests should be in place. Several rapid diagnostic tests (RDTs) for cryptosporidiosis are nowadays available and we have evaluated three different brands: RIDA QUICK for *Cryptosporidium/Giardia* Combi (Produced by R-Biopharm, Germany); CRYPTO/GIARDIA DUO-Strip (Produced by Coris BioConcept, Belgium); GIARDIA/CRYPTOSPORIDIUM QUIK CHEK (Produced by TechLab, USA). All three test are based on the lateral flow principle, able to detect both *Cryptosporidium* as well as *Giardia* and are sold as complete test kits. Of each test a single lot was evaluated. Stool samples of children suspected of having a protozoan infection causing diarrhea were used for diagnostic evaluation. Depending on the test used, the incidence of *Cryptosporidium* varied between 9.6% to 18.3%. The incidence of *Giardia* was higher (12.2% to 20%) and in line with previous observations. A small number of children harbored mixed infections: 1.7% to 3.5%. The agreement between the three different tests was determined. The agreement between the Rida test and the Duo-strip test was the best (K-value, as measure to express agreements between diagnostic tests, was 0.721). Also the agreement between Quik Chek test and the Duo -strip test was good, with a k-value of 0.689. The

agreement between the RIDA test and the Quik Chek test was moderate, k-value 0.593. Test performance in terms of sensitivity and specificity will be further assessed by PCR and reported.

## 1257

### THE PREVALENCE AND ASSOCIATION WITH DISEASE OF CRYPTOSPORIDIUM SPECIES AT URBAN AND RURAL SITES IN BANGLADESH

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Three *Cryptosporidium* species are in circulation in Bangladesh: *C. hominis*, *C. parvum* and *C. meleagridis*. A pan-*Cryptosporidium* qPCR assay was run on surveillance and diarrheal samples collected from infants in an urban slum of Dhaka (Mirpur) (n=250), and at a rural location (Mirzapur) (n=258). These children, enrolled at birth into a longitudinal study cohort, were visited at home twice weekly by field staff and brought to the study clinic once a month for a health assessment. Both diarrheal and surveillance samples were collected (Mirpur: 5133 surveillance and 1193 diarrheal; Mirzapur: 5838 surveillance and 263 diarrheal). Positive samples collected at  $\leq 60$  day intervals were considered part of the same infection and designated diarrheal or asymptomatic based on the phenotype at the time of the first positive sample. In Mirpur, 62% of the children were positive for *Cryptosporidium* and multiple infections were common (29% experienced two and 9% three). In Mirzapur, 43% of the children were infected once but only 8% were infected twice. Species specific qPCR assays for *C. parvum*, *C. hominis* and *C. meleagridis* were used to characterize a subset of the samples. *C. hominis* and *C. meleagridis* caused the majority of the infections but the distribution was highly regional. *C. meleagridis* was the major species present at the rural site (98%: 90/92) and was predominant in the surveillance samples (89/90) whereas *C. hominis* was predominant in the urban samples (93%: 118/127) and associated with diarrhea ( $p=0.0002$ ). *Cryptosporidium* diarrhea constituted 6% of year one diarrheal burden and 16% of that in year two. In summary, *C. hominis* but not *C. meleagridis* infection was associated with diarrhea in the first two years of life of infants in Bangladesh.

## 1258

### HISTOPATHOLOGIC DETECTION OF TOXOPLASMA GONDII INFECTION USING A MURINE MODEL UNDER IMMUNOSUPPRESSION

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Toxoplasmosis, a parasitic disease caused by *Toxoplasma gondii*, is a worldwide distributed zoonoses. One third of the population is believed to be infected with *T. gondii*, but pregnant women and immunosuppressed patients (like HIV infected people) are the most affected. In the intermediate hosts, including humans, the parasite reproduces asexually and cysts are formed in different tissues. The aim of this study was to evaluate the tissue damage caused by the Me49 strain of *T. gondii* in immunosuppressed Swiss mice using histopathology. In order to do this, we used twenty female Swiss mice who were two-months old orally infected with 20 Me49 strain cysts. The animals were divided in four groups (five mice per group): infected and immunosuppressed mice (group A), infected but not immunosuppressed mice (group B), not infected but immunosuppressed mice (group C) and not infected and not immunosuppressed mice (group D). Immunosuppression was done using

Dexamethasone (2 mg/kg). We sacrificed the animals fifteen, thirty and forty-five days' post-immunosuppression. During the necropsy, we took samples of brain, heart, liver, lung, diaphragm, spleen and lymph nodes. The samples were fixated using 10% formol-PBS and processed using the standard histology technique for Hematoxylin and Eosin (H&E) staining. In groups A and B, the brain was the most affected tissue and the parasite was found mostly in the cerebral cortex (gray substance). In the lungs and livers, we observed inflammation. Additionally, the mice of the group A showed higher number of cysts with an average size of 10-23 µm which were surrounded by an inflammatory reaction (neutrophils and plasmatic cells). In group C, we found calcifications in the gray substance of the brain. We present these results as relevant and comparative information to improve the understanding of the histopathologic changes caused by *Toxoplasma gondii* under immunosuppressive conditions in which the murine model simulates what can happen in human patients. We recommend to use immunohistochemistry and molecular tools to improve the detection of the parasite in tissues.

## 1259

### WHAT'S THE COST? PEDIATRIC CRYPTOSPORIDIOSIS IN PERU, BANGLADESH AND KENYA

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For children under two years of age, infection with *Cryptosporidium* has emerged as a leading cause of diarrhea-related morbidity and mortality in resource-poor regions. Our study quantified direct medical and non-medical costs as well as indirect costs incurred for *Cryptosporidium*-infected children aged 0-23 months in Kenya, and those younger than 12 months in Peru and Bangladesh. Variables factored into the model included age-specific cryptosporidiosis incidence, treatment costs, health care seeking behaviours, travel costs, caregiver productivity losses, mortality costs, and costs associated with growth faltering. Data inputs were extracted from government reports, internationally validated reference tools, peer-reviewed literature, and expert opinion. According to our analysis, medical costs per episode were higher in Kenya (\$27.10 USD) than in Peru (\$9.55) or in Bangladesh (\$2.44); however, the total annual economic burden was highest in Peru (\$28.5M), followed by Kenya (\$24.2M) and Bangladesh (\$5.5M). Indirect costs in each country far outweighed direct costs. This study not only highlights important data gaps impeding more comprehensive economic analyses, but also emphasizes the large economic burden that cryptosporidiosis causes for health systems in high prevalence areas and for caregivers of infected children. There is a critical need to invest in new solutions for *Cryptosporidium* prevention, diagnostics and treatment in order to address the current medical and economic barriers that prevent children from receiving appropriate and timely care in low resource countries.

## 1260

### COMBINATION EFFICACY OF CLOFAZIMINE AGAINST PIROPLASMOSIS

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In the present study, we evaluated the growth-inhibitory effects of clofazimine, currently used for treating leprosy, against *Babesia bovis*, *B. bigemina*, *B. caballi*, and *Theileria equi* *in vitro* culture, and *B. microti* in mice. The IC50 values of clofazimine against the *in vitro* growth of *B. bovis*, *B. bigemina*, *B. caballi*, and *T. equi* were 4.5, 3, 4.3, and 0.29 µM, respectively. In mice infected with *B. microti*, treatment with oral administration of 20 mg/kg clofazimine resulted in a significant lower

peak parasitemia (5.3%) as compared to a control group (45.9%), which was comparable to subcutaneous administration of 25 mg/kg diminazene aceturate. However, the growth of parasites was observed in mice after blood transfusions from clofazimine-treated mice on day 40 post-infection when parasites were not found in the blood smears. These results suggest that clofazimine has excellent inhibitory effects against *Babesia* and *Theileria* *in vitro* and *in vivo*, but it could not completely eliminate parasites in the host. Therefore, we evaluated the combination treatment with clofazimine and diminazene aceturate against piroplasms both *in vitro* and *in vivo* for the development of a novel chemotherapy with high efficacy and safety against animal piroplasmosis. The clofazimine-diminazene aceturate combination showed to have additive or synergistic effects on *in vitro* growths of *Babesia bovis*, *B. bigemina*, *B. caballi*, and *Theileria equi*. The lower dosages of clofazimine-diminazene aceturate combination showed to have a higher chemotherapeutic efficacy against *B. microti* in mice as compared to clofazimine or diminazene aceturate monotherapy. *B. microti* was not detected in blood, brain, spleen, and heart DNA samples by PCR from combination therapy on day 51 post infection. Furthermore, the parasite did not grow in blood-transfused mice from combination therapy groups. All results suggest that the clofazimine-diminazene aceturate combination chemotherapy will be a better choice to treat animal piroplasmosis instead of diminazene aceturate monochemotherapy.

## 1261

### GENETIC DIVERSITY OF BLASTOCYSTIS SUBTYPES IN PATIENTS WITH CHRONIC URTICARIA

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*Blastocystis* sp is a protozoan commonly found in human and animals. Pathogenic and biological aspects of this organism are still unknown but have been related as a possible etiologic agent of gastrointestinal and extraintestinal manifestations such as irritable bowel syndrome, as well as participation in the pathogenesis of chronic urticaria. Several subtypes (1-9) have been described in human samples. However, it remains unclear whether a given subtype may be unrelated to clinical manifestations. Genetic studies could help to establish links between phenotypic properties of the isolates and their subtypes. The aim of the present study was to investigate *Blastocystis* STs in samples from patients with chronic urticaria without definite cause attended at the Hospital das Clínicas of the Faculdade de Medicina da Universidade de São Paulo (HC-FMUSP). Stool samples diagnosed at the Section of Parasitology of the Central Laboratory (HC-FMUSP) were used for DNA isolation. Polymerase chain reaction (PCR) was performed using specific primers targeting the small-subunit rRNA gene of *Blastocystis* sp. DNA sequences were then aligned and compared to other sequences obtained from the GenBank and MLST database. We observed the occurrence of three STs: ST1 (38.5%), ST2 (23%) and ST3 (38.5%). The 18S alleles were retrieved for each subtype where ST2 and 3 showed the highest number of alleles. The present study is one of the few providing ST data from the human population in South-America and which evaluates the possible relationship of subtypes with chronic urticaria. Studies of allelic variation within species provides fundamental insights about the evolution and ecology of organisms. Financial Support FAPESP (2015/18213-6)

## 1262

**ANTISCHISTOSOMAL ACTIVITY OF PYRIDOBENZIMIDAZOLE DERIVATIVES**Godfrey Mayoka<sup>1</sup>, Jennifer Keiser<sup>2</sup>, Kelly Chibale<sup>1</sup><sup>1</sup>University of Cape Town, Cape Town, South Africa, <sup>2</sup>University of Basel and Swiss Tropical and Public Health Institute, Basel, Switzerland

Tropical diseases continue to plague developing countries, hampering economic development in already impoverished regions of the world. Malaria and schistosomiasis, two of the most prevalent parasitic infections, have their transmission exacerbated by geographical factors, poor hygiene and sanitation. Chemotherapy is the backbone of control and treatment of these infections with resistance to current medication posing a threat to the gains made towards eradicating these diseases. Concerted efforts to discover and develop new drugs using both phenotypic whole cell assays and target based approaches are, therefore, warranted. The long development time-lines, high costs and risk of attrition, further compounded by the low projected financial returns discourage research investments in these therapeutic areas. Drug repositioning offers a cheaper and quicker alternative to address this dilemma. In this approach, in which there is usually underlying biochemical or physiological similarities in the therapeutic areas under consideration, a drug or lead compound developed for one disease is used as a template for subsequent medicinal chemistry optimisation to identify a drug for another disease. In this context, based on similarities in the haemoglobin degradation pathway in schistosomes and plasmodia, we evaluated the antischistosomal activity of analogues based on a pyridobenzimidazole hit identified after a whole cell screening assay against human *Plasmodium falciparum*. Several compounds with potent *in vitro* activity, suitable pre-clinical pharmacokinetics and physicochemical properties were identified and prioritised for *in vivo* efficacy evaluation in a murine model of schistosomiasis. Further medicinal chemistry optimisation is ongoing to progress these compounds as prospective antischistosomal leads.

## 1263

**MAGNETIC BEAD-BASED SAMPLE PREPARATION FOR LOW-RESOURCE ENHANCEMENT OF ULTRASENSITIVE LATERAL FLOW ASSAY FOR DETECTION OF SCHISTOSOMA BIOMARKER CAA**Christine F. Markwalter, David W. Wright  
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Current parasitological methods for *Schistosoma* detection are not sensitive enough to detect low-density infections. Thus, there is a pressing need for highly sensitive and specific schistosomiasis diagnostics to determine prevalence in low-transmission areas and for verification of disease elimination. Recently, GJ van Dam and colleagues developed a urine-based up-converting phosphor lateral flow assay (UCP-LFA) for the *Schistosoma* biomarker circulating anodic antigen (CAA) that was shown to detect CAA for *Schistosoma* burdens as low as one worm pair. However, the UCP-LFA for CAA deviates from conventional point-of-care lateral flow assays because its high sensitivity relies on sample pre-processing that requires laboratory equipment not available in low-resource settings. We have developed a simple-to-use magnetic bead-based sample concentration method and device to enhance the UCP-LFA for CAA by concentrating CAA from larger sample volumes and delivering the enriched CAA to the lateral flow assay. Magnetic beads functionalized with a CAA binding ligand capture the biomarker from large-volume samples. These functionalized beads are deposited onto the UCP-LFA using a stationary magnet within a field-deployable bead-delivery device. A modified running buffer releases the biomarker from the beads, delivering an enriched CAA sample to the test and producing a positive signal at the test line for samples that would otherwise be undetectable.

## 1264

**THE USE OF GEOGRAPHIC INFORMATION SYSTEM AS A TOOL FOR SCHISTOSOMIASIS SURVEILLANCE IN THE PROVINCE OF DAVAO DEL NORTE, PHILIPPINES**Chiqui de Veyra<sup>1</sup>, Vicente Belizario Jr.<sup>1</sup>, John Paul delos Trinos<sup>1</sup>, Berne Silawan<sup>2</sup>, Agapito Hornido<sup>3</sup>, Hansel Amoguis<sup>4</sup>, Dominic Basalo<sup>5</sup>, Cherry Dema-ala<sup>6</sup>, Irenn Mantilla<sup>4</sup>, Rosele Layan<sup>3</sup><sup>1</sup>Neglected Tropical Diseases Study Group, National Institutes of Health, Manila, Philippines, <sup>2</sup>Provincial Planning and Development Office, Davao del Norte, Philippines, <sup>3</sup>Provincial Health Office, Davao del Norte, Philippines, <sup>4</sup>Department of Health Regional Office XI, Davao City, Philippines, <sup>5</sup>Rural Health Unit of Carmen, Davao del Norte, Philippines, <sup>6</sup>Rural Health Unit of Braulio Dujali, Davao del Norte, Philippines

Schistosomiasis (SCH), caused by *Schistosoma japonicum*, remains endemic in the Philippines. Effective SCH control requires describing areas at-risk where control efforts may be focused. This study aimed to demonstrate the utility of geographical information system (GIS) as a tool for SCH surveillance in the province of Davao del Norte, Philippines. Qualitative and quantitative data on SCH determinants were obtained from concerned local government units, partner agencies, and institutions. These were standardized and incorporated in the GIS map. Areas at-risk for SCH were described by overlaying determinants, which include geography and climate, agriculture, poverty, sanitation, presence of intermediate and reservoir hosts, prevalence levels, and mass drug administration coverage rates. The GIS map showed that endemic barangays are located in lowland and flood-prone areas where major rivers and tributaries are found. New Corella has the highest poverty incidence among the reported SCH-endemic areas, as well as the highest number of confirmed snail colonies. Among known endemic localities, Tagum City is the only city in the province which met the poverty incidence target of below 16.6%. Clustering of SCH cases was reported in barangays of the municipalities of Asuncion, New Corella, Tagum City, Kapalong, Braulio Dujali, and Carmen ranging from 0.48% (8 out of 1,655) in Braulio Dujali to 2% (25 out of 1,405) to Asuncion. This study demonstrated the utility of GIS as a tool for SCH surveillance. The use of the GIS map in predicting and assessing risk for SCH may allow prioritization of allocating resources and delivery of services in areas at higher risk for SCH. Likewise, the GIS map may be useful in monitoring and evaluation of the control program through data visualization in space and possibly even time which may help improve program policy and planning for targeted surveillance and effective control. Development of an action plan for SCH prevention and control using data generated by the GIS may guide program policy and planning toward SCH control and prevention in the province and in other endemic areas.

## 1265

**PREVALENCE OF SCHISTOSOMIASIS AROUND THE KAINJI AND JEBBA DAMS**Henry A. Okoro-nwanja<sup>1</sup>, Ogonna N. Nwankwo<sup>2</sup>, Grace I. Nwankwo<sup>3</sup><sup>1</sup>World Health Organization, Ibadan, Nigeria, <sup>2</sup>University of Calabar Teaching Hospital, Calabar, Nigeria, <sup>3</sup>Federal Teaching Hospital Abakaliki, Calabar, Nigeria

Schistosomiasis is one of the commonest neglected tropical diseases worldwide with socio-economic and health impact on infected individuals. Dams though highly desirable for their socio-economic benefits have some unintended consequences on human health and the environment. It often leads to increase in the prevalence of diseases and multiplication of vectors associated with illnesses. Thus Individuals residing close to dams have higher risk schistosomiasis than others. Multi-prong programmatic approaches have been adopted in past years to reduce the impact of this diseases in a region hosting two major dams in Nigeria for which there is a need to continuously monitor and evaluate the impact of the programs to inform more policies and actions to prevent the diseases

hence the need for this study. This study aims to assess the prevalence of schistosomiasis amongst population close to the Kanji dam and Jebba dams of Nigeria. The study was carried out in settlements around the dams in nine LGA located in Kebbi, Kwara and Niger states of Nigeria. A parasitological methods was used to examine the urine sample of 1266 individuals from households recruited through a multi-stage sampling methods. Ethical clearance for the study was obtained from the National Health Research Ethical Committee (NHREC) of the Federal Ministry of Health. Over half (61.8%) of our cases were aged above 17 years with the median age at 24 years(4-94) with cases almost equally divided between males and females. urine microscopy result showed total prevalence of schistosomiasis in communities around the dams was 38.8% with ranges between 8%-60%. The study showed the highest prevalence was in the pre-adolescence age group of 6-10 years(76.5%) followed by the pre-school age group of under-5(71.8%) and declines with ages further away from adolescence. Furthermore, schistosomiasis prevalence is highest among the pupils (63.6%) followed by livestock farmers (41.7%), crop farmers (32.4%) and least amongst civil servants (7.7%). There is a need for continuous surveillance around dams and heightened community wide mass campaign treatment in this areas as the strategy for elimination.

## 1266

### COINFECTION OF SCHISTOSOMIASIS HAEMATOBIMUM AND SEXUALLY TRANSMITTED INFECTIONS IN PREGNANT WOMEN: KISANTU HEALTH ZONE, DEMOCRATIC REPUBLIC OF THE CONGO

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*Schistosoma haematobium* (urinary schistosomiasis) infection is a common cause of morbidity among women in schistosomiasis endemic regions. Trematode ova deposition in genital mucosa can lead to inflammatory granulomas, bleeding, pain, and abnormal discharge. Moreover, these lesions are hypothesized to pose a risk for sexually transmitted infection (STI) acquisition and transmission, as they modify the mucosal layer of the genital tract and may alter the local immunologic milieu of infected women. In Kisantu, Democratic Republic of Congo, the overall prevalence of schistosomiasis is 61.8%; however, there is limited information on the prevalence of *S. haematobium* specifically or of STIs in the region. We conducted a cross-sectional study in four clinics providing prenatal and HIV care in Kisantu health zone, DRC from October 2016 to March 2017. A standardized questionnaire was administered to 352 consenting pregnant women, and biological samples (urine and vaginal/ endocervical swabs) were collected and analyzed. *Chlamydia trachomatis* (CT), *Neisseria gonorrhoea* (NG) and *Trichomonas vaginalis* (TV) were then tested for via nucleic acid amplification of vaginal swab samples [Xpert® CT/NG Assay and Xpert® TV Assay (Cepheid, Sunnyvale, CA)], and *S. haematobium* was detected via urine microscopy. The prevalence of *S. haematobium* in the study population was 15.1% (53/352). Among women with schistosomiasis infection, 28.9% were seropositive for HIV, while 18.6% were infected with at least one other STI: two percent of women were found positive for both CT/ *S. haematobium*, 5% were positive for both NG/ *S. haematobium*, and 14% were positive for both TV/ *S. haematobium*. The high prevalence of *S. haematobium* and presence of co-infection with HIV, CT, NG and TV found here among pregnant women is concerning, especially amidst mounting evidence of adverse maternal health and birth outcomes resulting from infection during pregnancy.

Factors related to co-infection should be further explored in order to determine the temporality of co-infective species acquisition, risk factors for co-infection, and consequences of co-infection on pregnancy.

## 1267

### ADDITION OF SNAIL CONTROL TO ACHIEVE DISEASE CONTROL TARGETS SCHISTOSOMIASIS: A COST-EFFECTIVENESS MODELING STUDY

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Schistosomiasis affects over 240 million people globally. There is growing interest in adding snail control interventions, but this is not currently emphasized as a global strategy. We aimed to assess the potential cost-effectiveness of various snail control strategies to guide future policymaking for disease control of schistosomiasis. We extended previously published dynamic, age-structured transmission and cost-effectiveness models to simulate mass drug administration (MDA) and snail control interventions against *Schistosoma haematobium*. We calibrated the model to data from low-burden (15% prevalence) and high-burden (48% prevalence) communities in rural Kenya. We simulated a 10-year intervention program with: i) MDA targeting school-aged children and the entire community at 75% coverage (annual and biannual); ii) snail control (annual and biannual); and iii) combined strategies. We estimated direct programmatic costs from literature and expert opinion, and computed the incremental cost-effectiveness ratio (ICER) in 2016 US\$ per disability-adjusted life year (DALY) averted, with a base case comparison of no intervention. We defined strategies as highly cost-effective if the ICER was less than the Kenyan GDP per capita (US\$1,377 US\$/DALY). In the low-burden setting, annual community-wide MDA with annual snail control was optimally cost-effective (ICER: 971 US\$/DALY with 76% of simulations being cost-effective). In the high burden setting, annual community-wide MDA with more frequent biannual snail control was optimally cost-effective (ICER: 486 US\$/DALY with 97% of simulations being cost-effective). In both settings, using the optimally cost-effective strategy reduced total disability by an additional 45% compared to school-based MDA alone over a 10-year period. These findings were sensitive to some programmatic inputs, including the country-specific willingness-to-pay threshold, which should inform generalizability to other settings. These results support inclusion of snail control in the current global guidelines for schistosomiasis.

## 1268

### ARE WE ON OUR WAY TO ACHIEVING THE 2020 GOALS FOR SCHISTOSOMIASIS MORBIDITY CONTROL USING CURRENT WHO GUIDELINES?

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Schistosomiasis remains an endemic parasitic disease affecting millions of people around the world. The World Health Organisation (WHO) has set goals for controlling schistosomiasis morbidity by 2020, along with its elimination as a public health problem in certain regions by 2025. Current WHO guidelines have been established to determine the frequency of treatment for a region depending on its baseline prevalence with a reevaluation done after 5-6 years after which the frequency of treatment may be adapted. Recent mathematical models developed to study the transmission dynamics of schistosomiasis can inform implementation policy

for mass drug administration programmes. Here, we use these models to predict whether the recommended guidelines set by the WHO are on track for reaching their 2020 goals for control of morbidity. In general, we find that the guidelines are sufficient in low to medium prevalence settings, however in high prevalence settings they are likely to fail. It also becomes harder to reach the goals in regions where school-aged children carry most of the infection as here higher levels of treatment are needed. Additionally, we find that although the prevalence of heavy-intensity infections is reduced in some scenarios, the overall prevalence remains high. In cases where the guidelines fail to meet the goals, we suggest alternative strategies, such as an increase in coverage, including the expansion of school-based to community-based treatment, along with an increase in compliance, which could assist in eliminating the morbidity of this disease.

## 1269

### CAA AND CCA DETECTION IN SCHISTOSOMIASIS: ASSURED DIAGNOSTIC TOOLS TO BE EMPLOYED WHEN MOVING FROM CONTROL TO ELIMINATION

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The renewed interest in mapping, intensified control and elimination of schistosomiasis (World Health Assembly Resolution WHA 65.21) has put the need for highly accurate diagnostic assays high on the agenda. Based on the well-studied schistosome antigen detection (CCA and CAA) ELISA's, a visual, field-friendly point-of-care urine test for CCA and a quantitative, ultra-sensitive reader-assisted assay for CAA have been developed. The CCA test is commercially available and may replace the Kato-Katz for prevalence mapping of community-level *S. mansoni* infections using a single drop of urine and also allows quick evaluation within days of treatment efficacy. The recently developed test for CAA is applicable to serum or urine of all schistosome species at sub-pg levels, which allows finding single worm infections. The assay has been transformed into a robust, dry-reagent test, used in several low-resource settings in Africa. In combination with optimized sampling schedules the CAA could rapidly identify *foci* of low prevalence/intensity of all human schistosome infections. Recent studies using the 2 ml urine format show that in near-elimination settings in China, South-East Asia, Africa and Brazil, prevalence of active schistosome infections by egg microscopy may be underestimated up to 10-fold. Also, decrease of CAA serum and urine levels after treatment has been shown in all effectively treated cases, however complete cure rates are very limited. The CAA strip assay therefore presents itself as a highly accurate diagnostic tool, with a clear value for application in control and elimination settings.

## 1270

### ASSESSING THE IMPACT OF INTENSIFIED TREATMENT STRATEGIES AGAINST UROGENITAL SCHISTOSOMIASIS IN NIGER

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Schistosomiasis is endemic across all of Niger. The National schistosomiasis control program commenced with mass drug administration in 2003 and to date the area has received over a decade of treatments. Both *Schistosoma haematobium* and *S. mansoni* are present, although *S. haematobium* is more common. The highest levels of infection are found along the Niger River Valley, with proximity to irrigation canals a major risk factor. In 2011 the SCORE project commenced in Niger, with a goal of providing an evidence-base and tools for programmatic decisions on how best to gain and sustain control of schistosomiasis, and, ultimately, elimination. The primary objective of the SCORE Niger study was to understand which treatment strategy provided the best reduction in prevalence and intensity of *S. haematobium*. In total 225 villages were

allocated to a four-year treatment strategy that aims to evaluate the impact of twice yearly versus annual treatment versus treatment every two years for urogenital schistosomiasis, both in the context of communitywide treatment (CWT) and school-based treatment (SBT). The villages had been divided into three possible groups with varying treatment combinations:

- Group A: Communities of moderate endemicity (10-24% prevalence) where annual SBT (ssss), biannual SBT (ssssx2), biennial SBT (shsh) and biannual SBT every two years (shshx2).
- Group B: Communities of high prevalence (>25%) where annual SBT (ssss), biannual SBT (ssssx2), biennial SBT (shsh) and biannual SBT every two years (shshx2).
- Group C: Communities of high prevalence were annual CWT (cccc) or biannual CWT (ccccx2). The findings showed that PZQ treatment resulted in an overall reduction in *S. haematobium* infection from Year 1 to Year 5, where the average prevalence went from 15.8% to 9.89%. The absolute difference between prevalence at Year 5 and baseline was greatest in high prevalence villages, with respect to moderate prevalence areas. Furthermore, biannual treatment resulted in a significantly greater reduction in prevalence, with respect to annual treatment, resulting in 9.1% and 1.3% decrease in prevalence respectively.

## 1271

### DYNAMIC OF SCHISTOSOMIASIS PREVALENCE FROM 2011 TO 2016 COHORT STUDY IN KALIFABOUGOU MALI

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Both Schistosomiasis, *Schistosoma haematobium* and *Schistosoma mansoni* are endemic in Mali. MDA with PZQ is widely applied in Mali since 2005. In Kalifabougou MDA with PZQ have been scaled up since 2010. The goal of this study is to evaluate schistosomiasis's prevalence after PZQ MDA. We have performed a cohort study with repeated cross sectional surveys from 2011 to 2016. Sixty hundred nineteen five (n=695) volunteers aged from 3months to 25 years old living in Kalifabougou village were included. Prevalence's of *Schistosoma haematobium* and *S. mansoni* eggs excretion were measured by urines filtration (*S.h*) and stools Kato Katz technic (*S.m*). During our surveys, all schistosomiasis positive were treated with single dose of Praziquantel. *Student t test*, ANOVA and Chi Square were used to compare means and proportions, with an alpha risk of 0.05. Six hundred seventy six (676) volunteers were included in 2011 and followed until 2016. The prevalence rate of *S.h* varied from 2011, 2013, 2014 with a progressive reduction 10.2%, 5.32%, and 5.25% respectively. However there was an increasing prevalence rate in 2015. This increases of *S.h* prevalence in 2016 was due to the missing campaign of PZQ MDA in 2014. This was corrected in 2015 and *S.h* prevalence was reduce in 2016 at 5.4%. Only one case of *S. mansoni* has been diagnosed in 2011. Children aged from 6 to 10 years old were more infected with *S.h*. In conclusion, MDA – PZQ is an efficient strategy for schistosomiasis control in Kalifabougou. But social science and parasite resistance additional studies are needed to explain the remaining 5% of *S.h* prevalence. These results could help elimination strategies.

### PRELIMINARY OBSERVATIONS ON THE FEASIBILITY OF USING A MAGNETIC PROBE FOR ISOLATION OF SCHISTOSOME EGGS FROM URINE

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Genito-urinary schistosomiasis, caused by *S. haematobium*, is diagnosed by identification of eggs in urine samples. The aim of this study was to investigate the feasibility of using a high field gradient magnetic probe to rapidly concentrate and isolate schistosome eggs into 40- $\mu$ L droplets from 100-mL urine samples to enable detection of the eggs using an optical microscope. The probe consists of a device with a needle made from a metal that can be readily magnetized and demagnetized. In the magnetized state the tip of the needle becomes an attractor of any magnetized particles within an approximate 3-mm range. In the demagnetized state, any magnetic particles on the tip are released. To test the feasibility of using such a device for diagnosis of infection, twelve 100-mL urine samples were seeded with either 10, 20, 50, or 100 eggs. Each concentration was prepared in triplicate. Each 100-mL sample of seeded urine was allowed to sediment for 80 minutes in a sedimentation cup. The supernatant from each cup was discarded and 1 mL of urine remaining was transferred to an Eppendorf tube. A 1- $\mu$ L suspension of magnetic particles was added and the tubes were homogenized for 30 mins. Each tube was then stirred with the magnetized probe for 10 seconds. The probe was then withdrawn from the tube with material adhered to the tip. The material was then washed from the tip onto a glass microscope slide with 40- $\mu$ L of water with the probe in the demagnetized state. The droplet was then examined with an optical microscope. Slide examination took 3 to 5 mins during which eggs were counted. Eggs and magnetic particles were observed in all cases (ranging from as low as one egg per slide from the samples seeded with 10 eggs per 100 mL up to as high as 38 eggs per slide for the samples seeded with 100 eggs per 100 mL). In conclusion, the results demonstrate that the methodology has the potential for 100% sensitivity for rapid detection of eggs down to a concentration of 0.1 eggs per mL of urine and suggest further studies with field samples of *S. haematobium* infections are warranted.

### 1273

### SOCIAL DETERMINANTS OF PREVENTIVE CHEMOTHERAPY UPTAKE DURING MASS-DRUG ADMINISTRATION INTERVENTIONS FOR SCHISTOSOMIASIS IN SUB-SAHARAN AFRICA: A SYSTEMATIC REVIEW

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Achieving sustained high-uptake of preventive chemotherapy treatment is key for controlling and eliminating schistosomiasis (SCH) in Sub-Saharan Africa. Despite growing investment in mass-drug administration (MDA) interventions, low levels of coverage are often reported in the region. This review aims to identify socioeconomic and cultural determinants affecting at-risk populations' acceptance of, or compliance with, MDA of Praziquantel. The search strategy retrieved qualitative and quantitative studies published between 2002 and March 2017. Bibliographic databases Scopus, PubMed/MEDLINE, EMBASE, PsycINFO, CINAHL and Web of Science were examined. 2294 unique abstracts were identified, 61 underwent full-text screening and 27 were chosen for final review. Most studies came from Uganda (n=12). Quality assessment was conducted to assert strength of evidence. A framework synthesis following an

ecological model of health behavior was adopted to sort emerging themes. At the individual level, results indicate that fear of side effects, having an occupation associated with geographical mobility and limited knowledge of SCH infection dynamics or MDA details limit uptake. At the organizational level, community engagement in the selection of drug distributors (CDDs) and distribution strategies favor acceptance of MDA. Community-level barriers concern misrepresentations of medicine intake, mostly associated with disease-treatment rather than prevention, and of health interventions, considered to have a 'hidden' agenda due to historical distrust toward governments and foreigners. Pre-existence of legitimate community organizations facilitate MDA success. At the policy level, limited resource allocation for health training among CDDs seems to affect local trust whilst lack of material support constrains distributors' reach among distant households and large populations. Results call for widening sensitization campaigns beyond general awareness of SCH and expanding community engagement in MDA schemes. Additional support for frontline workers is needed. Regional bias in review's sources calls for further research.

### 1274

### INFLAMMATORY BIOMARKERS ARE RELATED TO CRITICAL PREGNANCY OUTCOMES RELATED TO CRITICAL PREGNANCY OUTCOMES

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We have previously demonstrated that maternal schistosomiasis is associated with elevated pro-inflammatory cytokines in maternal, placental, and cord blood. In this study, we evaluated the factors associated with shifts in maternal and newborn cytokine profiles, and their association with adverse perinatal outcomes, using samples from a recently completed placebo-controlled trial evaluating praziquantel treatment for schistosomiasis among pregnant women enrolled at 12 – 16 weeks' gestation. Cytokine levels in maternal, placental and cord blood were measured using a bead-based assay (IL-1, 2, 4, 5, 6, 8, 10, 12, and 13, IFN- $\gamma$  and TNF- $\alpha$ ). Cytokines >90th percentile were regarded as elevated. Regression models evaluated the relationships among placental and cord blood cytokines, perinatal outcomes, praziquantel treatment and helminth coinfection. In maternal peripheral blood, IL-6 and IL-8 decreased significantly from 12 to 32 weeks' gestation in both arms, while IL-13 increased only in the praziquantel arm. In addition, the proportion of women with elevated TNF- $\alpha$  at 32 weeks' gestation and placental IL-6 was higher in the praziquantel arm ( $P=0.03$  and  $P=0.04$ , respectively). Hookworm infection was associated with elevated placental IL-4 ( $p=0.023$ ), IL-8 ( $p=0.028$ ) and IFN- $\gamma$  ( $p=0.022$ ). Regarding the association with cytokines and adverse birth outcomes, elevated cord blood IL-2 was associated with the risk of LBW (RR: 2.35; 95% CI: 1.06 – 5.19). The risk of SGA was greater when IL-8 (RR=1.74; 95% CI: 1.11 – 2.72) and IL-13 (RR=1.67; 95% CI: 1.07 – 2.61) in placental blood, and IL-8 (RR=2.37; 95% CI: 1.27 – 4.40) and IL-2 (RR=1.80; 95% CI: 1.01 – 3.21) in cord blood were elevated. Neonatal iron deficiency was associated with elevated cord blood IL-1 (RR=8.77; 95% CI: 1.19 – 64.5). Taken together, these data strengthen the evidence that cytokines in maternal and cord blood may be related to poor intrauterine growth, and helminth coinfections may significantly influence cytokine concentrations. Studies to better define when to treat women for helminth infections that may impact placental and newborn health are needed.

## 1275

**AN EVOLUTION OF PARASITOLOGICAL- AND SEROLOGICAL-BASED METHODS FOR DIAGNOSIS OF INTESTINAL SCHISTOSOMIASIS IN HIGH-LOW ENDEMIC SETTINGS**

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Infection with intestinal schistosomiasis is typically common in children, particularly within regions of sub-Saharan Africa where environmental water contact is high and access to adequate sanitation is poor. Traditional parasitological methods of diagnosis that visualise parasite ova underestimate true prevalence and as control programmes progress, infection egg-tallies may also decline. Consequently, there is a need to develop better methods for detection of schistosomiasis, especially in countries such as Uganda where ongoing school-based control has taken place for over a decade. Against this country-backdrop, we investigate the application of novel diagnostics to shed light on current levels of infection across 5 primary schools within Buliisa District, Lake Albert. We evaluated parasitological- and serological-based methods alongside real-time PCR and focused upon examination of children of school-age (i.e. 5-10 years). PCR-based methods explored the use of Taqman® assays on faecal samples. We assessed the diagnostic congruence between methods and report that intestinal schistosomiasis is still pervasive within this lakeshore environment.

## 1276

**EFFICACY AND SAFETY OF PRAZIQUANTEL IN PRESCHOOL-AGED AFRICAN CHILDREN WITH INTESTINAL OR URINARY SCHISTOSOMIASIS - AN INDIVIDUAL-PATIENT DATA META-ANALYSIS**

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Preschool-aged children are recognized as vulnerable group for schistosomiasis, but cannot be treated easily with praziquantel because the drug is registered only for children >4 years-old and can be given routinely only to those >60 cm-tall, in the absence of a paediatric formulation. Moreover, uncertainties persist as to the right dose of praziquantel that is effective and safe in young children, although a recent aggregated-data meta-analysis including 1694 preschool-aged children found no significant difference in efficacy with respect to school-aged children, as reported previously. We conducted an individual-patient data meta-analysis of 4 studies enrolling a total of 1600 children, of whom 899 were preschool-aged (<6 year-olds, mean age 3.8 years) (458 boys, 441 girls); of these, 417 were infected with *S. mansoni* (baseline arithmetic mean

ranging 32-80 eggs/gram of faeces in 4 studies) and 99 with *Schistosoma haematobium* (baseline arithmetic mean 24 and 118 eggs/ml of urine in 2 studies). After praziquantel 40 mg/kg treatment, 352 and 90 respectively had a follow-up visit at which they were found to have arithmetic mean egg reduction rate (ERRam) of 21% (180-d follow-up), 49% (42-d follow-up), 99% (84-d follow-up) and 100% (42-d follow-up) in the four trials of *S. mansoni*, and 93% and 100% in the 2 *S. haematobium* trials. Efficacy of praziquantel 40mg/kg in preschool-aged children varied.

## 1277

**PREVALENCE OF SCHISTOSOMA MANSONI INFECTION AND OTHER PARASITIC DISEASES IN PERIPHERAL AREAS OF BARRA MANSA, RIO DE JANEIRO, BRAZIL**

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Schistosomiasis mansoni and other intestinal parasites are chronic infections widely spread in the world. Globally, about two billion individuals are infected with parasites and most of them live in resource-poor conditions. Schistosomiasis mansoni continues to be one of the most serious world public health problems and it occurs in 76 countries worldwide. In Brazil, about six million individuals are infected with *Schistosoma mansoni*. The city of Barra Mansa (RJ) is a low transmission area with an estimated prevalence of 1%. The aim of this study was to determine the prevalence of *S. mansoni* infection and other intestinal parasites in five peripheral areas of Barra Mansa/RJ. This was a cross-sectional study conducted from March 2011 to February 2012. The samples were randomly selected from the population. A total of 650 individuals who freely agreed to participate were selected, and we obtained 610 fecal samples. All samples were assayed by using two slides with the Kato-Katz (KK) method using the Helm Test® Kit (Fiocruz) and two slides for Hoffman, Pons & Janer (HH). The slides were analyzed by the Laboratory of Clinical Parasitology of the Central Laboratory Division of the Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, Brazil. At the end of the analysis, the results were sent to the Secretary of Health of Barra Mansa, responsible for reporting them and treating the infected individuals. The age of participants ranged from 10 to 91 years, represented by 40.8% males and 59.2% females. The prevalence of parasites found were: *Endolimax nana* 17.4% (n=106); *Blastocystis* spp. 10.8% (n=66); *Entamoeba coli* 4.7% (n=28); *Giardia intestinalis* 1.8% (n=11); *Strongyloides stercoralis* 1.5% (n=9); *Enterobius vermicularis* 0.8% (n=5); *Schistosoma mansoni* 0.8% (n=5); *Ascaris lumbricoides* 0.5% (n=3); *Trichuris trichiura* 5% (n=3); *Taenia* spp. 0.3% (n=2); *Iodamoeba butschlii* 0.2% (n=1). The global prevalence of intestinal parasites was 27.9% (n = 170). The results of this study show the information available to date on these parasites in this population and represent a communication strategy to control these infections.

## 1278

**REVEALING BIOTIC DIVERSITY: HOW DO COMPLEX ENVIRONMENTS OFFER NOVEL WAYS TO CONTROL HUMAN SCHISTOSOMIASIS?**

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The growing discipline of disease ecology emphasizes understanding the biotic context in which disease transmission occurs. Such a perspective

is important for understanding transmission of human schistosomiasis in complex tropical aquatic habitats. In western Kenya, *Schistosoma mansoni* is transmitted by *Biomphalaria pfeifferi* in streams and by *B. sudanica* on the shoreline of Lake Victoria. We have undertaken bi-monthly surveys at each habitat where we collect data on abiotic factors, snail densities, and prevalence of larval trematode infections. Our study has revealed that stream transmission sites harbor dynamic populations of 8 different snail species, some of which may compete with *B. pfeifferi* or serve as dead-end hosts for *S. mansoni*. In Lake Victoria shoreline transmission sites, 11 snail species are noted and comprise a different subset of species than found in streams. Habitat use also differs dramatically with respect to vertebrate hosts, with cattle being particularly prominent along streams and with several wild bird species being conspicuous at lakeside habitats. The communities of digenetic trematodes inhabiting stream and lakeshore habitats have also been shown to differ considerably, including the species colonizing *Biomphalaria* snails as first intermediate hosts. In addition to *S. mansoni*, molecular analysis has shown *B. pfeifferi* to harbor 14 additional trematode species, and local prevalence, particularly because of the abundance of cattle-transmitted amphistomes, can rise to 25%, with overall *S. mansoni* prevalence of 4.9%. *Biomphalaria sudanica* transmits 18 species of digenetic trematodes in addition to *S. mansoni*, but the overall prevalence of infection is lower (12%), as is the prevalence of *S. mansoni* (0.4%). We have also established a dominance hierarchy among the trematode species infecting *Biomphalaria* that includes consideration of both predatory interactions and facilitation among the species involved. Further studies will document the extent to which *S. mansoni* transmission may be dampened by the complex webs of competing snails and schistosomes present in typical transmission sites.

## 1279

### EXAMINING THE IMPACT OF THE SCHISTOSOMIASIS CONTROL INITIATIVE ON PRAZIQUANTEL COVERAGE IN SUB-SAHARAN AFRICAN SCHOOLCHILDREN

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Schistosomiasis, a two millenia-old neglected tropical disease caused by parasitic worms, is prevalent in marginalized communities without access to safe drinking water and adequate sanitation. *Schistosoma mansoni* and *Schistosoma haematobium* are the two species of blood fluke in Africa. It is estimated that 218 million people required preventive treatment for schistosomiasis in 2015 and 90% of those requiring treatment live in Africa. Praziquantel, an anthelmintic drug, is recommended to treat schistosomiasis infections as it is safe and effective with few adverse effects. Praziquantel coverage consists of distributing the drug to infected parts of the population and putting adequate monitoring in place to assure that the treatment reaches the individuals and is taken properly. In examining the impact of the Schistosomiasis Control Initiative (SCI) on praziquantel delivery and coverage, we seek to analyze the SCI's implementation methods and strategic challenges present in executing control programs in Sub-Saharan Africa. The SCI was established in 2002 with the goal of reducing the global burden of neglected tropical diseases in sub-Saharan Africa by 2030 in accordance with the United Nations Sustainable Development Goals. SCI's role has been to identify country recipients, provide funding to governments for implemented programs, provide advisory support, and conduct monitoring and evaluation on the control programs. Strategic challenges faced by SCI include prioritizing resources, inadequate health care facilities and staff, environmental challenges, public health measures, and sustainability. The SCI predominantly focuses on facilitating vertical mass drug administration, an intervention that historically has had limited benefits in controlling schistosomiasis when used alone. Interrupting transmission through preventative chemotherapy, snail control, improved sanitation and access to safe water, or increased health education are alternative methods to accelerate the elimination of schistosomiasis. We hope this review of SCI's past methods can serve as a pathway to improved control programs in the future.

## 1280

### MOVING FROM CONTROL TO ELIMINATION OF SCHISTOSOMIASIS IN SUB-SAHARAN AFRICA: TIME TO CHANGE AND ADAPT STRATEGIES

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Within the past decade, significant progress has been made on large scale treatments for the control of schistosomiasis. Today, treatment with praziquantel is cost-effective and 'preventive chemotherapy' is the strategy of choice, endorsed by WHO. In sub-Saharan Africa, the landscape is becoming ever more heterogeneous as there are several species of *Schistosoma* that respond in different ways to ongoing preventive chemotherapy. The major focus of preventive chemotherapy is delivery of praziquantel by mass drug administration to those shown to be, or presumed to be, at-risk of infection and disease. In some countries, regional progress may be uneven but in certain locations there are very real prospects to transition from control into interruption of transmission, and ultimately elimination. To manage this transition requires reconsideration of some of the currently deployed diagnostic tools used in surveillance and downward realignment of existing prevalence thresholds to trigger mass treatment. Current strategies were designed primarily for the control of morbidity due to schistosomiasis and were formulated over two decades ago when the availability of praziquantel was limited. Moving from control to elimination of schistosomiasis is a paradigm shift that creates several challenges. The interruption of schistosomiasis transmission requires significant changes in the approach, design and strategies with a focus on reducing transmission and preventing reinfection. This involves several challenges such as implementation of intensified interventions, expansion of treatment coverage, use of alternative strategies, improvement of clean water supply, sanitation and hygiene, health education, reaching hard to reach and vulnerable communities, adapting treatment to transmission dynamics, mapping quality and uncertainties, redefining disease endemicity and focality for eligibility for MDA, monitoring and evaluation, and strengthening of institutional capacities. The presentation will highlight and discuss some of the key challenges and requirements for schistosomiasis elimination in sub-Saharan Africa.

## 1281

### AN IMPACT EVALUATION OF LARGE-SCALE WATER, SANITATION AND DRAINAGE INFRASTRUCTURE IMPROVEMENTS IN LUSAKA, ZAMBIA: PRELIMINARY FINDINGS IN THE DRAINAGE CATCHMENT AREA

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The Government of Zambia is upgrading and extending water, sanitation, and drainage infrastructure in Lusaka. As part of a planned pre- and post-evaluation of these upgrades and extensions, we conducted a baseline household survey to estimate the impact of the rehabilitation of Lusaka's central drainage on incidence of disease, flooding frequency and duration, household travel time, and employment and expenditure patterns. From January-May 2016, we surveyed 3,142 randomly selected households within 250 meters of the drain. We found that 19% of households had at least one member who had diarrhea (at least three loose stools in a 24 hour period) or respiratory illness [fever and (cough and/or sore throat)] in the 7 day period prior to interview. The incidence of diarrheal illness was 3.0% overall and 7.8% for children <5 years; 6% of individuals with diarrhea reported blood in their stool. Median duration of illness was 3 days (range 1-32). Respiratory illness incidence was 2.4% overall and 7.0% for children <5 years. Median duration of illness was 4 days (range 1-40). Of 1,064 children for whom an under-5 vaccination card was available,

68% were fully vaccinated against rotavirus (had received two doses); 6% had received one dose, and 24% had not been vaccinated. Sixty percent of households reported flooding in the month prior to the interview. Mean duration of flooding was 3.3 days, median 2 days (range 1-31). Median reported travel times were significantly longer during periods with flooding compared to periods without, for both school (15 minutes vs. 25 minutes,  $p < 0.0001$ ) and work (30 minutes vs. 40 minutes,  $p < 0.0001$ ). The majority of households (90%) had at least one adult member employed. Surveyed households spent a mean of \$157 per month on food items. This is similar to the mean of \$161 per month for non-food items (which includes rent). Relatively large proportions of household expenditures on food are indicative of low-income households. Given these baseline measurements, it is anticipated that improvements in drainage in central Lusaka could have a notable effect on the incidence of disease, flooding frequency, household travel time and time use.

## 1282

### WATER TREATMENT FOR THE REMOVAL OF *SCHISTOSOMA CERCARIAE*: A REVIEW AND IDENTIFICATION OF RESEARCH NEEDS

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Schistosomiasis control currently focuses on preventive chemotherapy with praziquantel, which is effective, safe and inexpensive. However, this treatment does not prevent subsequent reinfection. As schistosomiasis control targets become more ambitious and move towards elimination, interest is increasing in the potentially complementary roles of water, sanitation and hygiene (WASH) interventions which may disrupt transmission of the parasite, thereby slowing reinfection following treatment. In particular, water treatment for schistosomiasis control seeks to eliminate viable *Schistosoma* cercariae from water. A systematic review was carried out to summarize the existing knowledge on the effectiveness of water treatment for the removal or inactivation of cercariae, by processes including chlorination, filtration and ultraviolet disinfection, as well as water storage. This is the first review of its kind and provides a concise summary of what is known to-date regarding water treatment and cercariae of different *Schistosoma* species. The review also identifies gaps in knowledge and provides crucial and timely guidance for the control and elimination of schistosomiasis, by highlighting the requirements for designing effective, sustainable water infrastructure that minimises re-exposure to cercariae in water.

## 1283

### ENVIRONMENTAL PATHOGEN IDENTIFICATION TO CHARACTERIZE SANITATION LEVELS IN LOW AND MIDDLE INCOME COUNTRIES

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The provision of clean water and access to sanitation is central to the goals set in 2015 by the Sustainable Development Goals (SDGs). The reason for the prominence of water and sanitation in the SDGs is that 2.4 billion people still lack access to basic water and sanitation facilities, placing them at higher risk of diarrheal diseases that remain one of the leading causes of death for children under 5. Current approaches to prevent diarrheal disease and chronic sequelae, however, focus primarily on increasing access to sanitation facilities and less on minimizing transmission of enteric or waterborne pathogens. In light of recent mixed results from randomized control trials evaluating sanitation interventions, it is necessary to take a systems level approach when examining intervention strategies. This systems approach is further necessitated by the fact that enteric pathogens have multiple and often interdependent transmission pathways. An important input of a systems level approach is the accurate measurement of environmental pathogen loads. TaqMan Array Cards provide an efficient means of collecting genomic pathogen identification data from the

environment that is accurate, reliable and allows for the quantification of environmental pathogen concentrations. We present a study plan that used TaqMan Array Cards to characterize the spatio-temporal contamination levels of 40 enteric pathogens in Ethiopian and Mexican communities. We sampled sample both home and shared environments to characterize how sanitation interventions impact pathogen loads in both these environments.

## 1284

### EFFECTS OF A COMBINED WATER QUALITY, SANITATION, HANDWASHING AND NUTRITIONAL INTERVENTION ON TELOMERE LENGTH AMONG YOUNG CHILDREN IN RURAL BANGLADESH

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Early life exposures to stress may increase susceptibility to disease later in life. Telomere length (TL) is a prognostic marker of cellular aging and various diseases and could play a causal role in health or disease. Most studies suggest that faster childhood TL attrition is an indication of early life adversity. However, the trajectories of infant TL in low-income settings, the potential effects of childhood interventions on TL, and whether TL is a robust marker to evaluate stress-reducing interventions in children are all unknown. We evaluated whether combined water, sanitation, handwashing, and nutritional interventions improved TL during the first two years of life. We conducted a cluster-randomized trial in rural Bangladesh where geographical clusters of pregnant women were randomized into individual or combined interventions (ClinicalTrials.gov, NCT01590095). This substudy included children in the control and the combined water, sanitation, handwashing, and nutrition arms. Outcomes include TL at 1 and 2 years after intervention and the change in relative TL between years 1 and 2. Analysis was intention-to-treat. We randomized 720 clusters in the control and intervention arms; of these, we measured TL in 662 children at Year 1 (age ~14 months) and 713 children at Year 2 (age ~28 months). TL was normally distributed, and the mean  $\pm$  SD whole blood TL was  $1.43 \pm 0.23$  telomere to single-copy gene ratio (T/S) units at Y1 ( $6729 \pm 549$  base pairs) and  $1.45 \pm 0.24$  T/S units at Y2 ( $6763 \pm 586$  base pairs). We are currently performing the blinded analyses of intervention effects and will unblind after analyses are completed in May 2017. Understanding the critical molecular pathways contributing to TL and its attrition during early life would highlight the potential use of TL as a prognostic marker for future health risks and provide a potential target for future intervention development and evaluation.

## 1285

### ANTIBIOTIC RESISTANT *E. COLI* IN DRINKING WATER SAMPLES FROM RURAL ANDEAN HOUSEHOLDS IN CAJAMARCA, PERU

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Antibiotic resistance in pathogenic bacteria is a serious public health issue. The growing threat is a cause for concern and action to prevent the emergence of new resistant strains and the spread of existing ones to humans via the environment. This study was aimed at identifying faecal pathogens in drinking water obtained from rural Andean households from Cajamarca, Peru and measuring the antibiotic resistance profile of *Escherichia coli*. The study was embedded within a community-randomised controlled trial among 102 communities in the northern highlands of the Cajamarca Region, Peru. Generic *Escherichia coli* was isolated from drinking water of 320 households at baseline measurements. The antibiotic resistance pattern was determined against twelve commonly used antibiotics, using the Kirby bauer method. Out of 314 samples collected, 55.4% (n=174) were positive for thermotolerant coliforms. *E. coli* was isolated in 74% of these (n=117), *Klebsiella* spp. in 15.8% (n=25), *Enterobacter* spp. 10.1% (n=16) and *Citrobacter* spp. in 5% (n=8). We isolated multiple thermotolerant bacteria in 14% of the samples. The *E. coli* antibiotic resistance profile showed highest resistance against tetracycline (37.6%), followed by ampicillin (34.2%), sulfatrimethoprim (21.4%), and naladixic acid (13%). The high prevalence of faecal contamination in drinking water highlights the importance of household water treatment methods. Likewise, the high levels of antibiotic resistance found, urges to identify the origins of potential environmental contamination or misuse of antibiotics. Elucidating means for the safe disposal of antibiotic containing waste in this context and promoting such practices is essential.

## 1286

### WATER, SANITATION AND HYGIENE EDUCATION IN SCHOOLS TO PREVENT NEGLECTED TROPICAL DISEASES IN ANGOLA: A PROGRAM REVIEW

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Angola hosts many endemic NTDs that have a detrimental impact on child health, school attendance and quality of life. Concurrently, much of the country suffers from inadequate access to improved water sources and other essential WaSH services. Since 2015 The MENTOR Initiative have been implementing a WaSH education (WASHE) program alongside the government across three provinces in Angola (Huambo, Uige and Zaire). The program aims to increase handwashing practice in school-aged children and incorporate WaSH into the national primary school curriculum - striving for behavior change in schools and empowering the local community. In those three provinces the baseline KAP survey showed that on average, 86% of schools had no handwashing facilities at all and 49% had no access to safe drinking water. Sanitation levels were more promising with 48% of schools with access to basic sanitation, although 31% reported open defecation. The main program activities comprise teacher training and follow up supervisions. The training teaches good WaSH practices and develops a skill-based health education. Supportive supervisions monitor the impact of the training and work with schools to apply its teaching in context. During 2015, 1,082 teachers and directors were trained through the WASHE program - education centered on inclusion, participation and active learning. The attendance rate was excellent and teachers improved their test results by nearly 100% during the training. The program indicators show that, on average, all provinces

improved in all WaSH elements after training, especially in handwashing. Zaire province had the greatest improvement in water and handwashing practices, whilst unfortunately Uige has shown no improvement in sanitation from the baseline. The preliminary program results compare with international WaSH indicators and service ladders, although it is too soon to conduct a full impact assessment.

## 1287

### THE ROLE OF GENDER INEQUITY IN COMMUNITY-LEVEL SOCIAL ORGANIZATION AND REDUCED ENTERIC INFECTION IN RURAL, COASTAL ECUADOR

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Despite dramatic reductions in childhood mortality in the past decade, diarrhea remains a major cause of preventable childhood deaths worldwide. Well known measures to prevent diarrheal infection include good water, sanitation, and hygiene (WASH) practices. These behavioral practices, however, are influenced by a multitude of factors, including social organization. Women experience a continual tradeoff in daily tasks, particularly in low-resource settings, and play a unique role in influencing community-level social organization. Previous studies conducted on coastal Ecuadorian population have identified that a greater density of social ties between individuals in remote communities may lead to the spread of WASH practices, both individual and collective, and reduced diarrheal disease, however the role of gender was not examined. This study identifies how gender roles at the individual- and community-level are related to social organization in the context of WASH in 18 communities in rural, coastal Ecuador. From August to November 2016, we conducted in-depth interviews with men and women (5 per gender), and 4 focus groups in each community. Focus groups were purposively conducted with community leaders, adult men, adult women, and youth (13-18 years of age). Using a grounded theory approach, the study team transcribed, coded, and discussed interviews for a thematic analysis. We found that women experience a distinct set of stressors, largely, environmental, social, and sexual, and men play a critical role in creating gender equity within communities. Communities with higher agency amongst women, experience high social organization. Furthermore, both men and women identify water insecurity as a primary stress and deterrent to social organization. The intensity of the stressors experienced by women were modified by ethnicity, living environment, and access to a natural water source. This study has the potential to inform context-specific and gender-sensitive interventions.

## 1288

### THE MODERATING EFFECT OF SOCIAL CAPITAL ON WATER AND SANITATION RELATED ADVERSE PREGNANCY OUTCOMES

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Recent research found that women in rural India who open defecate or who bathe in public surface waters experience higher levels of preterm birth and low birth weight outcomes than women who use a latrine or bathe with an improved water source. Pregnancy outcomes could be caused by the daily physical stress of fetching water or leaving the home to seek a safe, private location to defecate and bathe. Addressing hygiene needs in public places also exposes women to social stressors, which may be more common in communities with high crime or harassment of women, or low social cohesion or social efficacy. This retrospective cohort study examined the effect of social capital on the association between water and sanitation access and pregnancy outcomes in 6,453 women who gave birth to a child between 2004 and 2011 waves of the India Human Development Survey. The prevalence of preterm birth or low

birth weight infancy was 14.2% and 15.0%, respectively. Unadjusted logistic regression analysis found that open defecation (vs private latrine use) was associated with preterm and low birth weight, sharing an in-building latrine was associated with preterm birth, and spending > 2 hours per day fetching water (vs <2 hours) was associated with low birth weight. Improved water access was not associated with either outcome. In fully adjusted models, fatigue and anemia accounted for the observed associations between open defecation and in-building shared latrines with preterm birth, but public latrine use remained negatively associated with preterm birth (Odds Ratio (OR)=0.16; 95% Confidence Interval (CI)=0.08, 0.33). This may be due to skewed levels of wealth among public latrine users. Of social factors, harassment of women was associated with preterm birth (OR=1.70; CI=1.39, 2.07) and low birth weight (OR=1.24; 95% CI=1.02, 1.51). The effect of harassment of women on adverse pregnancy outcomes in Indian women suggests stress-moderated pathways. Anemia and fatigue-mediated sanitation effects on preterm birth could reflect stress or helminth infection pathways. More research on how social factors and water and sanitation access affects pregnancy outcomes is needed.

## 1289

### SOAP ON A ROPE HALL PASS: A RANDOMIZED CONTROLLED TRIAL OF A DISRUPTIVE CUE TO IMPROVE HANDWASHING BEHAVIOR IN NAMWALA DISTRICT, ZAMBIA

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The Zambian Ministry of General Education has made strides in improving sanitation across schools, which is known to improve student health, decrease absenteeism and improve learning. Extensive work has been done to reshape policies and guidelines for school water, sanitation and hygiene (WASH). However, access to handwashing facilities and behaviours continue to lag. Several partners in the Zambian WASH sector have used a variety of interventions in schools to improve handwashing, including the provision of handwashing stations. We are conducting a school-randomized trial to evaluate the effectiveness of disruptive cues to improve observed handwashing among primary school-aged children in Zambia. The disruptive cue in this evaluation is a bar of anti-bacterial soap threaded with a piece of rope to be used as a hall pass as students leave the classroom to use pit-latrines. The primary outcome for the evaluation to determine the effectiveness of the trial intervention will be the proportion of school-aged children that wash their hands upon exit from the latrine. This outcome will be measured through latrine-exit observation by an unobtrusive observer at three time points: pre-intervention, 1-month post-intervention, and 3 months' post-intervention for both intervention and control groups. Baseline data collection is scheduled to occur in May, 2017, with the intervention occurring shortly thereafter. Follow-up data collection will occur in June and August 2017, with final analyses conducted by October 2017.

## 1290

### IMPACT OF IMPROVED WATER AND SANITATION PRACTICES ON DIARRHEAL INCIDENCE IN CHILDREN <5 IN A MOUNTAINOUS NORTHEAST PAKISTANI VILLAGE

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From 1989-1996, diarrhea was the second highest cause of childhood mortality in Oshikhandass, Gilgit-Baltistan, where the main water source was untreated glacier melt and toilet facility the traditional pit latrine

(*chukan*). Interventions (water filtration plants, composting pit-latrines, flush-toilets) were introduced from 1996-2002. From 2011-2014, a socioeconomic household (HH) survey (SES) on toilet type and water source and treatment was conducted, and diarrhea incidence re-assessed. Lady Health Workers (LHWs) trained in diarrhea management (WHO-IMCI) did weekly surveillance of children <5 years. LHWs classified diarrheal episodes by severity, gave treatment (ORS, zinc, antibiotics if bloody) or referred, and followed up until recovery. LHWs and social scientists collected SES data on water and sanitation practices. Improved water use meant use of a functional water filtration plant or mineral water and water treatment (boiling, chlorination). Sanitation improvements meant use of flush toilets or new pit latrines. In 1989-96, incidence of diarrhea was 65 episodes/100 child years; in 2011-14, incidence was similar at 68. Diarrheal incidence was lower in households with only new toilets than in those with old toilets (83 vs. 105, Incidence rate ratio 0.8, 95% CI 0.7-.9). Incidence was lower in households using new water sources than in those with old sources (73 vs. 97, incidence rate ratio 0.75, 95% CI 0.66-.86). Treatment of water by boiling or chlorination made no difference to incidence of diarrhea in HH using either old or new water sources. Households that used new toilets had significantly lower diarrheal incidence compared to households with old ones. Households that used new water sources also had significantly lower diarrheal incidence, while treatment of water was not found to have a protective effect. This implies that functional water filtration plants have greater impact on diarrheal incidence than water treatment at home. This study will inform policymakers and NGOs to implement improved community-based water and sanitation practices to include water filtration plants and flush toilets/new pit latrines.

## 1291

### A QUALITATIVE ASSESSMENT OF MOTIVATORS AND BARRIERS TO HANDWASHING BEHAVIORS IN AN EMERGENCY SETTING IN NORTH KIVU, DEMOCRATIC REPUBLIC OF CONGO

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Diarrhea and acute respiratory infections (ARI) account for about 30% of deaths among children displaced due to humanitarian emergencies. A wealth of evidence supports the positive impact of handwashing promotion for prevention of both diarrhea and ARI. While socially and emotionally driven factors are proven motivators to handwashing in non-emergency situations, little is known about how to improve handwashing behavior in emergency settings. To identify motivators and barriers to handwashing, we conducted a qualitative investigation from June to August 2015 in a camp for internally displaced persons with a population of 6360 in eastern Democratic Republic of Congo. We held key informant interviews with 9 NGO and camp officials, in-depth interviews with 18 mothers of children <5 years, and 4 group discussions with camp residents. Handwashing using either soap or ash was observed to occur after 10% of latrine use events. Messages to promote handwashing were focused on disease transmission and prevention and disseminated regularly since the camp opened. Hardware for communal and home handwashing stations was reported to be old, unavailable or culturally unacceptable. Residents who engaged in day labor outside the camp had limited exposure to hygiene messages and handwashing technology. Mother-respondents identified health and illness prevention as the primary reason for handwashing. Further probing elicited emotionally and socially related motivators, including the desire to maintain a clean appearance and fresh smelling hands, which respondents stated evoked pride and status in the community. Respondents also alluded to social pressure to

follow hygiene practices recommended in the camp. A substantial deficit in handwashing practices with soap highlights the need to improve handwashing promotion strategies. The focus on health-related motivators and failure to use emotive and social drivers for handwashing may have led to missed opportunities of communication approaches shown to be effective in other settings. Findings illuminate that it is essential to develop and maintain culturally relevant handwashing strategies.

## 1292

### EVALUATION OF COMMUNITY-DERIVED ECOLOGICAL SANITATION TECHNOLOGY IN THE PERUVIAN AMAZON

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Ecological sanitation (EcoSan) offers a promising alternative to pit latrines in low-resource settings that lack options for the safe disposal of human waste. EcoSan manages waste by reducing human exposure to infectious pathogens while recycling valuable nutrients back into the environment as fertilizer. The overall goal of this longitudinal study is to assess the effectiveness of a community-based compost toilet system in the Peruvian Amazon implemented by a local non-governmental organization, *Instituto de Formación de Adolescentes y Niños Trabajadores* (INFANT). This system utilizes a small network of toilets located in community centers to collect human waste mixed with sawdust in sturdy bags, which are subsequently transported to a centralized, protected storage structure to facilitate pathogen attenuation. To reduce pathogens, bags are stored statically for 6 months and then transferred to an actively-mixed compost heap with waste vegetation for 6 additional months. Our specific aims are: 1) to survey pathogen reduction of waste collected from toilets already deployed in the field over the six-month static storage period, and 2) to model the static bags using controlled experimental conditions to determine the factors affecting pathogen reduction. To achieve these aims, we have taken monthly samples from field bags (n=5) from INFANT's compost toilets, as well as from model bags (n=10) that were created using defined amounts of inputs of stool, sawdust, and urine. Pathogen reduction was estimated using most probable number analysis of indicator organisms, total coliform and *E. coli*. At each sampling event, temperature, weight, pH, and field observations were also measured. Preliminary results indicate that the six-month static storage period resulted in pathogen reduction through a combination of desiccation and anti-microbial action of the sawdust amendment. Data from the controlled study component suggest that modifying the compost toilet design to utilize a urine diversion system may improve compost potential and reduce environmental contamination via leachate.

## 1293

### KNOWLEDGE, ATTITUDES AND PRACTICES (KAP) RELATED TO DIARRHEA IN A RURAL CARIBBEAN POPULATION WITH POOR WATER, SANITATION AND HYGIENE CONDITIONS

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Water, sanitation and hygiene (WaSH) conditions are closely linked with the presence of several infectious diseases that increase the mortality rates and health expenditures globally. Each year about 842,000 diarrhea deaths were estimated to be caused by poor WaSH conditions. Rural areas are more vulnerable to acquire these types of diseases, thus the assessment of the knowledge, attitudes and practices (KAP) represent an important point to cope it. In this sense, it was carried out a study in Barú village, a rural Colombian Caribbean community where WaSH indicators and KAP regarding diarrhea were evaluated. A total 124 dwellings were included. This community showed poor WaSH conditions: none dwelling presented water supply, instead this was obtained through adapted tanker ship (inadequate for human consumption). The community also have not

sewage system. Most of household used septic tank system 71.2% (n=89), the other 28.8% (n=35) use alternatives as throwing sewage into open field. About KAP, 73.4% (n=91) of respondents reported at least one episode during the previous year; "children" were reported as the family members who suffers episodes most frequently (36.3%, n=45), followed by "mother" (23.4%, n=29), and "father" (21.8%, n=27), the remaining 18.5% (n=23) corresponded to "grandfather/mother", "other adults" or "other children". Concerning, knowledge about causes of diarrhea, food poisoning was identified as the most frequent suspected etiology (25%, n=31), followed by unsafe water (16.1%, n=20), only 9.7% (n=12) of answer corresponded to intestinal parasite infections. 17.7% (n=22) of subjects mentioned unknowing the cause, and the remaining 25.8% (n=32) reported other diverse causes. Hydration was the most frequently treatment reported (43.6%, n=96). 20.9% (n=46) of families commonly employ antibiotics as the main treatment against the disease. WaSH conditions represent an important point in the improvement of health conditions in vulnerable populations. This community present not only poor WaSH conditions but also have high risk of present diarrhea cases due to insufficient knowledge about the disease.

## 1294

### A NON-KELCH13 MOLECULAR MARKER OF ARTEMISININ RESISTANCE IDENTIFIED BY IN VITRO SELECTION OF RECENTLY-ADAPTED WEST AFRICAN *PLASMODIUM FALCIPARUM* ISOLATES

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Drug resistance is one of the most pervasive challenges for malaria control, and the recent emergence and spread of artemisinin resistance in Southeast Asia is cause for serious concern. Identifying the genetic determinants of artemisinin resistance in *Plasmodium falciparum* is crucial for understanding this phenomenon and tracking the spread of resistant parasites. In 2011, prior to publication of the kelch13 resistance marker by Ariey and colleagues, we began *in vitro* selection experiments to identify the genetic determinants of artemisinin resistance. Here we present the characterization of three independently generated artemisinin-resistant parasite lines, selected on a background of recently culture-adapted Senegalese isolates. Using intermittent and step-wise *in vitro* selection methods, we pulsed the selections with steadily increasing concentrations of dihydro-artemisinin (DHA) for four years. Selected lines Pikine A, Pikine B, and Thies A all show a significant increase in their 0-3hour Ring Stage Survival Assay (RSA) survival percentage (6%, 7.9%, and 9.6%, respectively) as compared to their sensitive parents (0-3hRSA < 1%). These same lines show no change in their EC50 response to DHA, artemisinin, or other antimalarials in a 72-hour dose-response assay. Propeller domain kelch13 mutations were not found in the selected lines, suggesting a non-kelch13 mechanism of resistance. Whole genome sequencing identified mutations in Pf coronin (PF3D7\_1251200) that were associated with artemisinin resistance in all three selected lines. Functional evaluation using CRISPR-Cas9 gene editing will confirm the role of these candidate mutations in conferring artemisinin resistance *in vitro*. This study identifies additional molecular markers of artemisinin resistance, increases our understanding of how this resistance is acquired, and will provide insights into the molecular mechanism of artemisinin resistance in *P. falciparum*.

## 1295

### PHENOTYPIC CHANGES AND DRUG SENSITIVITY ASSOCIATED WITH AN *ATG18* MUTATION IN *PLASMODIUM FALCIPARUM*

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Delayed parasite clearance half-life for *Plasmodium falciparum* parasites treated with artemisinin derivatives was first reported in 2007 in Southeast Asia. This artemisinin resistance has been partly attributed to single nucleotide polymorphisms (SNPs) in the *KELCH* gene; however, these mutations occur on a genetic background of mutations in other genes. SNPs in autophagy related gene 18 (*ATG18*) that are associated with artemisinin delayed parasite clearance half-life have been found independently in Cambodia (T38I) and along the China/Myanmar border (T38N). Given that the Atg18 protein binds to PI3P in other organisms and that PI3P is thought to be involved in artemisinin resistance, we decided to investigate the contribution of a SNP in *ATG18*. Using CRISPR-Cas9 editing, we added the T38I SNP to a Dd2 laboratory line with a *KELCH* mutation (R539T). We intend to compare phenotypic changes in the *ATG18* T38I line vs. *ATG18* WT line. Further, we will determine the relative sensitivity of these lines to artemisinin derivatives using the ring survival assay as well as their sensitivity against an FDA drug library using IC50 assays.

## 1296

### MULTIPLEX COMPETITIVE GROWTH ASSAYS FOR MEASURING THE BIOLOGICAL IMPACT OF FITNESS IN DRUG-RESISTANT *PLASMODIUM FALCIPARUM*

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The repeated emergence of drug resistance in *Plasmodium falciparum* underscores the importance of understanding the genetic architecture of current resistance pathways, as well as any associated fitness costs. Parasite fitness is likely to play a key role in driving the epidemiology of drug resistance, but it is infrequently studied in a laboratory setting. Competitive head-to-head assays comparing fitness between strains are typically laborious and limited in throughput, and suffer from issues when comparing growth between strains growing in different wells or flasks. We present a high-throughput method to profile fitness of multiple parasite lines in parallel in a single flask using barcode sequencing. CRISPR-Cas9 genome editing was used to insert a unique barcode into multiple parasite lines of different genetic backgrounds that possess well-described sensitivities to several standard antimalarials. These barcoded lines were then mixed together and grown in a single culture, with relative growth rates in the presence and absence of drug accurately quantified by next-generation sequencing of the barcodes. We are now using this method to study the effects of parasite background on fitness in artemisinin resistant strains isolated from Southeast Asia, using genome editing on these parasites to examine the importance of specific alleles of candidate genes. Overall, we describe a new approach that combines CRISPR-Cas9 editing and next-generation sequencing to address the biological impact of fitness in multiplex competitive growth assays.

## 1297

### A MALARIA GENETIC CROSS GENERATED IN A HUMANIZED MOUSE INDICATE MULTI-GENE CONTROL OF RESISTANCES TO ARTEMISININ AND PIPERAQUINE

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Repeated emergence of drug resistant parasites in response to antimalarial drugs has rendered many drugs less effective, including chloroquine and mefloquine. Artemisinin (ART) and its derivatives are the last line of defense against multi-drug resistant malaria. The slow clearance rate of parasites in response to treatment with ART and its derivatives is of grave concern. Resistance to partner drugs of ART, such as piperazine, is also emerging, raising concerns for the future of ART combination therapies (ACTs). To preserve the efficacy of ACTs there is an urgent need to determine the genetic causes of resistance and understand the mechanism by which resistance has developed. Quantitative genetics techniques, such as quantitative trait loci (QTL) mapping in *Plasmodium falciparum* experimental genetic crosses, have been successful in defining resistance mechanisms but past genetic crosses are not relevant for these recently emerged resistances. We analyzed 33 progeny from a recently developed genetic cross between an ART resistant clone with no mutations in the coding regions of K13 (PF3D7\_1343700), the putative resistance gene, and an ART sensitive clone that was generated in a humanized mouse model. Proof of concept QTL mapping using chloroquine phenotype data produces a strong peak on chromosome 7 coinciding with the *pfcr* locus, validating our genetic mapping approach. Dihydroartemisinin, the active metabolite of ART derivatives, was analyzed for both IC50 results and the ring stage survival assay (RSA). QTL mapping of RSA data identifies a suggestive statistical peak on chromosome 14, not linked to the well-known chromosome 13 locus carrying K13. Piperazine and mefloquine, both drugs used in ACTs were also mapped using IC50 data resulting in peaks on a locus containing plasmepsins II (PF3D7\_1408000) and III (PF3D7\_1408100). Interestingly, we find no evidence for copy number changes in these plasmepsins between the two parents. This work highlights biological and evolutionary implications of previously unidentified loci responsible for ART and piperazine resistance.

## 1298

### ISOLATION OF PIPERAQUINE RESISTANT RODENT MALARIA PARASITE FROM MUTATOR MALARIA

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While antimalarial drugs have been employed in prevention and treatment of malaria, emergence of drug resistant parasites remains a major issue for the achievement of malaria elimination. Defining the molecular mechanisms of drug resistance is crucial for designing new drug and prevention of emergence and spread of drug resistant parasites. However, molecular dissection of parasite drug resistance is hindered by scarcity of available genetic tools for Plasmodia. We developed a transgenic *P. berghei* parasite, malaria mutator, which shows over 80 times higher mutation rate than wild type parasite because of an artificially lack of proof reading activity of DNA polymerase. It is anticipated that the malaria mutator produces many mutants with various traits including drug resistance and thus their analysis would widen our knowledge for the mechanisms of parasite drug resistance. Here we provide a proof of concept by showing that resistant parasite to piperazine (PQ), one of the partner drugs

for artemisinin-based combination therapies, was quickly isolated from malaria mutator. Whole-genome sequence analysis revealed several SNPs shared among PQ-resistant parasite clones, which included a novel type of mutation in *Pbcrt* gene. An introduction of this mutation into the *Pbcrt* gene by CRISPR/CAS9 technology conferred PQ resistance to a susceptible parasite. We will discuss PbcRT-mediated PQ resistance and provide our view for malaria mutator as a novel research tool of drug resistance.

## 1299

### CURRENT STATE OF MALARONE RESISTANCE IN CAMBODIA AND ITS IMPLICATIONS ON THE TREATMENT OF *PLASMODIUM FALCIPARUM* IN SOUTHEAST ASIA

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Atovaquone-proguanil (AP) is an attractive treatment option for malaria in Southeast Asia as it is generally well-tolerated and effective against *Plasmodium falciparum* resistant strains. However, there are concerns over emergence of atovaquone resistance during therapy. We have analyzed atovaquone and cycloguanil markers of resistance in parasite isolates from 205 patients with uncomplicated *P. falciparum* or mixed *P.falciparum/P. vivax* malaria infections who participated in a therapeutic efficacy study of AP with or without oral artesunate (AS) at two sites in Cambodia. At enrollment, all 205 samples samples (157 from Anlong Veng and 48 from the Kratie site) evaluated at the *Pf* cytochrome b (*cytb*) Y268 locus by Sanger sequencing were wild type. Of 14 *P.falciparum* recrudescences, only one carried the Y268C mutation from volunteer at the Anlong Veng site, treated with AP without AS, whose parasite clearance time was 64 hours and recrudescence parasitemia on day 36 days following treatment. Amplicon deep sequencing targeting *cytb* confirmed the presence of the Y268C mutation in 99.6% of the sequence reads at recrudescence, but did not detect the mutation pre-treatment or 24 hours into treatment, even at a minor allele frequency down to 0.25% (33,267 and 6,047 reads at D0 and D1). This suggests de novo development of atovaquone resistance through treatment rather than expansion of a pre-existing parasite subpopulation. All isolates from the Anlong Veng and Kratie sites had normal atovaquone IC50 but with significant elevation of the cycloquanyl IC50 (2,987 nM, 95% CI = 2415 – 3559) in the 44 isolates tested to date, consistent with high prevalence of DHFR mutations which may have contributed to treatment failures. There was no efficacy benefit from adding AS and nearly all isolates carried K13 mutations. Analysis of pharmacokinetics data and its contribution to treatment failures will also be presented. Deployment of AP under triple combination therapy may be a more viable option against MDR parasites, but clinical efficacy studies are needed to identify safe and effective drug combinations without cross resistance between partner drugs.

## 1300

### TRANSCRIPTIONAL RESPONSE OF *PLASMODIUM VIVAX* PARASITES TO CHLOROQUINE IN VIVO

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Resistance to antimalarial drugs is challenging the effort to eradicate malaria worldwide. This issue is particularly problematic for *Plasmodium vivax*; since this parasite cannot be cultured *in vitro*, the mechanisms of resistance remain largely unknown. Here, we used RNA-seq to characterize the transcriptional changes in *P. vivax* parasites from Cambodian vivax malaria patients following chloroquine (CQ) treatment. Using stranded RNA-seq, we generated 3-29 million paired-end reads mapped to the *P. vivax* genome (10-43% of the total reads) before treatment. Eight hours after CQ treatment, the proportion of reads mapping to the *P. vivax* genome was dramatically reduced in all patients, with an average reduction of 71.0%, 95% CI [65.8-76.2]. Using paired analyses, we identified 570 genes that were significantly differentially expressed post CQ treatment (FDR<0.01). Heat-shock proteins and genes associated with gametocytes were induced after CQ treatment, while vir genes, tryptophan-rich antigens, and RNA-binding proteins were downregulated. PvCRT (chloroquine resistance transporter), which has been implicated in CQ resistance, showed decreased expression following treatment (q-value=0.004). Additionally, we observed variable extent of retention of the 9th intron of PvCRT: the parasites that retained this intron were more susceptible to CQ than parasites with complete splicing (though all infections were successfully cleared after 72 hours). Our study shows that rigorous gene expression profiles can be generated directly from vivax malaria patients without short-term culture or other processing. Our findings highlight the diversity and complexity of parasite gene expression and provide a unique perspective on the molecular response of the parasite to antimalarials *in vivo*. By investigating changes in gene expression, splice isoforms, and noncoding RNAs, we will better understand how this parasite responds to antimalarial drugs and potentially develops resistance.

## 1301

### ESTIMATING HEALTH IMPACT OF RAPID DIAGNOSTIC TESTS FOR MALARIA

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Scaling up access to diagnostic testing is a cornerstone of malaria control policy. Rapid Diagnostic Tests (RDTs) for malaria have a potential of providing universal access to highly specific malaria diagnostic services. The potential health impact of RDTs for malaria however, is determined by a multitude of factors including disease epidemiology, natural history, care-seeking patterns and patient and provider adherence to the test results. We construct a decision model that explicitly parameterizes these epidemiological and behavioral factors to estimate the impact and effectiveness of scaling up RDTs, and perform global sensitivity analysis to evaluate the relative importance of these factors in modulating RDT impact and effectiveness in malaria endemic countries. We find that compliance to disease diagnosis, uptake of RDTs by providers and patients during scale up, co-incidence of malaria and bacterial pneumonia, and the overall effectiveness of Artemisinin Combination Therapies (the first line treatment for malaria) are the most important factors influencing the impact and effectiveness of RDTs on under-five malaria and pneumonia burden in 24 malaria endemic Sub-Saharan African countries. Further, we find that scaling up RDTs in public and private health facilities in malaria endemic countries with moderate RDT uptake and compliance to test results and medication could increase appropriate treatment of malaria and pneumonia by 20 million cases, which would avert 56,000 under-five deaths, or 1.5 million DALYs annually.

## 1302

### EVIDENCE OF CHANGING CASE MANAGEMENT BEHAVIORS FOLLOWING AN INTERVENTION TO INTRODUCE MALARIA RAPID DIAGNOSTIC TESTS TO PRIVATE PHARMACIES IN KINSHASA

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DRC DFID Abstract ASTMH 2017 Fever case management in Kinshasa centres on the private sector, where 70% of childhood fevers seek care. While diagnostic testing is available in clinics, RDT services have not historically been provided in private pharmacies or drug shops. As drug shops had 81% antimalarial market share in 2015 this leaves a critical gap in testing access. A policy change in November 2015 permitted RDTs to be used in pharmacies with qualified pharmacists nationwide, expanding possible testing channels. We conducted pre- (Oct 2015) and post- (Dec 2016) exit interview and post-only mystery client surveys to evaluate an intervention training pharmacists and supplying free RDTs after the policy change. Eligible exit interview clients were adults seeking treatment for fever for themselves or on behalf of someone else. We captured information on previous sources of care, diagnostic services and medicines received or prescribed during the current visit. Brand names were recorded from packaging when available. In 2016, confirmed RDT-negative mystery clients with no reported fever in the past four weeks were additionally recruited to assess provider adherence to algorithms for test-negative patients. Clients bought all medicines prescribed and data were captured shortly after exiting the pharmacy. 421 clients from 44 pharmacies were interviewed in 2105, and 398 from 83 sites in 2016. Reported mRDT testing increased from 0% in 2015 to 32% in 2016. In 2015 under presumptive treatment, 41% of clients received any antimalarial and 7% received an ACT. In 2016, 79% of positive clients received an ACT; presumptive antimalarial use was stable (38%). 127 mystery client visits were conducted at 64 pharmacies in 2016. 98% clients were tested for malaria. 32% of clients were told they were given a positive diagnosis and all mystery clients receiving a negative diagnosis went on to be prescribed an antimalarial. These data suggest mRDTs have been keenly adopted by pharmacists. However, prescribing practices for presumptive treatment appear slower to change and there is more work to be done to adhere to negative test results and ensure correct treatment.

## 1303

### IMPACT OF A MALARIA RAPID DIAGNOSTIC TEST DETECTING PLASMODIUM FALCIPARUM-SPECIFIC HISTIDINE-RICH PROTEIN-2 (RDT-PfHRP2) ON THE MANAGEMENT OF FEBRILE CHILDREN UNDER-5 YEARS OF AGE IN A HIGH SEASONAL MALARIA TRANSMISSION AREA

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The WHO recommends to screen febrile patients by microscopy or malaria rapid diagnostic test such as *Plasmodium falciparum* histidine-rich protein-2 (RDT-PfHRP2) before start of treatment. But antigen persistence after successful antimalarial treatment can affect the performance of this RDT and may result in missing true cause of fever in a malaria endemic area. The aim of our study was to determine the impact of RDT-PfHRP2 on the management of other causes of fever in children under-5 years of age. A prospective etiology study was conducted in 2015 among febrile children under 5 years (axillary temperature  $\geq 37.5^{\circ}\text{C}$ ) in the Nanoro Health District (Burkina Faso). In order to assess the impact of RDT-PfHRP2 testing

on the management of febrile children, the malaria RDT was performed at health facility levels and the matching blood smear was independently assessed by microscopy at the laboratory of the Clinical Research Unit of Nanoro. Additional blood, urine and stool samples were collected to perform bacterial and viral testing in the laboratory. In total 683 blood samples were analyzed with microscopy and RDT-PfHRP2. *Plasmodium falciparum* malaria was diagnosed in 49.78% cases by microscopy compared to 69.55% by RDT-PfHRP2. The RDT-PfHRP2 reported 29.68% (141/475) false positive and 1.76% (6/340) of false negative cases. The RDT-PfHRP2 had a high sensitivity (98.2%) and NPV (97.1%), but a low specificity (58.9%) and PPV (70.3%). Almost 50% of alternative cause of fever were diagnosed by laboratory testing in the RDT false positive malaria group. The corrected accuracy of the RDT by removing all alternative cause of fever diagnosed and children with 2 weeks' malaria treatment antecedent did not affect the sensitivity (98.2%) and NPV (96.4%) but improve the specificity (63.0%) and the PPV (77.22%) of RDT-PfHRP2. The use of a malaria RDT-PfHRP2 in a malaria endemic area may cause misdiagnosis of the actual cause of fever due to false positive test results. The development of practical diagnostic tool to screen for other causes of fever in malaria endemic areas is required to save lives and to improve the performance of the RDT.

## 1304

### ASSESSING THE FIELD SENSITIVITY OF MALARIA ANTIGEN DETECTION TESTS USING AN ULTRA-SENSITIVE BEAD-BASED ASSAY

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The large-scale introduction of antigen detection tests has revolutionized malaria diagnosis in malaria-endemic countries, allowing for the change in policy to universal confirmation of suspected malaria cases. In 2015, over 70% of malaria testing in sub-Saharan Africa was done using rapid diagnostic tests (RDTs), most of which detect the presence of the *Plasmodium falciparum* histidine rich protein 2 (HRP2). Several RDT manufacturers are developing highly sensitive RDTs (hsRDTs), which promise a limit of detection (LOD) orders of magnitude lower than conventional RDTs. However, it is not known how sensitive current RDTs are in field circumstances, as there is no single standard HRP2 limit of detection (LOD) for malaria RDT product testing. The HRP2 concentration in samples from patients attending outpatient clinics in one high- and one low-transmission province in Angola was measured quantitatively using a recently-developed ultrasensitive bead-based assay, capable of detecting HRP2 concentration at low picogram levels. Samples were analyzed from patients presenting with and without febrile illness. The number of patients with HRP2 concentration above the LOD for a conventional RDT was compared to the number of patients HRP2-positive by the ultrasensitive assay. The distribution of HRP2 concentrations was bimodal in both provinces in both afebrile and febrile patients. Conventional RDTs detected 81% of all HRP2-positive febrile patients and 52-77% of HRP2-positive afebrile patients. The added utility of an hsRDT would be greater in afebrile patients, where an hsRDT with a LOD of 200pg/mL would detect an additional 40-50% of HRP2-positive persons compared to a conventional RDT with a LOD of 1000pg/mL. In febrile patients, the hsRDT would detect an additional 10-20% of cases. The added utility of a hypothetical hsRDT in outpatients did not vary by transmission setting. Conventional RDTs already capture over 80% of symptomatic HRP2-positive individuals, regardless of underlying transmission intensity. The LOD of an hsRDT would need to approach 200 pg/mL to provide a substantial benefit over conventional RDTs in this setting.

### PREVALENCE AND OUTCOMES OF *PLASMODIUM FALCIPARUM* INFECTIONS DETECTED ONLY BY ULTRA-SENSITIVE PCR IN SCHOOL CHILDREN IN SOUTHERN MALAWI

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New ultra-sensitive PCR (us-PCR) techniques can detect *Plasmodium falciparum* infections below the limit of detection (LOD) of traditional molecular methods. In areas where elimination is considered, identifying these infections is important because low density infections are common and may help perpetuate transmission. However, the utility of these more labor-intensive methods is not clear in higher transmission settings. We tested specimens from a primary school-based study to evaluate the impact of using us-PCR to estimate infection prevalence in a high transmission setting. Students were interviewed about symptoms and treatment, axillary temperature was measured, and samples were collected for microscopy, PCR, rapid diagnostic test (RDT) and hemoglobin at baseline and after 1, 2 and 6 weeks. Our standard real time PCR detected *Pf*LDH with a LOD ~2.6 parasites/ $\mu$ l and our us-PCR, targeting *Pf*18S ribosomal DNA and RNA, had a LOD of ~0.02 parasites/ $\mu$ l. We used logistic regression adjusting for school and sample weighting to compare outcomes between students who at baseline had infections detected only by us-PCR and uninfected students. Infection prevalence was estimated as 39% using traditional PCR vs. 49% using us-PCR. The proportion of infections detected only by us-PCR was inversely correlated with prevalence by traditional PCR, i.e. the highest proportions of us-PCR infections were in the lowest prevalence schools. Infections detected only by us-PCR were not associated with fever, treatment or anemia at the time of the evaluation, or in the subsequent six weeks. However, students with us-PCR-detectable infections at baseline were more likely to have infections detected by RDT, microscopy, and traditional PCR during follow-up than were students with no infection detected at baseline. Thus, using us-PCR did not substantially alter our understanding of the epidemiology of infection in this population. Infections that are below the LOD of standard PCR would likely be detected by subsequent sampling. Our results do not suggest a need for ultra-sensitive techniques to detect infection in high-burden settings.

### SENSITIVITY COMPARISONS AMONG MOLECULAR DIAGNOSTIC TOOLS FOR MALARIA DIAGNOSIS REQUIRED FOR MALARIA ELIMINATION IN MADAGASCAR

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Sensitive and accurate diagnosis is required for malaria elimination. Facing the limits of detection of microscopy and RDT, PCR assays have been developed showing a variable level of sensitivity depending on the methodology and targeted gene. The 18S RNA gene (18S) PCR

assay remains the standard for malaria diagnosis. However, the 18S PCR is time-consuming and labour intensive making its usage difficult for high-volume sample processing. Our aim here was to design the most sensitive PCR diagnostic assay for use at the Madagascar NMCP by targeting the cytochrome b (cytb) gene that presents 40 to 100 copies more than 18S. For this we compared the cytb approach with two PCR methods: the Snounou-18S nested PCR and the 18S-based LDR-FDA multiplex assay. 200 samples were processed from Ankililoaka commune in southwest Madagascar collected in April 2016 after seasonal malaria chemoprevention. 18S and cytb PCR assays were performed in the NMCP laboratory; blinded assessment of this comparison was performed by LDR-FDA at Case Western Reserve University. Results showed that 18S PCR detected a prevalence of 18.5% while cytb PCR detected a prevalence of 23% with 5 *P. falciparum* (Pf) and 2 *P. vivax* (Pv) concordant samples. 6 samples were Pv positive with 18S PCR while 22 were diagnosed as Pv by cytb PCR. No mixed infections were detected by 18S PCR while 4 were detected by cytb PCR. Cytb PCR showed a full concordance with Pf positive microscopic samples whereas 18S PCR detected only 25% of them. Among 133 samples negative by RDT and microscopy, 18S and cytb PCR assays detected *Plasmodium* in 24 and 32 samples, respectively, indicative of submicroscopic infections. In contrast to other published studies the cytb PCR assay achieved better sensitivity than the 18S assay. Results of the LDR-FDA diagnosis of these 200 samples allow an independent evaluation of both the cytb and 18S PCR assays. Implementation of sensitive PCR assays is crucial for epidemiology studies and accurate surveillance for supporting the elaboration of appropriate strategies towards malaria elimination in Madagascar.

### REAL-TIME DETECTION OF DEFECTIVE MRDTS IN THE FIELD: THE APPLICATION OF A SURVEILLANCE-RESPONSE SYSTEM FOR POST-MARKET SURVEILLANCE OF DIAGNOSTICS

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It is estimated that >350 million malaria RDTs were used in 2015 and utilization continues to increase. Malaria RDTs are primarily procured by NGOs, aid agencies and governments to implement the WHO recommendation for treatment of parasite confirmed cases. Selection of RDTs have been guided by FIND's RDT lab evaluations. However, RDTs are primarily used in remote areas where information about the RDT quality and performance is limited, and no system for post-marketing surveillance exists. Fionet (Fio Corp, Toronto) has been designed to strengthen quality control both directly at the user interface in facilities and remotely through a web-based portal. Fionet includes a mobile device that performs automated interpretation of RDTs, captures a high-resolution image of RDTs and collects patient data. Information is summarized in portal reports that can be monitored in real-time. Quality features of the system include immediate feedback to the user on processing errors, and portal reports on cumulative errors stratified by facility, user, type of error and time. In June 2016, a deployment of Fionet begun in 30 health facilities located in two states of Nigeria. Within two weeks, an abnormal frequency (~11%) of malaria RDTs with no control line detected was apparent. The RDT manufacturing defect was observed across multiple facilities and remained constant over subsequent months - during which >2500 malaria RDTs were processed. Notification from the portal prompted personnel to conduct site visits and confirm the RDTs came from multiple lot numbers, had not expired, and had been properly stored and processed. The manufacturer issue has been confirmed in subsequent laboratory testing. The system ensured that decision-makers, those procuring RDTs, were aware of a quality control issue occurring in remote health facilities. Subsequently, Fionet has detected this defect in two additional deployments, located in Kenya and Nigeria, with all three

deployments sourcing malaria RDTs from the same manufacturer. Post-market surveillance is crucial to ensure the quality and effectiveness of malaria RDTs in the field.

## 1308

### DRIED BLOOD SPOTS ALLOW FOR EFFICIENT, LARGESCALE SURVEYS OF HEPATITIS C VIREMIA

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Efficient viral load testing is needed for hepatitis C (HCV) surveillance and diagnosis. HCV viral load testing using dried blood spots (DBS), made with a single drop of finger-prick, whole blood on filter paper, is a promising alternative to traditional serum- or plasma-based approaches. We adapted the *m2000* instrument (Abbott Molecular, Des Plaines, IL) for high-throughput HCV viremia testing using dried blood spots with simple specimen processing and applied these methods to estimate the national burden of infection in the Democratic Republic of Congo (DRC). We tested DBS collected during the 2013-2014 DRC Demographic and Health Survey (DHS), including 1,309 adults  $\geq 40$  years of age. HCV-positive samples underwent targeted sequencing, genotyping, and phylogenetic analyses. This high-throughput screening approach reliably identified HCV RNA extracted from DBS prepared using whole blood, with a 95% limit of detection of 1196 IU/mL (95% CI 866-2,280 IU/mL) for individual 6mm punches and 494 IU/mL (95% CI 372-1,228 IU/mL) for larger 12mm punches. Fifteen infections were identified among samples from the DRC DHS; the weighted country-wide prevalence of HCV viremia was 0.9% (95% CI 0.3-1.6%) among adults  $\geq 40$  years of age and 0.7% (95% CI 0.6-0.8%) among HIV-positive subjects. All successfully genotyped cases were due to genotype 4 infection. In conclusion, DBS-based HCV testing represents a useful tool for the diagnosis and surveillance of HCV viremia. 100,000-200,000 adults  $\geq 40$  years of age in the DRC may have active infection and be eligible for treatment.

## 1309

### A NOVEL ROLE OF SCHLAFEN4 IN WEST NILE VIRUS REPLICATION AND PATHOGENESIS

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West Nile virus (WNV) is a neurotropic flavivirus that has emerged globally as a significant cause of viral encephalitis in humans. No effective therapies exist for treating individuals with WNV infection, and pathogenesis of WNV encephalitis (WNVE) is not completely understood. Addressing the fundamental questions regarding host proteins recruited by the WNV to establish infection in the primary target cells is an integrated and indispensable part of the effort towards development of effective therapeutics. Schlafen4 (SLFN4) is a poorly characterized but important member of the Schlafen family that includes several mouse and human genes. Role of SLFN4 in virus infections is yet to be determined. Herein, we demonstrate that WNV infection induces a dramatic up-regulation of SLFN4 in mouse brain, as well as in mouse primary cells, including mouse embryonic fibroblasts (MEFs), macrophages, dendritic cells, astrocytes, and neurons. Similarly, we found that WNV infection of human brain cells induced up-regulation of human orthologs of mouse SLFN4, namely SLFN12 and SLFN12L. Furthermore, knocking down of SLFN4 in MEFs resulted in significant decrease of WNV infectivity titers, suggesting that

SLFN4 is required for WNV replication. Additionally, mRNA and protein expression of WNV-induced interferons, chemokines, and cytokines were significantly decreased in SLFN4-knockdown MEFs. We next used SLFN4-deficient mice and an established murine model of WNVE to determine the role of SLFN4 in WNV disease pathogenesis. After subcutaneous inoculation with 100 plaque forming units WNV, SLFN4-deficient mice exhibited significantly higher survival percentage than wild-type mice. Increased survival in SLFN4-deficient mice was associated with significantly reduced viral burden in circulating blood compared to wild-type mice. Collectively our data for the first time indicate the novel role of SLFN4 in WNV replication and pathogenesis.

## 1310

### A CROSS-SECTIONAL STUDY OF NEUROCOGNITIVE OUTCOMES POST-WEST NILE VIRUS INFECTION

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With West Nile virus (WNV) now endemic throughout North America, with upwards of 3 million cases, it is critical to document the long-term clinical outcomes. Among a large cohort of WNV patients in Houston, Texas, we found up to 40% of those who presented with clinical disease continued to experience WNV-related morbidity up to 8 years post-infection. This percentage was up to 70% for those who initially presented with encephalitis. There is a need to understand the long-term neurological outcomes resultant of WNV infection. From 2002-2012, a total of 262 patients were enrolled as research participants in this longitudinal cohort. Of the 262 potential participants, 117 took part in neurologic and neurocognitive evaluations, with 30 of those patients receiving MRIs to evaluate cortical thinning and regional atrophy. Correlations of clinical syndrome with neurologic and neurocognitive dysfunctions were evaluated, as well as correlations of neurocognitive outcomes with MRI results. Across the entire cohort, almost half (49%; 57/117) of patients had some type of abnormal neurological exam finding, with most abnormalities being unilateral in nature. The most common abnormalities included decreased strength (26%; 30/117), abnormal reflexes (14%; 16/117), and tremors (10%; 12/117). We observed a 22% overall rate of impairment on the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS), with the domains of immediate (31%) and delayed memory (25%) being the most frequently impaired. WNV patients showed significant thinning as compared to controls in both the left and right hemispheres, primarily in the frontal and limbic lobes. Significant regional atrophy was identified in the cerebellum, brain stem, thalamus, putamen, and globus pallidus. This study provides valuable new information regarding the neurologic neurocognitive changes resultant of WNV infection and how those changes relate to cortical thinning and regional atrophy.

## 1311

### EVOLUTION OF STRUCTURAL VARIATION IN THE UNTRANSLATED REGIONS OF THE WEST NILE VIRUS GENOME

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The RNA genome of West Nile virus (WNV) is flanked by 5' and 3' untranslated regions (UTRs), that fold into structures required for replication. These structures appear to be highly conserved across WNV isolates, however analysis to date has relied on consensus sequences,

which may miss variation within the viral quasispecies. To analyze structural dynamics in WNV quasispecies, we passaged WNV99 in triplicate in 3 bird species (crow, robin, house sparrow), sampling at each passage (N=45), and subjected these populations to next-generation sequencing. Ten reads from each UTR that carried at least 1 mutation from the consensus sequence were sampled at random from each population and the impact of each mutation on structure was analyzed using Mfold. We hypothesized that: (i) mutations in double-stranded (DS) regions would be more likely to cause structure change compared to mutations in single-stranded (SS) regions, (ii) mutations would be more common in SS than DS regions, (iii) mutations producing major structural changes would be less common than those producing minor changes, and (iv) mutations producing major structural changes would be most common in robins and least common in crows, which sustain the lowest and highest viremia, respectively. Over the course of passaging, quasispecies diversity did not increase or decrease for all bird-passaged WNVs. In general, mutations in DS regions were more likely to result in structure change than mutations in SS regions. Contra hypothesis (ii), mutations were more frequent in DS than SS regions of the 3' UTR, while the distribution was not different from the expected in the 5' UTR. The frequency of mutations that produced major, minor, or no structure change was similar in both UTRs. The frequency of major structure change was not related to viremia. The 5' UTR consensus sequences for passage 5 were conserved for all three bird-passaged viruses when compared to the WNV NY99. However, the robin-passaged 3' UTR consensus sequence at passage 5, contained several substitutions that result in minimal structure change.

## 1312

### THE IMPACT OF INTRODUCTION OF JAPANESE ENCEPHALITIS VACCINE IN INDIA - THE SUCCESS STORY

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In 2005, a massive Japanese encephalitis (JE) outbreak occurred in many districts of Uttar Pradesh and Bihar, claiming the lives of more than 1,500 children. Based on requests from many states, the Government of India (GoI) decided to initiate JE vaccination campaigns in the endemic districts to protect vulnerable children from future disease outbreaks. The strategy was a one-time mass JE vaccination campaign with a single dose of live attenuated SA 14 14-2 JE vaccine for children between 1-15 years. This was to be followed by integration of JE vaccine into routine immunization in the campaign districts for the new cohort aged 16-24 months. Viewing PATH's expertise in the field of JE vaccination, the GoI requested PATH to provide technical support in planning and implementation of the JE vaccination campaigns in all the JE-endemic states of India. Hundred and four districts of 11 states were selected for JE vaccination campaigns in a span of 5 years in a phased manner. The Government of India also made efforts for strengthening the acute encephalitis syndrome (AES)/JE surveillance system and established 121 sentinel sites. Capacity of health personnel was built in all the states for lab testing and improving the surveillance system. This has contributed to more states/districts reporting AES/JE cases thereby increasing the number of endemic states to 22 and districts to 216. From 2006 to 2016, through the successful JE vaccination program, around 148 million children have been immunized and there has been a significant decline in JE positivity rate from 58% to 11.4%. Following JE vaccination in the pediatric population, an age shift has been observed amongst children above 15 and adults. This led to the decision by the Government of India to introduce JE vaccination for the age group of 16- 65 years in selected JE endemic districts. Based on evidence, the government is expanding JE vaccination to new areas. This presentation will showcase the success story and the lessons learned through JE vaccination.

## 1313

### IMPAIRING THE INFECTION PROCESS OF YELLOW FEVER VIRUS IN *Aedes aegypti* BY MANIPULATING THE MOLECULAR HINGE REGION OF THE ENVELOPE PROTEIN

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The re-emergence of yellow fever virus in Africa and Latin America since 2015 has created a significant human public health threat to tropical regions worldwide. The shortage of the live-attenuated 17D vaccine limits disease control capabilities in response to outbreaks of yellow fever and highlights the importance of global vaccine stockpiles. Whilst it is well-accepted that genetic mutations in the 17D vaccine strains are responsible for the loss of viscerotropism in humans and reduced efficiency in infection and dissemination in *Aedes aegypti*, the attenuation mechanisms caused by genetic mutations remains unknown. Without such knowledge, the rationale-based design of efficacious vaccine candidates against YFV and other related flaviviruses cannot be achieved. In this study, the attenuation of YFV in *Ae. aegypti* caused by the vaccine-associated G52R mutation, located in the molecular hinge region between the domain I and domain II of the envelope (E) protein, was evaluated. *Per os* challenge of *Ae. aegypti* was performed to determine the phenotypic difference between the wildtype and mutant viruses. The results demonstrate the contribution of the G52R mutation to the attenuation of YFV and the functional importance of the molecular hinge region of YFV E protein for the infection process in *Ae. aegypti*.

## 1314

### A PAN-VIRAL CAPTURE SEQUENCING APPROACH TO ELUCIDATE THE VIROME OF ACUTE FEVER AND ENHANCE VIRAL SURVEILLANCE IN WEST AFRICA

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Recent public health emergencies, such as those caused by Ebola and Zika viruses, have highlighted vulnerabilities in public health systems and the need to greatly expand our ability to rapidly identify and stop these threats. The 2013-16 Ebola outbreak expanded in part because many regions lacked local diagnostic capacity to rapidly detect and respond to the virus. Despite the burden of infectious disease in sub-Saharan Africa, little is known about the spectrum of viral infections responsible for disease in many regions. Recent advances in next generation sequencing (NGS) have the potential to transform viral detection and discovery by enabling unbiased pathogen detection in clinical samples. However, the low titer of virus and high human background present in many clinical samples greatly reduces the efficiency and increases the cost of this approach, posing a major challenge for the application of these technologies locally for surveillance and diagnostics. Hybrid capture techniques can address these challenges by enriching for viral nucleic acids, though this conventionally requires a priori knowledge of the viral targets. To address this, we designed and validated a pan-viral hybrid capture panel containing > 300 viral taxa known to infect humans. We show that this approach significantly improves virus detection and genome assembly in both mock and clinical samples from a panel of viruses, including viral co-infections and genetically diverse strains. We next used this pan-viral capture approach to identify viruses present in a cohort

of serum samples collected from individuals with an acute fever from Senegal. This work is the first genome sequencing-based viral surveillance effort in Senegal and demonstrates the utility of our pan-viral hybrid capture approach for this application. We will discuss the metagenomic findings from our studies.

## 1315

### WUCHERERIA BANCROFTI INFECTION IS LINKED TO SYSTEMIC ACTIVATION OF CD4 AND CD8 T CELLS

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Susceptibility to HIV has been linked to CD4+ T cell activation in cohorts of seronegative individuals with high HIV-exposure. Our group recently described an increase of HIV transmission in individuals infected with *Wuchereria bancrofti*, the causative agent for lymphatic filariasis (LF). However, the underlying reason for this phenomenon needs further investigation. Previous studies revealed an increase in HLADR/CD38 positive CD4 cells for soil transmitted helminths (STH), especially *T. trichiura* (TT) infection, but this has not yet been evaluated for *W. bancrofti*. Methods: Two-hundred and fifty HIV negative participants above 18 years of age from the Worm HIV Interaction study (WHIS) were grouped on the basis of their helminth infection status, according to Trop Bio ELISA (LF), Kato Katz and stool based RT-PCR. FACS analysis of the peripheral blood was used to measure T cell activation markers (HLA-DR, CD38), differentiation markers (CD45, CD27) regulatory markers (FoxP3, CD25) and HIV co-receptor (CCR5) expression. Results: Five groups of different helminth infection status were defined and compared: Forty-six individuals were free of helminths, 20 were infected with LF, 27 with TT and 146 participants with other STH. We noticed a significantly higher percentage of HLA-DR positive cells among CD4+ T cells in patients with LF (11.1%) and TT (10.1%) infection compared to helminth free individuals (8.0%) or participants infected with other STH (8.1%) (LF versus helminth free;  $p=0.001$  after adjustment for age and sex). Furthermore, the percentage of CCR5 expressing regulatory CD4 T cells was significantly higher in LF infected, compared to helminth free individuals (61.2% versus 53.2%,  $p=0.041$ ) or other helminths (61.2% versus 55.2%,  $p=0.049$ ). Interpretation: A higher level of CCR5 positive CD4 T regulatory cells in addition increased immune activation as demonstrated by HLADR/CD38 cells was found in LF positive individuals when compared to participants infected with other helminths. This could explain the increased susceptibility to HIV infection of *W. bancrofti* infected individuals.

## 1316

### MODULATION OF HUMAN INNATE LYMPHOID CELL FUNCTION BY IL-10 AND TGF-BETA

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Chronic human filarial infection is associated with an immune-modulatory state mediated largely by IL-10 (and to a lesser extent TGF- $\beta$ ), cytokines that alter antigen-specific effector T cell responses. These filarial infections are also associated with an expansion of innate lymphoid cell (ILC) subsets that mirror in many ways the CD4+ helper cell (Th) subsets: ILC1s (IFN- $\gamma$ ), ILC2s (IL-5 and IL-13) and ILC3s (IL-17A or IL-22). Human ILC subsets can easily be detected in the circulation accounting for only 0.21% (range 0.023-3.58%) of lymphocytes under homeostatic conditions. Despite the low frequency of ILCs in circulation, *ex vivo* experiments have demonstrated that these ILCs release extremely large per cell quantities

of cytokines following activation and, if left unchecked, these ILC-derived cytokines can have deleterious effects on the host. To explore the interface between ILC expansion and the regulation of ILC activation ILC subsets were isolated from the peripheral blood of healthy donors by flow cytometry-based sorting and activated in the presence or absence of IL-10. ILCs stimulated in the presence of IL-10 had a marked reduction (range from 2-3.6 fold) in cytokine production when compared to ILCs activated without IL-10. Similar cytokine regulation was seen with TGF- $\beta$ . To explore the mechanisms underlying the inhibition of ILC cytokine production, we examined the expression of IL-10R on ILC subsets and found that ~20-45% of each subset expressed IL-10R and did so at the same per cell intensity (GeoMFI=839) as did human monocytes, known to express IL-10R. Studies are currently underway to explore the downstream effects of IL-10R engagement on ILCs and to identify additional mechanisms that regulate ILC cytokine production, including the role of the related cytokines IL-19 and IL-24 known also to regulate T cell responses in filarial infections.

## 1317

### BIOMARKERS OF ACTIVE INFECTION WITH ONCHOCERCA VOLVULUS

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Onchocerciasis (river blindness) is a neglected tropical disease that has been targeted for control and elimination through mass drug administration (MDA) of ivermectin that ultimately interrupts transmission. As efforts shift from control to disease elimination, better tools are needed to identify the presence of viable *Onchocerca volvulus* (*Ov*) parasites that can be used in surveillance and eventually for certification of elimination. To identify potential biomarkers that could be used in an immunologically-based rapid diagnostic assay, we utilized the stage-specific transcriptomes and proteomes of *Ov* and shortlisted 30 candidates as potential biomarkers based on *Ov* specificity and over representative (or unique) expression in the adult female stage. From these 30, we were able to develop immunoassays to quantify 2 of the most promising biomarkers OVOC8995 and OVOC12838. Antisera against peptides derived from OVOC8995 and OVOC12838 were raised in rabbits and then the IgG from each were affinity purified using specific peptide affinity columns. We then developed immunoassays that can reliably detect OVOC8995 and OVOC12838 in human serum with lower detection limits of 1ng/ml and 0.1ng/ml respectively. Using sera from microfilariae (mf)-positive *Ov*-infected individuals and sera from uninfected healthy controls or from those with other non-*Ov* filarial infections (e.g. *W. bancrofti* and *L. loa*) as comparators, we were able to detect OVOC12838 and OVOC8995 in *Ov*-infected samples only with very minimal to no reactivity in the various control sera. Given the high degree of *Ov*-specificity shown by OVOC12838 and OVOC8995 and the sensitivity of the assay to detect sub-nanogram levels of circulating antigen in *Ov*-infected individuals provides a promising platform to detect low-level infections during surveillance. Ongoing analyses with larger cohorts comprising of samples across geographical regions and pre- and post-treatment sera samples are currently underway for the validation of these two assays.

### AN INVESTIGATION OF *ONCHOCERCA VOLVULUS* GEOGRAPHIC POPULATION-SPECIFIC SECRETED MIRNA PROFILES

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Studies of geographic differences in *Onchocerca volvulus* (Ov) biology and pathogenesis may facilitate eradication efforts. It has recently been shown that Ov secretes microRNA (miRNA) into the host circulation and that Ov from differing geographic locations secrete different miRNA species. In the present study, we investigated the diversity of circulating Ov microRNAs in pools of 12 sera each from microfilariae-positive patients with onchocerciasis from the Americas, West Africa, Central Africa and East Africa and from Ov-uninfected individuals. RNA was extracted from these pools and single-end 50 base-pair sequencing reads were obtained using an Illumina HiSeq Rapid approach. These reads were then mapped to the Ov genome, the human genome, and the Nematoda miRNA sequences in miRBase v21, revealing 116 putative Ov-specific miRNA species that had not been previously identified. Alignments of the RNA-Seq reads showed that single nucleotide polymorphisms (SNPs) were present in a subset of the miRNAs identified. Comparative analysis of RNA-Seq data from the four regions studied also suggested geographic variation in the miRNA species secreted. Thus, we have found novel secreted Ov miRNAs with considerable diversity in their sequences and geospatial presentation. Taken together, these findings support a model for Ov population diversity reflecting geographic origin.

### DEVELOPMENT OF AN ANTIGEN-CAPTURE IMMUNOASSAY FOR THE DIAGNOSIS OF ACTIVE *LOA LOA* INFECTION

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Antigen-capture immunoassays are needed for the diagnosis of loiasis. We recently developed assays to quantify two microfilaria (mf) specific antigens whose quantity in sera reflects *Loa loa* mf counts. However, having an immunoassay that could detect all actively infected patients with loiasis, irrespective of their mf load, is of additional importance. To this end, we developed a capture (sandwich) ELISA assay to quantify *L. loa* SXP1 (LI-SXP1), an 148 amino-acid antigen protein expressed in all stages the *L. loa* parasite. Using purified polyclonal anti-LI-SXP1 IgG as a capture antibody, we were able to show that LI-SXP-1 antigen can be detected in both mf positive (n=28) and amicrofilaremic (n=20) *L. loa*-infected patients, but not in *L. loa*-uninfected (n=22) sera. Because of the significant conservation of sequence of the SXP1 protein across the various filariae, there was some level of antigen cross-reactivity in sera from mf positive patients with *Wuchereria bancrofti* and *Onchocerca volvulus* although the quantities measured were markedly lower than what was seen in *L. loa* infection. We next generated monoclonal antibodies to *L. loa*-SXP1 and found that 2 of these, when used as capture antibodies, significantly improved both the sensitivity and specificity of the capture ELISA assay. We are now assessing the alterations in LI-SXP1 levels following definitive treatment of *L. loa* with diethylcarbamazine. Taken together, circulating LI-SXP1 protein may be a very promising biomarker that can be exploited in a point-of-care immunoassay for the diagnosis of active *L. loa* infections.

### COMPARISON OF PCR-METHODS FOR *ONCHOCERCA VOLVULUS* DETECTION IN SKIN BIOPSIES FROM THE TSHOPO PROVINCE, DRC

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Current WHO criteria for stopping mass drug administration (MDA) for onchocerciasis allows skin snip PCR to rule out infection if less than 10 children have positive OV-16 serology results. Negative skin snip PCR results allow the OV-16 serology results to be considered false positives. PCR methods need to be optimized for this use. We compared the performance of two PCR methods for *O. volvulus* detection, a real-time PCR targeting the O150 repeat region (qPCR) and the O150-PCR ELISA (PCR-E), in 471 residual skin snip biopsy samples. Limits of detection (LOD) were compared using dilutions of qDNA of adult *O. volvulus*. PCR+E results were considered positive if the optical density (OD) > 0.1 and repeat testing was positive. Receiver Operator Characteristics (ROC) analysis was used to evaluate the performance of PCR-E. The LODs for qPCR and PCR-E were 20 fg and 5pg, respectively. qPCR detected a significantly higher number of positive skin snips (224/471 or 47.5%) than PCR-E (127/471 or 27%) ( $p < 0.0003$ ). A total of 161/471 (34.2%) samples were initially positive by PCR-E. However, 34/471 (7.2%) were negative upon repeat testing, and 24 of those 34 (70.6%) were qPCR+. Compared to qPCR, PCR-E had a sensitivity and specificity of 56.7% and 100%, respectively. ROC analysis for PCR-E showed the optimal threshold at OD=0.055, with a sensitivity of 83% and specificity of 90.7%. Because PCR-E missed 43.3% of qPCR+ samples, the qPCR assay may be more appropriate for evaluating the snips of OV-16+ children. As qPCR may not be feasible in all settings and detection of *O. volvulus* DNA in snips is used to make decisions about stopping MDA, improving the performance of other PCR methods as an alternative is critical. Adjusting the PCR-E OD cut-off could improve sensitivity but at a cost to specificity. The LOD of the conventional PCR method using qPCR primers was 20 fg - comparable to qPCR, thus incorporating this PCR method into the PCR-E might increase test sensitivity. All these options should be considered and further validated when defining the appropriate PCR assay to detect *Onchocerca* DNA in skin snips.

### IDENTIFYING "WINDOWS OF OPPORTUNITY" FOR THE DETECTION OF PARASITE MATERIAL IN THE EXCRETA/FECES OF VECTOR AND NON-VECTOR MOSQUITOES

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Previous experiments have demonstrated that the DNA of various human parasites, including *Brugia malayi*, can be detected in the excreta/feces (E/F) of both vector and non-vector mosquito species. Such detection has the potential to enable alternative E/F-based molecular xenomonitoring approaches, allowing for the improved throughput of testing in low prevalence settings. However, if field-based utilization of this novel approach to testing is to occur, clear definition of the E/F-based windows of parasite detection must be defined for each mosquito species of

interest. As factors such as vector competency, and metabolic differences impact the timing of the deposition of parasite material into the E/F, identification of such time periods will be critical for proper study designs, and will shape critical decision making processes regarding target species, and trapping methodologies. Accordingly, utilizing both real-time PCR and digital PCR techniques, we have identified the corresponding detection windows for a series of vector and non-vector mosquito species. This testing has enabled us to determine the time frame during which the deposition of parasite DNA occurs, with vectors demonstrating a narrow window of detection in comparison to non-vector species. Similarly, the post-blood meal time point at which parasite DNA becomes most readily and easily detectable has also been identified for each species tested. We believe that this information provides a critical step forward in elucidating a field-applicable methodology for E/F collection and testing.

## 1322

### IMPACT OF TWO ANNUAL CYCLES OF MASS DRUG ADMINISTRATION ON TEMPORAL TRENDS OF CLINICAL MALARIA

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Though a substantial decline in malaria burden has been observed in The Gambia, areas of on-going transmission still persist in the country. We evaluated the impact of Mass Drug Administration (MDA) on clinical malaria. Residents of twelve villages across The Gambia were enrolled into a prospective cohort were followed up from June 2013 to December 2015. Two rounds of MDA with dihydroartemisinin-piperazine (DHAPQ) were conducted at the start of the transmission seasons (TS) in June 2014 and May 2015. Follow-up surveys were conducted monthly for 6 months in each TS where finger prick samples were collected for haemoglobin estimation and *P. falciparum* detection. Clinical malaria was detected by passive case detection during the TS. DHAPQ coverage was 80.9% (3784/4678) in 2014 and 84.3% (4394/5210) in 2015. In 2014 during the first 21 days post MDA, prevalence of clinical malaria was 0.2%, (5/2500) compared to the prevalence at the end of the previous TS in December 2013; 1.75%, (58/3301). *P.falciparum* individuals at the end of the 2013 TS who took DHAPQ had a higher odds OR=1.94 (95% CI: 1.4 - 2.8) of clinical malaria subsequently in the 2014 TS between August to December. The prevalence of clinical malaria two months post MDA was 0.14%, (5/3549). Following the second annual round in 2015, no case of clinical malaria was detected in the first 21 days post MDA. Only 3 cases (0.1%, 3/2999) were detected two months post MDA, compared to the prevalence in December 2014; 0.4% (12/3135) at the end of the TS. The incidence rate of malaria in 2014 was 0.07 episodes (95% CI: 0.07-0.08) PPY (per person per year at risk) compared to 0.4 episodes PPY (95% CI 0.38-0.39) in 2013. Individuals had a lower risk of developing clinical disease in 2014 compared to 2013, an incidence rate ratio (IRR) = 0.8 (95% CI: 0.68-0.83). In 2015 the incidence rate of clinical malaria was 0.5 episodes PPY (95% CI 0.46-0.52). The risk of developing clinical malaria was higher in 2015 compared to 2013; IRR=1.3 (95% CI: 1.2-1.4). A second annual cycle of DHAPQ is protective from malaria within the first 21 days however, single annual MDA with DHAPQ is not sufficient to interrupt transmission of clinical malaria.

## 1323

### SPEEDING UP MALARIA ELIMINATION; A CLUSTER RANDOMIZED CONTROLLED TRIAL OF MASS DRUG ADMINISTRATION IN SOUTHEAST MYANMAR, AN AREA WITH ARTEMISININ RESISTANCE

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Artemisinin resistant malaria is present throughout Southeast Asia. It has been followed by partner drug resistance in some areas, where artemisinin based combination therapy is now failing. With no new drugs on the horizon elimination of artemisinin resistant *P. falciparum* may be the only way to prevent further spread. Mass drug administration (MDA) has successfully eliminated malaria in some areas in the past. We conducted a paired cluster-randomised controlled trial to determine the effectiveness of 3 rounds of MDA of dihydroartemisinin-piperazine plus a single low dose primaquine in addition to routine malaria control activities as part of a pilot project to speed up malaria elimination. The study assessed the feasibility, acceptability, safety and its effect on malaria. Malaria prevalence was assessed using ultrasensitive PCR before and 3, 5, 10 and 15 months after MDA. Sixteen clusters (population 8721) were enrolled, paired and randomised. MDA coverage was 90% (4164/4622). It was well tolerated with few (151), mostly mild, adverse events reported. *P. falciparum* prevalence dropped from 14.2% at baseline to 1.4% ( $p < 0.001$ ) 3 months post MDA in the intervention group, while it dropped from 15.6% at baseline to 10.4% in control villages in the same period. Over time the difference in malaria prevalence between MDA and control clusters diminished, possibly as a result of migration and re-introduction of new infections from neighbouring villages where MDA was not provided. Kelch 13 mutations associated with artemisinin resistance comprised 57% (54/94) of samples at baseline and 54% (7/13) at month 3 post MDA. MDA can be considered safe and effective to reduce *P. falciparum* prevalence in low transmission areas with artemisinin resistance.

## 1324

### REACTIVE CASE DETECTION WITH TARGETED MASS DRUG ADMINISTRATION: INTERRUPTING MALARIA TRANSMISSION AND ACHIEVING ELIMINATION BEYOND INTERVENTION AREAS IN NORTHWESTERN PERU

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Reactive case detection (RCD) with targeted mass drug administration (tMDA) was previously found to be an effective strategy to support malaria elimination initiatives in Tumbes Peru, a region with a predominance of vivax malaria. We assessed the effect of RCD/tMDA on malaria incidence in intervention areas of Tumbes and the surrounding region of Piura. A pilot malaria elimination program based on RCD/tMDA was rolled out in the two most highly malaria-endemic districts (nine reporting units) in Tumbes from 2009-2010 and then scaled up to 11 additional districts (34 reporting units) from 2011-2014. Non-intervention areas in Piura were evaluated from 2011-2016. Malaria cases were passively detected, and followed-up within 24 hours together with household contacts (excluding elders, pregnant women, and chronically-ill subjects). The primary study endpoint

was a temporospatial reduction in weekly parasite incidence (WPI = total malaria cases per week/ 1,000 inhabitants) across all reporting units, analyzed by proximity to the intervention area's reporting unit. During the study period (2009-2014) we analyzed a total of 9102 autochthonous malaria cases. During the pilot program we estimated a mean reduction in WPI across Tumbes intervention areas of 99% (97–100) and 85% (77–94) at 12 and 24 months (m), respectively; and non-intervention areas of 34% (27–40) and 86% (76–93) at 12m and 24m, respectively. During the same period, we estimated a mean reduction in WPI at the near, mid and far range Piura districts of 63%, -73%, and -420% at 12m, and 91%, -126% and 91% at 24m, respectively. After scale up, we found a negative association between the mean WPI reduction and proximity to intervention area across the Piura districts, at both 24m ( $p=0.04$ ) and 48m ( $p<0.01$ ). During years 2015 and 2016, neither area in Tumbes nor in Piura has reported any autochthonous malaria cases. The RCD/tMDA strategy has shown to effectively interrupt malaria transmission in the Piura region and may support malaria elimination initiatives in other settings with a high predominance of vivax malaria.

## 1325

### REACTIVE CASE DETECTION FOR MALARIA IN AMHARA NATIONAL REGIONAL STATE, ETHIOPIA: DESCRIPTIVE AND IMPACT EVALUATION ANALYSIS

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Case investigation (CI) of malaria cases and reactive case detection (RCD) of malaria infections are strategies that can contribute to malaria elimination in low-transmission settings. In Amhara region, Ethiopia, CI with RCD was conducted in 37 villages during the malaria transmission seasons in 2015-2016. *P. falciparum* or mixed malaria cases diagnosed at the health posts were considered index cases and were investigated (regarding socioeconomic characteristics, travel history, etc.). RCD with focal test and treat (FTAT) was conducted in the index case household and the ten closest neighboring households within a 100 meter radius. During the FTAT, all individuals in the targeted households were tested with a rapid diagnostic test (RDT) and all positives were treated with an antimalarial drug. Preliminary results from April 2015 to August 2016 indicate that there were 179 index cases, of which 47% were investigated. Of these, 58% were male and only 23% were children younger than ten years of age. During the FTAT, 548 households were visited, of which 96% were investigated. Of the 2,372 individuals in those households, 85% were tested, of which 1.7% were positive (56% *P. falciparum*, 18% *P. vivax*, 26% mixed). Risk factors for RDT-positivity included travel history, reported or measured fever, sleeping under a bednet, and recent antimalarial treatment. There were different transmission patterns, with several villages having very few cases, some villages having mostly imported cases, and others having mostly local cases. To evaluate whether CI with RCD had an impact on malaria incidence in the intervention villages, a quasi-experimental design with a comparison group of villages from the same regions—matched by pre-intervention incidence and altitude—will be used. A difference-in-differences analysis using negative binomial regression will be conducted. Final descriptive and impact evaluation results will be presented.

## 1326

### EVALUATING THE EFFICIENCY OF REACTIVE CASE DETECTION TO ACHIEVE MALARIA ELIMINATION IN RURAL SOUTHERN ZAMBIA USING FOLLOW-UP HOUSEHOLD VISITS AND PARASITE GENOTYPING

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Targeted interventions to identify and treat the asymptomatic reservoir have been implemented in areas approaching malaria elimination. Reactive case detection is currently conducted in Southern Province, Zambia to enhance surveillance and clear the asymptomatic reservoir. After an index case is confirmed with malaria by a rapid diagnostic test (RDT), household members and neighbors residing within 140-meters are tested with an RDT and treated with ACT if positive. The efficiency of this strategy to detect and treat *P. falciparum* infections was evaluated in the catchment area of Macha Hospital in Southern Province. A subset of index cases was evaluated by a study team who administered a questionnaire, performed an RDT, and collected a blood sample on filter paper for detection of *P. falciparum* DNA by qPCR. As part of the study, the screening radius was extended to 250-meters and follow-up visits were performed 30 and 90 days after the initial visit. From March 2016-January 2017, 139 households with 935 residents participated. Parasite prevalence and transitions between RDT and qPCR positivity were compared between residents of index and neighboring households over the follow-up. Parasite genetic relatedness between persistent infections within individuals and new infections within households were analyzed using a *P. falciparum* 24 SNP molecular barcode. Overall, parasite prevalence was higher in index households (1.4% by RDT, 6.9% by qPCR) compared to neighboring households (0.7% by RDT, 2.7% by qPCR). Parasite prevalence by RDT and qPCR decreased over follow-up visits but *P. falciparum* infection persisted and was not eliminated in study households. Persistent infections were detected by qPCR among those negative by RDT. Identical molecular barcodes were detected among persistent infections within individuals and among newly identified infections within households. The efficiency of reactive case detection in this setting is limited by the low sensitivity of the RDT and continued focal transmission after the intervention. Focal drug administration in the index household may be a more efficient strategy to achieve elimination.

## 1327

### ACHIEVING INTERRUPTION OF LOCALLY TRANSMITTED PLASMODIUM FALCIPARUM MALARIA CASES THROUGH PILOTING A BASIC ESSENTIAL PACKAGE OF ACTIVITIES FOR MALARIA ELIMINATION IN THE CONTEXT OF ARTEMISININ RESISTANCE, 2015-2017

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The Cambodian/Thai border area has long been an area of concern for malaria control efforts due to the emergence and spread of chloroquine-resistant and more recently artemisinin-resistant *Plasmodium falciparum* malaria parasites. This recent spread led international health organizations to suggest a move from containment to elimination strategies. Subsequently, the Cambodia Strategic Plan for Malaria Elimination 2011-2025 was developed, which calls for elimination of *P. falciparum* by 2020 and all malaria species by 2025. A basic essential package of activities for

malaria elimination was developed following the 1-3-7 model developed in China (notification within 24 hours, investigation within 3 days, and response within 7 days). The package used rural health facilities and community health workers to form response teams for conducting active surveillance in their catchment areas. Simultaneously, management capacity at district and central levels was strengthened. The model was implemented in 3 administrative districts in Battambang Province from July 2015 to January 2017. Results show an increase in notification, investigation, and response of reported cases from 52%, 20%, and 36%, respectively, to 100% in all categories by May 2016. Response activities included health education to 2,492 people, 242 insecticide treated nets distributed, and 1,377 individuals screened through reactive case detection (RACD). RACD identified no cases among index and surrounding households, however 2.53% of co-travelers were infected. There was a reduction in the average monthly *falciparum* incidence from 0.089 (SD=0.055) during July 2015 - April 2016 to 0.017 (SD=0.012) during May 2016 - January 2017. Additionally, the percentage of locally transmitted cases during the same periods above dropped from 10.3% to 0%, with no locally acquired cases reported since March 2016. The data illustrates the potential of the model to increase the quality of programmatic activities and reduce malaria incidence. The model will be reevaluated and revised as necessary in April 2017 after which implementation will continue in the study site.

## 1328

### A MULTI-COUNTRY INITIATIVE TO ACCELERATE ELIMINATION BY REDUCING CROSS-BORDER IMPORTATION OF MALARIA

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The importation of pathogens, including malaria, through population movement has long been recognised as a contributor to the transmission of infectious diseases and an obstacle to disease elimination. The Elimination Eight (E8) is a multi-country Southern African regional initiative to coordinate the elimination of malaria in four frontline countries (Botswana, Namibia, South Africa and Swaziland) by 2020, and pave the way for elimination in four second line countries (Angola, Mozambique, Zambia and Zimbabwe) by 2030. The frontline four countries have reduced malaria incidence to pre-elimination levels; their highest burden areas are generally adjacent to borders with second line countries. The region is experiencing a steady rise in both regular (formal) and irregular (informal) migration and travel. The persistence of malaria transmission in the pre-elimination countries is partially caused by ongoing importation of infections from across the borders, where malaria transmission is higher, and access to health services is limited. As they reduce malaria incidence, E8 countries therefore remain vulnerable and receptive to malaria outbreaks triggered by imported cases from their neighbors. To counter this risk, E8 countries, working through their National Malaria Control Programs, have established a mix of approximately 40 static and mobile border health facilities on 5 key international borders between high and low transmission districts of E8 countries. The goal of these health posts is to improve access to malaria testing and treatment services, among mobile and migrant populations and underserved resident communities in border districts. All health posts provide core diagnosis and treatment of malaria, whilst static facilities additionally provide a basic package of primary health care services. An evaluation study will be carried out to assess the impact of these border posts on mobile and resident populations. This presentation will describe the E8 border health post project and its evaluation.

## 1329

### IMMUNE PROFILING AND NETWORK MODELING OF ZIKA VIRUS INFECTION IN CHILDREN WITH OR WITHOUT PRIOR EXPOSURE TO DENGUE VIRUS IN A COHORT STUDY IN NICARAGUA

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Zika virus (ZIKV) is an emerging, mosquito-borne flavivirus that is responsible for the recent pandemic in the Americas. ZIKV usually causes a mild febrile illness; however, it has been linked with Guillain Barré Syndrome in adults and congenital birth defects, including microcephaly, when infection occurs during pregnancy. However, much remains to be defined regarding the human immune response to ZIKV infection. In this study, we collected blood in PAXgene solution and plasma at acute (days 1-3 and days 4-6 post-onset of symptoms) and convalescent (day 14-16) time-points and peripheral blood mononuclear cells (PBMCs) at day 4-6 and convalescence from symptomatic ZIKV-infected pediatric cases in a 13-year ongoing cohort study in Managua, Nicaragua, who presented to the study health center. Patients were documented to be either previously exposed to DENV (n=45) or DENV-naïve (n=45). Comprehensive innate immune responses were investigated by CyTOF, Luminex cytokine assays, and RNA-seq. Initial CyTOF analysis revealed significant differences between acute and convalescence in the cytokine and chemokine profile, and differences in frequencies and phenotype of specific circulating leukocytes subsets, including significantly elevated expression of CD169 on monocytes in convalescent samples. Luminex results revealed that levels of CCL2, CCL5 and CXCL10 are significantly higher in DENV-immune patients compared to DENV-naïve individuals on days 4-6 of infection. Type I and II interferon levels were significantly higher on days 1-3 of infection compared to other time-points along with several other interleukins and chemokines. We also performed RNA-seq analysis during the acute (days 1-3) and convalescent phase of ZIKV infection to identify differences in gene expression at the transcriptomic level. These data are being analyzed and integrated into our network modeling and key driver analysis. This study will provide the most comprehensive immune profiling and network analysis of the human response to ZIKV infection to date, will illuminate the effect of prior DENV infection, and will help inform future diagnostics and drug therapies.

## 1330

### LABORATORY IDENTIFICATION OF PERSISTENT ZIKA VIRAL RNA IN SEMEN OF A U.S. COHORT

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Zika virus (ZIKV; *Flavivirus*) is an emerging mosquito-borne virus that is capable of sexual transmission. ZIKV infection during pregnancy can cause congenital disease. In order to assess the risk of sexual transmission, the duration and frequency of ZIKV shedding in semen was evaluated through

a prospective study of men in the U.S. with symptomatic, laboratory-confirmed ZIKV infection. 225 men were enrolled in the study, and home-collected semen and urine were provided every 2 weeks for up to 6 months after symptom onset. Samples were tested for ZIKV RNA by real-time RT-PCR, and a subset of samples were tested by plaque assay for infectious virus. In the first 30 days post-onset of disease, 56% of men tested (23 out of 41) had at least one sample with detectable ZIKV RNA in semen, and 7% (3 out of 41) had at least one sample with detectable infectious ZIKV in semen. By 65 days post-onset of disease, the proportion of semen samples with detectable ZIKV RNA had declined 2-fold, and by 211 days, 99% of men had undetectable ZIKV RNA levels in their semen. To date, the latest day post-onset of disease with detectable ZIKV RNA was 256 days. The latest day post-onset of disease with detectable infectious virus was 25 days, and the lowest RNA level corresponding to detectable infectious virus was 7.3 log<sub>10</sub> RNA copies/mL semen. Semen from vasectomized men (n=30) also were positive for ZIKV RNA but at significantly lower levels. The study results suggest that ZIKV RNA shedding in semen is common and long-lasting, even in vasectomized men. However, the implications for sexual transmission remain somewhat unclear, as infectious virus was not detected from any tested samples that contained less than 7.0 log<sub>10</sub> RNA copies/mL semen. Further studies to determine the cell populations in the male urogenital tract that are persistently infected and to identify immune correlates of persistent ZIKV RNA shedding will provide additional insights into the sexual transmission risk potential from ZIKV infected men.

### 1331

#### STAGING EARLY AND LATE EVENTS IN ACUTE ZIKA VIRUS INFECTION USING RNA+ PUERTO RICAN AND CONTINENTAL U.S. BLOOD DONORS

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Due to the risk of blood transfusion-transmission, nucleic acid amplification testing (NAT) of the blood supply for Zika virus (ZIKV) RNA was implemented during 2016 in the U.S. and its territories. At the peak of the epidemic in Puerto Rico (PR) over 1.5% of blood donors were detected as infected by ZIKV NAT, with ~350 total infected donations interdicted. Fifty-one ZIKV+ donors identified in 2016 from both PR and the continental US were enrolled into longitudinal follow-up studies. Using the index donation and follow-ups we were able to classify eight distinct stages of acute infection based on plasma viral load (VL), anti-ZIKV IgM seroconversion and the persistent viral RNA in blood compartments. These stages begin with IgM seronegative donors with VLs below or above 300 IU/ml (stages I and II, respectively), progress to IgM seropositive stages with VLs >300 (stage III) and VLs <300 with either repeat testing reactive or non-reactive ID-NAT results (stages IV and V, respectively). Follow-up samples could be classified into a further stage with negative NAT on plasma, but with detectable persistent viral RNA in packed red blood cell (pRBC) preparations (stage VI). Finally, RNA non-reactive eclipse and convalescent stages book-end the six stages with detectable viral RNA. Early in the epidemic most of the index donations were stage I and II donors. All of the stage I and II donors seroconverted to IgM+ within 5-24 days of index donation. The duration of ZIKV IgM seroreactivity ranged from 40 to over 185 days. Overall, approximately 50% of plasma RNA+ donors fell into the IgM seropositive stages (III-V) at index, with a higher percentage of tail-end stage IV and V infections observed towards the end of the epidemic. Many of the stage IV and V donors rapidly progressed to stage VI with non-detectable plasma viremia, but persistent viral RNA in the pRBC compartment. Further studies are required to demonstrate the risk to blood safety that donations from individuals in stage VI would

represent. Using the data collected in this study we will approximate the duration of the various stages and the length of the window for transmissibility.

### 1332

#### PRIOR DENGUE VIRUS EXPOSE SHAPES T CELL IMMUNITY TO ZIKA VIRUS IN HUMANS

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While progress has been made in characterizing humoral immunity to Zika virus (ZIKV) in humans, little is known regarding the corresponding T cell responses to ZIKV. Here we investigate the kinetics and viral epitopes targeted by T cells responding to ZIKV and address the critical question of whether pre-existing dengue virus (DENV) T cell immunity modulates these responses. We find that memory T cell responses elicited by prior infection with DENV recognize ZIKV-derived peptides. This cross-reactivity is explained by the sequence similarity of the two viruses, as the ZIKV peptides recognized by DENV-elicited memory T cells are identical or highly conserved in DENV and ZIKV. We further show that DENV exposure prior to ZIKV infection influences the timing and magnitude of the T cell response. ZIKV-reactive T cells in the acute phase of infection are detected earlier and in greater magnitude in DENV-immune patients. Conversely, the frequency of ZIKV-reactive T cells continues to rise in the convalescent phase in DENV-naïve donors, but declines in DENV pre-exposed donors, compatible with more efficient control of ZIKV replication and/or clearance of ZIKV antigen. Finally, we discovered that ZIKV structural proteins (E, prM and C) are major targets of both the CD4 and CD8 T cell responses, whereas DENV T cell epitopes are found primarily in nonstructural proteins - thus highlighting a fundamental difference between the immunodominant antigens targeted by human T cell responses against DENV and ZIKV viruses.

## 1333

**INDEX CLUSTER STUDY OF ZIKA VIRUS INFECTION IN MANAGUA, NICARAGUA**

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Zika virus (ZIKV) recently caused major epidemics in the Americas and is linked to congenital neurological birth defects and Guillain-Barré Syndrome. A pilot Zika index cluster study was conducted from August 31 to October 21 of 2016 in Managua, Nicaragua, towards the end of the initial ZIKV epidemic and during government vector control activities targeting Zika-positive households. Real-time RT-PCR (rRT-PCR)-confirmed symptomatic Zika cases from the study Health Center were selected as index cases (n=33), and contacts (household members) were enrolled during a home visit the next day (n=109). Enrollment and 4 follow-up visits (days 3-4, 6-7, 9-10, and 21) included blood, urine and saliva collection, along with clinical, demographic, socio-economic status (SES) and risk factor questionnaires. Samples were analyzed by the ZCD rRT-PCR assay, which detects Zika, dengue, and chikungunya viruses; only ZIKV was detected during the study period. ZIKV serological responses were assessed by a highly ZIKV-specific in-house ZIKV IgM capture ELISA (IgM) and a standardized in-house ZIKV Inhibition ELISA (IE), using enrollment and day-21 serum samples. An entomological household survey was performed on day 10. At enrollment, 11 (31%) contacts were ZIKV-positive (4 by rRT-PCR and 7 by rRT-PCR and IgM), and 3 showed IgM seroconversion and 2 a ≥4-fold rise in IE titer during the study. In addition, 23 (21%) were positive by IgM, indicating a recent ZIKV infection. Analysis of clinical information enabled estimation of the symptomatic to asymptomatic (S:A) ratio of 5:11 (1:2.2.) among the contacts. Of the 11 asymptomatic ZIKV-positive contacts, 6 were rRT-PCR-positive and 5 were positive by serology. Entomological analysis revealed no rRT-PCR ZIKV-positive *Aedes aegypti* mosquitoes. Initial evaluation of ZIKV viral load kinetics indicated that ZIKV is detectable up to visit 3 (days 6-7) in saliva and up to visit 4 (day 9-10) in urine. SES and household data is being analyzed to identify potential protective and risk factors for ZIKV infection. Overall, these data inform the development of public health decisions that target prevention of ZIKV transmission.

## 1334

**PREVALENCE AND INCIDENCE OF ZIKA VIRUS INFECTION AMONG HOUSEHOLD CONTACTS OF ZIKA PATIENTS, PUERTO RICO, 2016-2017**

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Little is known about prevalence or incidence of Zika virus (ZIKV) infection among household contacts of infected patients or risk factors for infection, including sexual practices. The Zika virus Persistence (ZiPer) study is an ongoing cohort study in Puerto Rico, begun in May 2016. After presenting to care, index patients with ZIKV RNA in blood or urine and their household contacts were offered study participation, including a survey and diagnostic testing for: a) ZIKV RNA in serum, urine, saliva,

vaginal fluid, and semen; and b) anti-ZIKV IgM antibody in serum. ZIKV RNA- and IgM-negative contacts were offered additional testing at a 4-month follow-up visit. We assessed ZIKV prevalence (RNA or IgM) in contacts, and factors correlated with prevalence with bivariate prevalence ratios (PR), 95% confidence intervals (CI). Associations with sexual contact were assessed by evaluating reported sex with the index patient, and a social-network method that evaluated all pairwise household relationships using a matched odds ratio (mOR). Incidence among retested contacts was measured with rates per 100 person-days (PD) and 95% CI. Among 352 household contacts of 167 index patients, prevalence of ZIKV infection was 35% (CI: 30-40%). Age, sex, and other demographics were not associated with prevalent infection. Sexual contacts of index cases did not have significantly higher prevalence than non-sexual ones (PR=1.25, 95%CI: 0.89-1.76), and the network approach found no association (mOR=1.01). Household factors associated with infection included reporting ever leaving windows open (PR=2.33, CI: 1.33-4.06) and not having window screens (PR=1.59, CI: 1.20-2.10). Among 118 ZIKV-negative members retested, 10 (8%) had incident infections (rate: 0.11/100PD, CI: 0.05-0.19). One-third of household members of Zika patients had prevalent infection, and nearly one-in-ten became infected within four months. For this population there was insufficient evidence to implicate sexual transmission as a major mode of ZIKV transmission. In regions with ongoing ZIKV transmission, personal and household measures to prevent ZIKV infection should be sustained.

## 1335

**VIRAL LOAD DOES NOT SUPPORT ADE HYPOTHESIS IN DENGUE-PRIMED ZIKA-INFECTED PATIENTS**

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Most studies on the pathogenesis of severe dengue disease focus on the process of antibody-dependent enhancement (ADE) as a primary risk factor. With the circulation of ZIKV in DENV-endemic areas, many people infected by ZIKV were likely exposed to DENV. Currently, the influence of such exposure on Zika disease outcomes remains unknown, despite several *in vitro* data suggests that ADE may play a role in Zika pathogenesis. We investigated whether patients previously exposed to dengue exhibited higher viremia when exposed to a subsequent, heterologous dengue or Zika infection than those patients not previously exposed to dengue. We measured viral loads and cytokines profile during patients' acute infections. Neither dengue nor Zika viremia was higher in patients with prior DENV infection. Of the ten cytokines measured, only one significant difference was detected: levels of IL-1β were lower in dengue-infected patients that had experienced a previous dengue infection than patients infected with dengue for the first time. Taken together this data shows no signs of ADE were observed *in vivo* in patients with acute ZIKV infection who had prior exposure to DENV.

## 1336

**HOST BLOODMEAL REMNANT ANALYSIS DEMONSTRATES THE VARIABLE CONTRIBUTION OF WHITE FOOTED MICE TO ENZOOTIC TRANSMISSION OF LYME DISEASE SPIROCHETES**

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The public health burden of Lyme disease continues to increase. Modes of intervention include those that target the putative main reservoir mouse, *Peromyscus leucopus*, but such interventions are not consistently effective in reducing the entomological inoculation rate. One explanation

for varying results is that in some sites, mice may not be the sole source of infected ticks. The incrimination of reservoirs for the agent of Lyme disease has been largely based on indirect measures, such as relative tick burdens and infectivity of diverse hosts. Bloodmeal analysis of host-seeking ticks would directly and definitively incriminate the reservoir as it does for mosquito-borne infections. To date, bloodmeal remnant analysis of ticks (particularly nymphs) has been limited because assays lack sensitivity; typically only about half of the samples yield results. We developed a sensitive real-time PCR assay targeting host-specific retrotransposons that yields a signal in over 98% of the ticks tested. Retrotransposons are genetic elements that can integrate themselves into and typically are present as thousands of copies per genome. In addition, they have coevolved with diverse mammalian families making it possible to design host-specific PCR primers. We designed primers to identify the most likely hosts for subadult deer ticks in New England: white-footed mouse, meadow vole, cottontail rabbit, squirrel/chipmunk, skunk/raccoon, deer and shrew. Host-seeking nymphal deer ticks from Martha's Vineyard were individually homogenized and analyzed for evidence of infection by *Borrelia burgdorferi*; host bloodmeal remnants were determined for those that were infected. Host DNA was detected in 98% of the ticks tested. During 2016, *P. leucopus* had served as the source of infection for 64% [95% CI 51-76] of the infected ticks and rabbits 22% [12-35]. During 2015, only 43% [29- 57] of the infected ticks had fed as larvae on *P. leucopus*; rabbits and shrews each fed 22% [12-36]. We conclude that although *P. leucopus* serves as a major source of *B. burgdorferi* for larval deer ticks, other animals significantly contribute to maintaining enzootic transmission.

### 1337

#### COMPARISON OF AN ULTRA-SENSITIVE PCR-BASED ASSAY TO TWO-TIER SEROLOGY IN THE DIAGNOSIS OF EARLY LYME DISEASE

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Lyme disease is a tick-borne infection caused by the bacteria *Borrelia burgdorferi*. Current diagnostic testing relies on a two-tier ELISA and western blot serology performed during acute and convalescent phases. Limitations of serology include both insensitivity for early and non-specificity for later infection. The purpose of this study is to compare performance of an assay for direct detection of *B. burgdorferi* in skin and whole blood to two-tier serology among a sample of patients diagnosed with early Lyme disease. Thirty-nine patients from Maryland with physician-documented erythema migrans rash were enrolled. A 2mm skin biopsy sample and 20ml of whole blood were obtained and tested using an ultra-sensitive, nucleic acid-based PCR assay employing isothermal amplification and multiple PCR primers. Acute and convalescent serologies were measured by a large commercial laboratory and interpreted by CDC criteria. Group comparisons were determined by chi-square or Fisher's exact tests for categorical and Wilcoxon Rank Sum test for continuous variables. At time of diagnosis, thirty participants (76.9%) were skin PCR positive (S+) for *B. burgdorferi*, 10/39 (25.6%) were blood positive (B+), and 5/39 (12.8%) were serology positive. Nine participants (23.1%) were skin biopsy and blood PCR negative (S-/B-), and all 9 were also serology negative. No participants were (S-/ B+). There was a statistically significant difference in the proportion serology positive at the time of diagnosis between the S+/B+ and the S+/B- groups (40% vs. 5%, respectively,  $p=0.03$ ), and a statistically significant difference in median number of new-onset symptoms reported at the time of diagnosis between the three groups (S+/B+: 10, S+/B-: 5, S-/B-: 2,  $p=0.002$ ). Skin biopsy PCR is a more sensitive diagnostic test for early Lyme disease than whole blood PCR or two-tier antibody testing. We did not find PCR or serologic evidence of infection with *B. burgdorferi* among a subgroup of patients

with physician-diagnosed erythema migrans. At time of initial diagnosis, a higher proportion of those with evidence of bacterial blood stream dissemination had a positive serologic test.

### 1338

#### GENOMIC SURVEILLANCE AND DIAGNOSIS OF TICK-BORNE DISEASE *BABESIA MICROTI*

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*Babesia microti* is tick-borne disease that is an emerging threat to public health due to increasing prevalence and expanding geographic range. Despite ongoing research, the care of patients with tick-borne disease is frustrated by the limited number of diagnostic assays and surveillance tools. Leveraging the genetic diversity, we identified through our whole genome sequence (WGS) analyses of *B. microti*, we have developed a rapid lineage-specific qPCR-based diagnostic and single nucleotide polymorphism (SNP)-based high resolution melting (HRM) surveillance tool for *B. microti*. These tools were built on our experience in developing diagnostics for hemorrhagic fever viruses and *Plasmodium vivax* malaria. Our *B. microti* qPCR assay is robust and targets a conserved region within the mitochondrial gene cytochrome oxidase B and is highly sensitive, with a detection limit of 2pg of parasitic gDNA. In addition to detection, constant surveillance of hotspots of Babesiosis is imperative to diagnosis and to predict the spread of the pathogen. Using our WGS data we developed a SNP-based barcode using HRM. We identified candidate SNPs for our barcode by performing SNP calling using available sequence data (with quality scores of > 30) on 26 parasite strains from 4 geographic locations and identified 775 informative SNPs. Screening this set of informative SNPs, we developed a 40-SNP barcode consisting of 20 mitochondrial SNPs and 20 nuclear SNPs that capture high degrees of population diversity and differentiate geographically distinct populations. We used our HRM assay to identify *B. microti* infections in a pilot study of 50 clinical specimens with high reproducibility and sensitivity and to identify the geographic origin of a given strain. By implementing field-deployable genotyping tools, the HRM platform can be expanded to include new variants identified by WGS analysis such as drug resistance. Most importantly, this approach provides a technology that is easily transferred to researchers as many real-time PCR instruments are coupled with HRM analysis software, creating a rapid genotyping technique where analysis can be completed in 1.5 hours.

### 1339

#### NOVEL IMMUNO-DOMINANT *BABESIA MICROTI* ANTIGENS THAT INDUCE PROTECTIVE IMMUNITY AGAINST PARASITE CHALLENGE IN MICE

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Human babesiosis caused by *Babesia microti* is a major public health concern in the United States and is found in temperate regions throughout the world. While the disease is generally mild to moderate in children and young adults, it is more severe or even fatal in newborn infants, the elderly and immunocompromised individuals such as those with cancer, HIV, asplenia, and those who acquire the infection through blood transfusion. Additionally, asymptomatic persistent infection presents a serious risk to blood safety. A vaccine targeted against high risk populations could reduce disease severity and mortality in vaccinated individuals. Using a genomics approach, we have identified a battery of novel immunodominant antigens that are reactive against plasma/sera from *B. microti*-infected individuals.

We have chosen the nine highest ranking immunodominant antigens for vaccine efficacy studies using multiple rounds of immunoselection. BALB/c mice were immunized three times with these recombinant protein antigens formulated in Complete/Incomplete Freund's adjuvant while control group mice were given only adjuvant. Both groups were infected with *B. microti* parasites through syringe inoculation. We noted a significant reduction in parasite burden after vaccination with 3 of the 9 candidate antigens - BmSERA (Serine Repeat Antigen), BmMCFRP (Maltese-cross form related protein) and Bm3 (function unknown). The geometric mean of maximum percent parasitemia (95% CIs) was significantly reduced in the BmSERA, BmMCFRP and Bm3 immunized groups (0.3%, 1.5% and 1.6%, respectively) compared with the control group (2.9%). Furthermore, a significant delay in peak parasitemia was observed in the vaccinated versus control mice with the mean peak parasitemia (95% CIs) at 18.8, 18.4 and 17.5 days for the BmSERA, BmMCFRP and Bm3 immunized group, respectively, compared to 15.6 days for the control mice. Studies to determine the vaccination induced antibody and cellular immune mechanisms of protection are in progress.

### 1340

#### SCRUB TYPHUS NO LONGER RESTRICTED TO THE TSUTSUGAMUSHI TRIANGLE

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Scrub typhus, a vector-borne disease, has for many years been considered endemic to a region bordering central and eastern Asia, northern Australia and the Western Pacific. This no longer is true as cases of scrub typhus have been found in the Chile and United Arab Emirates. In addition, others have reported scrub typhus-like cases from Africa and orientia DNA from rodents of Europe and Africa. Moreover, serological evidence of orientia infection have been reported from Africa. Recent work in South America, including studies in Peru and Chile corroborate the findings that scrub typhus should no longer be restricted to the Tsutsugamushi Triangle.

### 1341

#### A SYSTEMIC FUNCTIONAL ANALYSIS OF THE PHOSPHOINOSITIDE METABOLIC PATHWAY IN *PLASMODIUM FALCIPARUM*

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Phosphoinositides (PIPs) are critical components of cellular membranes in eukaryotes, playing massive roles in signal transduction, cell motility, cytoskeletal reorganisation, DNA synthesis, cell cycle, adhesion, membrane transport, permeability and intracellular trafficking. Collectively, seven isoforms of PIPs, the protein effectors that bind them and enzymes that generate or modify them compose a remarkably complex protein-lipid signalling network. Despite their crucial role in eukaryotes little is known about PIP metabolism and functions in the malaria parasite *Plasmodium falciparum* where the PIP profile is much complex than in uninfected RBCs. Here, in this study we performed a comprehensive knock out screen, by employing the recently reported Selection-Linked Integration (SLI) method to select for targeted gene disruptions (TGD), wherein we attempted to disrupt the complement of the PIP metabolic pathway including PIP kinases, phosphatases, putative PIP effectors and PIP transfer proteins, a total of ~30 targets to identify the genes that are essential for asexual proliferation in *P. falciparum*. Integration of the SLI-TGD vector at the correct genomic locus was monitored by PCR products across the 5' and 3' integration junctions and the quantitative absence of the original WT locus. Our study shows that >50% of the genes in the PIP metabolic pathway could not be disrupted and are likely to be essential for the maintenance of parasite viability, which correlated well with the sparse essentiality data from homologs in *P. berghei* or *T. gondii*. This lack of

redundancy points not only to the vital role of a number of individual kinases/effectors/phosphatases but also to a potentially abundant source of anti-malarial targets. We will describe asexual growth, susceptibility to antimalarials and gametocytogenesis in the knock outs generated. Additionally, we have also employed the recently reported strategy of knock sideways to localize native proteins by fusing with GFP and functionally analyzing the essential PIP kinases at the protein levels by mislocalizing the native proteins.

### 1342

#### CAPTURING DIFFERENTIAL PROTEIN TURNOVER DYNAMICS IN ARTEMISININ RESISTANT *PLASMODIUM FALCIPARUM* USING PULSE-SILAC

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Malaria caused an estimated 438,000 deaths in 2015. Although the first-line antimalarial - artemisinins (ARTs) - have contributed to decreased mortality rates, ART resistance is a major threat. Several lines of evidence indicate that artemisinin resistance is mediated, at least in part, by altered protein turnover dynamics. To investigate how ART-resistant parasites are capable of overcoming artemisinin-induced toxicity we developed a pulse-SILAC (stable isotope labeling amino acid in cell culture) approach that is capable of quantifying the rates of protein synthesis, degradation and turnover across the proteome. We observe that following ART exposure, both sensitive and resistant lines have impaired protein turnover that affects all detectable proteins. However, ART-resistant lines begin recovering protein synthesis after 12 hours post drug exposure with increased turnover of proteins associated with protein folding, translation and response to oxidative stress. This difference in parasite recovery at 12-18 hours post-artemisinin treatment was independently validated with a firefly luciferase reporter assay. Here we present how this novel triplex pulse-SILAC approach can provide insight into investigating protein turnover dynamics across the intra-erythrocytic developmental cycle for artemisinin-resistant parasites. These findings indicate that long-lasting endoperoxides should overcome artemisinin resistance and we present our evidence for the enhanced efficacy against ART-resistant parasites of OZ439, an endoperoxide with enhanced pharmacokinetics.

### 1343

#### USING SINGLE-CELL TRANSCRIPTOMICS TO ELUCIDATE SEXUAL COMMITMENT AND DIFFERENTIATION IN *PLASMODIUM FALCIPARUM*

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Sexual reproduction is an obligate step in the *Plasmodium falciparum* life cycle, with mature gametocytes being the only form of the parasite capable of human-to-mosquito transmission. During the red blood cell stage of human infection, less than five percent of *P. falciparum* parasites commit to a sexual fate and become gametocytes. This occurs in a 3:1 ratio of females to males that mature through five distinct gametocyte stages over 10-12 days before being transmitted to the mosquito. Previous studies utilizing *Plasmodium berghei* indicate that male and female gene expression is highly distinct. But in *P. falciparum*, although over 300 genes are predicted to be gametocyte-specific, only a few genes have been postulated to be male or female specific. Since these markers are expressed at late stages and their gender-specific expression is debated, separating male and female populations remains technically challenging. Most importantly, there is a large amount of heterogeneity in the parasite population, especially at early gametocyte stages. To overcome these challenges, we have developed an unbiased single-cell approach to determine which transcripts are temporally expressed in males versus females. Using microfluidic technology, we have isolated over 300 single parasites at synchronized asexual and sexual stages to

compare the expression of 90 conserved gametocyte-specific genes. These analyses have identified gender-specific gene expression for mid-to-late stage gametocytes, including two male-specific candidate genes. These validated male-specific genes are being knocked out using CRISPR-Cas9 technology and will be tested for their functional roles in male gametocyte development and gamete exflagellation.

### 1344

#### CHARACTERIZING THE POTENTIAL BIAS WITHIN GENOMIC TOOLS FOR INFERRING CHANGES IN *PLASMODIUM FALCIPARUM* TRANSMISSION INTENSITIES

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Substantial progress has been made globally to control malaria, however there is a growing need for innovative new tools to ensure continued progress. In response, a number of genomic studies have been conducted detailing the potential for utilising measures of *Plasmodium falciparum* genetic diversity to infer changes in transmission intensity and the role of intervention policies. This work has revealed that the complexity of infection (COI), genetic relatedness and degree of clonality could be used as metrics with which to guide programmatic surveillance efforts. These studies, however, have noted a need to better understand both the interacting role of super-infection and cotransmission upon genetic epidemiological models and the impact of transmission heterogeneity within the population. Here we present a mathematical transmission model to identify potential bias that may be introduced by these processes upon the predictive accuracy of these genomic tools. Using an individual-based model of both human and mosquito dynamics, we incorporate a parasite genetic barcode model that identifies parasites based on 24 unlinked SNPs. Our modelling predicts firstly that heterogeneity within the human population can lead to bias in estimates of the mean population COI, with preferentially sampling clinical cases and young children leading to potential underestimates of transmission declines as a result of interventions. As a result we identify optimal sampling schemes that ensure sufficient representation of the genetic diversity of the parasite population for accurate inferences of transmission intensity. Additionally we characterise the impact of increased rates of cotransmission, and through sensitivity analysis identify transmission settings in which the role of cotransmission leads to overestimation of transmission intensity based upon genetic diversity inference. Through highlighting where potential bias exists, we further show support for how genomic tools could be deployed effectively to provide real-time inference upon the effectiveness of intervention programmes.

### 1345

#### THE CONSEQUENCES OF CENSORING NEW INFECTIONS WHEN DERIVING ANTIMALARIAL EFFICACY AGAINST UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA

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The primary endpoint in efficacy trials of uncomplicated *P. falciparum* is polymerase chain reaction adjusted failure defined as the reappearance of the same parasite which caused the initial infection (recrudescence). Competing risk event (CE) of a new infection can be observed during the follow-up. These are censored in Kaplan Meier (K-M) analysis, the currently recommended statistical method for deriving failure. In the presence of CE, K-M is known to result in an overestimate of cumulative failure compared to Cumulative Incidence Function (CIF). The overall aim of this work is to quantify the bias due to the use of K-M in estimating cumulative failure in studies with artemisinin combination therapies (ACTs) in uncomplicated *P. falciparum*. Cumulative failure for recrudescence were derived using:

1 minus K-M estimate where competing events were censored, and CIF which accounts for competing event by still maintaining them in the risk set. The discrepancy between the two estimates was expressed as absolute and relative difference. The impact of study duration, proportion of recrudescences and new infections on the difference was investigated. Data on 31379 patients treated with ACTs from 87 trials (1996-2014) in Africa, Asia and S.America were available. There were 682 (2.2%) recrudescences and 3,738 (11.9%) new infections as classified by study investigators. A total of 85% (199/233) of treatment arms reported at least one new infection. On absolute scale, the overestimation using K-M was small with a median of 0 [range: 0- 0.06]. The median relative overestimation was: 2.4% [range: 0- 67.1%] and this increased with study duration: 0.6% on day28, 4.7% on day42, and 9.3% on day63. The relative overestimation was correlated with the proportion of new infections which becomes greater with longer follow-up (correlation 0.67 [95% CI 0.61-0.73]). Censoring new infections in the K-M analysis led to a marginal overestimation of cumulative failure of recrudescence which increased with study duration. The CIF approach is ideally suited to derive cumulative failure estimate when the observed proportion of new infection is high.

### 1346

#### SINGLE CELL GENOMICS OF MALARIA INFECTIONS

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Single-cell genomics can provide a means to determine the genetic structure of complex communities of unicellular organisms. Genetic analysis of infections with malaria parasites are complicated by multiple parasite lineages. These cannot be unambiguously determined from bulk resequencing efforts, severely restricting the effectiveness of association studies, and of our understanding of gene flow through parasite populations. To better understand parasite ecology at the level of individual cells, we have developed a method to capture single-cell haplotypes. Our optimized approach uses fluorescence assisted flow cytometry to capture singly-infected red blood cells, followed by whole genome amplification and next generation sequencing. By focusing our single cell method on replicating parasites we improve the overall success rate of amplification reactions (from 65% to 95%) and routinely generate near complete capture of the 23Mb parasite genome (mean genome coverage 90%). We use this approach to sequence hundreds of single genomes from an area of intense malaria transmission in Chikhwawa, Malawi. This data allows us to build a fine scale picture of the population structure of malaria parasites, both within and between infections.

### 1347

#### VARYING IMPACT OF MALARIA INTERVENTIONS AT DISTRICT LEVEL - IMPLICATIONS OF A MATHEMATICAL MODEL FOR STRATEGIC PLANNING

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National Malaria Control Programs (NMCPs) are tasked with identifying the most efficient intervention packages and their optimal deployment strategies. Mathematical models can provide guidance in this process by predicting the impact of potential intervention packages in different settings. Tanzania is currently re-defining the national malaria strategic

plan and goals for 2020 which should be tailored to local variations in the impacts of different interventions. We calibrated a population-based dynamic differential model at sub-regional level to provide support for the update of the 2017-2020 national strategic plan for Tanzania. Estimates of prevalence and intervention coverage were obtained from various country-wide datasets including malaria indicator surveys, Insecticidal Treated Nets (ITN) distribution data and predictions from the Malaria Atlas Project (MAP). Models were fitted to prevalence data while estimating the baseline level of transmission as well as the relative ratio between indoors and outdoors biting mosquitoes. The impacts of a selection of potential interventions were simulated per district, projecting the prevalence in 2020 and the average number of cases averted between 2017 and 2020. In low transmission and urban areas, ITNs are expected to have limited impact on the transmission intensity compared to Indoor Residual Spraying (IRS). Irrespective of other interventions, in high transmission settings, ITNs are indispensable and ITN coverage must be at least maintained to avoid rebound of both transmission and burden. At moderate transmission intensity both IRS with a high coverage and the maintenance of the current ITN coverage are expected to lower the transmission. The greatest impact was predicted when these were combined with increasing case management coverage. The modelling results suggest that depending on the local setting, the most appropriate interventions will vary, and the most effective strategy therefore involves sub-national stratification. The integration of this modelling framework into the NMCP strategic planning should ensure that an appropriate strategy will be implemented.

### 1348

#### POTENTIAL FOR ZIKA VIRUS TRANSMISSION FROM MATERNAL CIRCULATION TO FETAL BLOOD STREAM BY A PARAPLACENTAL ROUTE ACROSS AMNIOCHORIONIC MEMBRANE AND FETAL SKIN

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Zika virus (ZIKV) infection in pregnancy leads to fetal infection and a syndrome of severe birth defects. We previously reported that American and prototype African ZIKV strains infect human cytotrophoblasts and other placental cells, consistent with transmission across placenta, but also that amniotic epithelial cells (AmEpCs) lining the amniochorionic membrane are highly susceptible (Tabata, et al. *Cell Host & Microbe*, 2016). By mid-gestation, amniochorionic membrane abuts parietal decidua opposite the placenta. We therefore hypothesized two routes of transmission - one placental, from basal decidua and the intervillous blood space, and one paraplacental, from parietal decidua across the amniochorionic membrane. Here we explore paraplacental transmission by examining AmEpC infection and modeling transmission to fetal skin. We show that American and prototype strains infect mitotic AmEpCs and that cytokines and interferon are produced, which could modulate infection in the tissue environment. We also show that mid-gestation fetal skin explants (3 donors of 14-17 weeks) are susceptible to infection by the American strain and produce infectious progeny. Infected cell types identified by immunofluorescence and flow cytometry include keratinocytes and CD14+ monocytes. Paraplacental transmission could therefore proceed from maternal blood vessels in parietal decidua to susceptible cell types, including cytotrophoblasts invading parietal decidua, trophoblast progenitor cells in the chorion, and mitotic AmEpCs. Cytokine and interferon responses by AmEpCs could suppress infection while enabling persistent infection. Transmission to fetal circulation could occur through infected CD14+ immune cells associated with fetal vessels in skin. Alternatively, infection of amniotic epithelium could be secondary to fetal infection but nonetheless increase fetal exposure by prolonging or amplifying virus production. Our results suggest that fetal infection might be reduced by therapies that target infection of amniotic epithelium and dissemination to fetal skin.

### 1349

#### RAPID DEVELOPMENT OF A REPLICATING VIRAL RNA VACCINE FOR ZIKA VIRUS

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Due to the immediate and on-going public health threat posed by Zika virus (ZIKV), there is an urgent need for a safe and effective vaccine. In addition, platform technologies that facilitate rapid responses to emerging diseases are needed to address future infectious disease epidemics in a timely manner. Nucleic acid-based vaccine platforms have the potential to revolutionize the vaccine industry due to reduced cost, less complexity of manufacturing, and the potential for rapid vaccine design in response to new disease targets. Plasmid DNA vaccines are showing promise in the clinic. However, antigen expression requires delivery to host cell nuclei for RNA transcription and regulatory agencies have expressed safety concerns over potential for genomic integration events. RNA-encoded vaccines alleviate this concern, only requiring cytoplasmic delivery. However, this approach has historically suffered from other hurdles, including relatively low RNA stability, difficult delivery, and insufficient expression levels due to immune detection and host-translation shut-off. We have developed an RNA based vaccine platform, which is comprised of novel delivery formulations to enhance stability and cellular uptake and subsequent expression of RNA-encoded antigens, and an alphavirus-based replicating viral RNA (rvRNA). The rvRNA effectively subverts the host innate immune response through virus-specific mechanisms and amplifies an mRNA encoding the vaccine antigen for robust expression and immunogenicity. We applied this platform technology towards the development of a ZIKV vaccine. In an immunocompetent murine model of ZIKV infection, we demonstrated high-titer neutralizing antibodies following a single intramuscular or intradermal dose of our formulated rvRNA encoding the ZIKV structural protein genes. Seroconversion is observed as early as 14 days after vaccination with 100-fold dose sparing compared to unformulated RNA. The rapid and robust immunogenicity observed warrants further development of this technology in the application of vaccines for emerging infectious diseases.

### 1350

#### DIFFERENCES IN PREVALENCE OF SYMPTOMATIC ZIKA VIRUS INFECTION BY AGE AND SEX

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During the Zika virus (ZIKV) outbreak in Puerto Rico in 2016, non-pregnant women aged 20-39 years were disproportionately identified with ZIKV disease. We used household-based cluster investigations to determine if this disparity was attributable to age- or sex-dependent differences in the rate of ZIKV infection or reported symptoms. We offered participation to residents of households within a 100-meter radius of the residences of a convenience sample of 19 laboratory-positive ZIKV disease cases. Participation included answering a questionnaire and testing for: a) ZIKV RNA in serum and urine; and b) anti-ZIKV IgM antibody in serum. Participants were defined as ZIKV-positive if either assay was positive. We used general estimating equations with a binomial distribution and assumed an exchangeable correlation matrix to model associations among individual and household characteristics and binary outcomes of ZIKV infection and symptomatic ZIKV infection (defined by reported

rash or arthralgia in the previous six months based on a sensitivity analysis). Among 367 study participants, 114 (31.1%) were laboratory-positive for ZIKV infection; 17 for ZIKV RNA, 87 for ZIKV IgM, and 10 for both. Age, sex, income, and education were not associated with ZIKV infection. However, ZIKV infection was significantly more common among participants who: reported a household member ill in previous three months (adjusted odds ratio [aOR] = 2.6; 95% confidence interval [CI] = 1.3, 5.2); and, lived in a cluster with a greater number of vacant houses (aOR for 1-unit increase = 1.2; 95% CI = 1.0, 1.3). Prevalence of symptomatic infection attributable to ZIKV was 30%. Female sex (aOR = 5.7; 95% CI = 2.0, 15.9), age <40 years (aOR = 10.4; 95% CI = 3.0, 36.2), and asthma (aOR = 3.4; 95% CI = 1.1, 10.7) were independently associated with symptomatic ZIKV infection. While neither female sex nor age were associated with an increased risk of ZIKV infection, both were associated with symptomatic ZIKV infection. Further investigation to identify a potential mechanism of age- and sex-dependent differences in reporting symptomatic ZIKV infection is warranted.

## 1351

### INCIDENCE OF ZIKA VIRUS INFECTION AND EFFECT OF PRE-EXISTING DENGUE VIRUS EXPOSURE ON ZIKA VIRUS INFECTION AND DISEASE IN A PEDIATRIC COHORT IN NICARAGUA

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Zika virus (ZIKV) was introduced into Brazil in 2015 and spread rapidly across the Americas. In Nicaragua, the first case was reported in January 2016, followed by an explosive epidemic in July-August of 2016. We developed sensitive and specific serological assays that enable detection of anti-ZIKV antibodies to determine the incidence of ZIKV infection in a prospective, community-based pediatric cohort study of dengue and Zika in Managua, Nicaragua. This study, ongoing since 2004, follows ~3,500 children aged 2-14 in a low- to middle-income area of Managua, the capital. Suspected Zika, chikungunya, and/or dengue cases and undifferentiated febrile illnesses are screened by realtime RT-PCR (rRT-PCR) for infection with all three arboviruses. Blood, saliva and urine samples are collected from suspected cases during the acute phase and at convalescence (2-3 weeks post-symptom onset). Additionally, annual blood samples are collected from all healthy subjects every March/April. A total of 314 Zika cases and only 4 dengue cases were confirmed by rRT-PCR in the cohort from January to October of 2016. To determine the incidence rate of ZIKV infection, we are analyzing 2016 and 2017 annual serum samples side-by-side using a highly sensitive and specific ZIKV NS1 blockade-of-binding ELISA as well as a ZIKV Inhibition ELISA to detect a ≥4-fold rise in antibody titer in paired samples. These data enable calculation of the Symptomatic to Inapparent (S:I) ZIKV infection ratio. We are also determining the effect of prior DENV exposure on ZIKV infection and disease incidence, S:I ratio, and disease severity by analyzing: 1) documented prior DENV exposure in the cohort, 2) number of DENV infections, 3) pre-existing cross-reactive anti-ZIKV antibody titers, and 4) pre-existing cross-reactive anti-DENV antibody titers. These measurements will enable estimation of the force of infection and Ro of ZIKV as well as evaluation of potential immune correlates of protection against and risk of ZIKV infection and disease.

## 1352

### ANALYZING THE IMMUNE RESPONSE TO ZIKA VIRUS USING REPORTER VIRUS PARTICLES, ANTI-ZIKV ANTIBODIES AND EPIOTOPE MAPPING

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We have analyzed the human immune response to Zika virus (ZIKV) using a number of novel tools. (1) Pseudoinfectious ZIKV reporter virus particles (RVPs) provide one round of infectivity in permissive cells, with a luminescent or fluorescent readout for infectivity. RVPs have been used to identify patient sera and individual monoclonal antibodies (MAbs) that neutralize ZIKV, and provide a rapid and reliable alternative to PRNT-based assays. We have also used RVPs to characterize MAb binding, including highly conformational MAbs that do not recognize ZIKV envelope protein expressed in cells. RVPs maintain the native antigenicity of wild-type virions allowing characterization of MAb binding by ELISA or biosensor. (2) Anti-ZIKV MAbs were isolated following immunization with DNA and sub-viral particles, and phage library panning with RVPs. We isolated 48 MAbs that bound specifically to conformational epitopes on prM/E from ZIKV, but not DENV, including a MAb that potently neutralized ZIKV RVPs (IC50 45 ng/ml). The MAbs are being used as critical reagents for ZIKV-specific immunodetection, diagnostic applications, and ZIKV neutralization studies. (3) An Ala-scan mutation library of ZIKV prM/E is being used to epitope map the immune response to ZIKV. We used Shotgun Mutagenesis technology to create a comprehensive ZIKV prM/E library of 672 single mutations arrayed in 384 well plates and expressed in human cells. We quantified immunoreactivity by high-throughput flow cytometry, enabling us to epitope map anti-ZIKV MAbs for a published study that included a highly neutralizing MAb, isolated from a Brazilian patient, that was protective in animal models of ZIKV fetal disease. The epitope location of this MAb suggested that it binds across adjacent protein E dimers, preventing rearrangements necessary for infectivity. We have also screened each individual prM/E variant for ZIKV particle budding and infectivity by applying the RVP system to the mutation library to produce ZIKV virions. This identified mutations that increased ZIKV RVP budding, which may be applicable to the design and production of anti-ZIKV vaccines.

## 1353

### ZIKA VIRUS CLINICAL ISOLATES REPLICATE IN HUMAN PROSTATE CELLS AND ORGANOID

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Zika virus (ZIKV) is an ssRNA Flavivirus, traditionally known to cause Zika fever but recently linked to Guillain-Barré Syndrome in adults and microcephaly in newborns. While ZIKV is mainly transmitted by *Aedes* species mosquitoes, numerous cases of sexual transmission have been reported during the current outbreak. Little is known about which host cell types or entry factors aid in mediating this sexual transmission. While ZIKV RNA has been detected in the semen of infected males as well as in the urine, saliva and plasma of their partners following sexual contact, ZIKV urogenital tract tropism remains poorly understood. Infected males have also reported symptoms including prostatitis and hematospermia, which indicate that the prostate may be involved in infection as a reservoir for viral replication. We are currently investigating ZIKV cell tropism in prostate cells using contemporary ZIKV clinical isolates from persons infected in Central and South America. To investigate whether ZIKV infects the prostate, we infected human prostate cells and organoid co-cultures with low passage ZIKV isolates. The prostate organoids are composed of both stromal mesenchymal stem cells and epithelial cells

to better recapitulate *in vivo* conditions. Using qRT-PCR to detect viral RNA and immunohistochemistry to detect active ZIKV replication, we determined that ZIKV infects and replicates in the organoids. Infectious virus was also cultured from infected organoids using limiting dilution assays. These results suggest low-level viral persistence can occur within the prostate over extended time periods and might ultimately explain the presence of ZIKV in semen. Virus produced by infected prostate cells may be introduced into the seminal fluids secreted with sperm cells during ejaculation. Currently, we are using flow cytometry to determine which of the known flavivirus attachment factors are expressed in the organoids. Assessing ZIKV urogenital tract tropism and elucidating host cell attachment factors are imperative to better understand the mechanism of ZIKV pathogenesis and sexual transmission.

### 1354

#### REPLICATION OF ZIKA VIRUS AND CYTOMEGALOVIRUS IN FIRST-TRIMESTER HUMAN PLACENTAS SHOWS DIVERGENT PATTERNS OF INFECTION AND PATHOGENESIS THAT COULD AFFECT TRANSMISSION

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Zika virus (ZIKV), a *Flavivirus* responsible for the recent American epidemic, causes Congenital Zika Syndrome, a spectrum of severe malformations in the fetus/infant including microcephaly rarely seen with other neurotropic viruses. The exception is human cytomegalovirus (HCMV), a *Herpesvirus*, the leading viral cause of congenital infection and birth defects in the U.S. How these viruses are transmitted from disparate infectious sources - mosquitoes and toddlers, respectively - to maternal circulation and how they disseminate to the fetus *in utero* is poorly understood. We reported that ZIKV replicates in primary cells isolated from human placentas and amniochorionic (fetal) membranes and in explants of first-trimester placentas and infectious progeny are produced (Tabata, *et al.* Cell Host & Microbe, 2016). Here we compared ZIKV and HCMV infection in anchoring villus explants, a functional model of the developing human placenta. Patterns of ZIKV infection revealed that the American Nicaraguan and prototype African ZIKV strains replicate in cytotrophoblasts and Hofbauer cells (fetal macrophages) and release infectious virus. Consistently reproducible infection was observed in proliferating cytotrophoblasts in cell columns and Hofbauer cells near villous cores. Among 355 villus explants dissected from 6 placentas 8 to 11 weeks in gestational age, productive ZIKV infection was consistently observed in proliferating cytotrophoblasts in cell columns and branching villi (23%), invasive cytotrophoblasts (46%) and Hofbauer cells (22%). In contrast, HCMV failed to infect Hofbauer cells and only pathogenic clinical strains replicated efficiently in villus explants but nonetheless failed to release infectious progeny. Our results suggest that ZIKV and HCMV reach the developing placenta by hematogenous dissemination in the intervillous blood space, but patterns of infection and pathogenesis differ suggesting that the mechanisms of transmission could also diverge *in utero*.

### 1355

#### PROTECTIVE EFFECTIVENESS OF SEASONAL MALARIA CHEMOPREVENTION IN NIGER: A PROSPECTIVE CASE-CONTROL STUDY

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Seasonal malaria chemoprevention (SMC) is recommended in the Sahel: monthly courses of sulfadoxine-pyrimethamine and amodiaquine (SPAQ) are given to children aged 3-59 months during the high transmission

season. Despite high SMC program coverage, malaria continues to overwhelm health structures in Magaria District of Niger, so we aimed to estimate the protective effectiveness of SMC (PESMC) in field conditions. We conducted a prospective case-control study, stratified by SMC distribution method (directly-observed vs. non-directly observed first doses). Cases of clinical malaria (fever+positive pLDH RDT) were enrolled. Three age-matched healthy controls were enrolled in the case's village of origin on the same day. Caregivers were asked about receipt of SMC, access to care, demographics, diet and socio-economic status. Thick and thin smears were prepared and blood was collected to measure plasma levels of amodiaquine. We estimated that 590 cases and 1770 controls would provide 90% power to describe PESMC of 50% with 5% precision. Conditional logistic regression was used to compare cases and controls; PESMC was calculated as (1-OR)x100%. 577 cases and 1700 controls were enrolled between 1 Aug and 2 Dec 2016. Among children with a card proving receipt of SMC, PESMC against clinical malaria was 85.1% [95%CI: 78.7-89.6]. When children without a program card but whose parents reported receipt of SMC are also considered, PESMC was 50.2% [27.6-65.7]. PESMC was significantly higher in the first-dose DOT zone than in the first-dose non-DOT zone: with card 96.8% [93.1-98.5] vs 59.1% [34.5-74.4],  $p < 0.001$ , and with card or verbal report 88.6% [77.7-94.2] vs 20.5% [-30.5-51.5],  $p < 0.001$ . Similar trends were seen for PESMC against microscopy-confirmed malaria and asymptomatic parasitemia. Among children with cards proving receipt, overall point estimates of PESMC were above 70% for 4 weeks after each SMC distribution. In conclusion, important differences in PESMC were seen with different distribution strategies. Analysis of plasma amodiaquine levels is ongoing and will provide important information about adherence to treatment.

### 1356

#### COMMUNITY-LED IMPLEMENTATION OF INTEGRATED MALARIA CONTROL IN SOUTHERN MALAWI

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Sub-optimal coverage of malaria interventions limits the potential for achieving malaria elimination. Additionally, bed nets and indoor residual spraying are not universally sufficient for elimination, especially in areas with outdoor transmission or insecticide resistance. Community engagement strategies may increase the use of interventions by populations at risk through increased acceptability and understanding. Integrating additional vector control interventions with current strategies could lead to further reductions in transmission. We have established a multi-disciplinary project with an emphasis on community engagement and locally-appropriate integrated vector management to address these challenges in a rural community of 25,000 people in southern Malawi. Community engagement has been facilitated by training community volunteers as health animators and supporting the organisation of village-level committees in collaboration with a non-governmental organisation working in the community. Larval source management (LSM) and structural house improvements (HI) are implemented as additional vector control interventions. We are measuring the effectiveness of LSM and HI using a randomised block, 2x2 factorial, cluster-randomised trial design from 2016 to 2018. Household-level cross-sectional surveys are conducted on a rolling, 2-monthly basis to measure parasitological and entomological outcomes over three years, beginning with one baseline year. The effectiveness of the community engagement is being assessed through participant observations, focus group discussions, in depth interviews, and standardized surveys. In the baseline year, the prevalence of parasitemia in

children aged 6-59 months varied seasonally, ranging from 5% to 65%. In the same year, malaria vector mosquito densities ranged from 0.0 to 0.5 *Anopheles* females per house per night. In this presentation, we will discuss lessons learned regarding community engagement and adaptation of LSM and HI to the local ecological and social setting.

### 1357

#### INSECTICIDAL WALL LINING FOR MALARIA CONTROL IN LIBERIA: RESULTS FROM A CLUSTER RANDOMIZED CONTROL TRIAL

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Control of malaria vectors in Liberia depends on pyrethroid-impregnated long-lasting insecticidal nets (LLINs). Despite regular mass distribution, LLIN usage is patchy, and pyrethroid resistance in the key vectors may further reduce their efficacy. Durable, insecticidal wall liners (DL) which create a long-lasting insecticidal surface on walls are a promising new vector control method. The current study is a cluster-randomized control trial of a prototype wall liner impregnated with Fenpyroximate and Avermectin. In 20 clusters, rooms of consenting homeowners received DL in addition to existing LLINs. In 20 control clusters, homeowners used only LLINs. Community-based teams were trained to install DL using locally-available fasteners and nailing strips. *Plasmodium falciparum* prevalence in children 2-59 months was measured by rapid diagnostic tests before DL installation and again 12 months later. DL retention was measured 12 months after installation. A qualitative study of recipient and installer perceptions was conducted during installation and again after 18 months. Malaria prevalence in intervention clusters after 12 months was 34.6% compared to 40.1% in control clusters ( $p=0.052$ ), despite reduced LLIN usage where DL was installed. Wall lining was installed in 69% of rooms surveyed 12 months after installation. Homeowners without the lining cited absence at the time of installation (35%) or the room being under construction (33%) as the most common reasons for not installing DL. Perceptions of the community-based installation process were frequently positive, though additional non-cash incentives were requested. Recipients of DL cited aesthetic improvements to their rooms and perceived reduction in nuisance mosquitoes. These results show that community-based teams can successfully deliver DL. Despite lower LLIN usage after DL installation, we measured a small reduction in malaria prevalence after 12 months. Together, these results suggest that DL is a scalable intervention for control of pyrethroid-resistant vectors in locations where high LLIN usage is difficult to achieve.

### 1358

#### IMPACT OF INDOOR RESIDUAL SPRAYING WITH ACTELIC 300CS IN MALI ON KEY ENTOMOLOGICAL INDICATORS OF MALARIA TRANSMISSION

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According to the 2015 malaria indicator survey, 93% of households in Mali owned an ITN, while 71% of children under five years old and 78%

of pregnant women used an ITN the previous night. PMI supported IRS at the start of the short seasonal rains in July in three districts in 2016; namely Barouéli, Koulikoro, and Fana using an organophosphate, Actellic 300CS. Overall 228,672 structures were sprayed, with 788,922 persons protected. Entomological monitoring was conducted in six villages, (3 sprayed and 3 adjacent unsprayed), between July and December. Monthly pyrethrum spray catches (PSC) were conducted in 20 houses per site to determine indoor resting densities. Monthly human landing catches (HLC) were conducted in 8 houses per village to determine biting rates, time of biting, species composition and entomological inoculation rate (EIR). The residual activity of Actellic 300CS was assessed with WHO cone bioassay using susceptible, insectary reared *An. gambiae* in 19 houses. The residual efficacy of IRS with Actellic 300CS in Mali was two months according to WHO criteria of mortality >80%, although mortality was >60% after 5 months. Molecular analysis indicated *An. coluzzii* as the most common species (71.6%) followed by *An. gambiae* (17.3%). Biting rates were particularly high in August and September both indoors and outdoors with a peak in biting late at night between 22:00 and 4:00am. Therefore, people who frequently use LLINs should be more protected. The three IRS areas had lower indoor resting densities of *An. gambiae* s.l. for 2 months compared to unsprayed areas. Similarly, the human biting rate was lower in sprayed areas, with a mean of 6.1 bites p/person/night, compared with 11.9 in unsprayed sites. The combined effect of lower biting rates and similar sporozoite rates (2.1% IRS districts, 2.9% unsprayed areas) meant the EIR was 84.5% lower in sprayed sites compared to unsprayed sites over five months. However, the EIR in IRS areas was still high with a mean of 7.0 infectious bites per person over 5 months. In areas with intense seasonal malaria transmission, additional prevention and control tools may be needed beyond LLINs and IRS.

### 1359

#### EVALUATION OF THE RESIDUAL EFFECTIVENESS OF FLUDORA FUSION WP-SB, A FORMULATED COMBINATION OF CLOTHIANIDIN AND DELTAMETHRIN, FOR THE CONTROL OF PYRETHROID-RESISTANCE MALARIA VECTORS ON BIKO ISLAND, EQUATORIAL GUINEA

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Insecticide resistance to malaria vectors has been identified in some 60 malaria endemic countries. This has posed a global health challenge in the fight against malaria. Pyrethroid-resistance is the most commonly reported. Unfortunately, pyrethroids are not only relatively safe and less expensive, but also the only class of insecticides currently recommended for use in insecticide-treated mosquito nets. The global decline of indoor residual spraying coverage in recent times has been attributed to pyrethroid-resistance. The quest for safer insecticides with different modes of action against malaria vectors is a priority. As part of its vector control monitoring strategies, the Bioko Island Malaria Control Project (BIMCP) in Equatorial Guinea conducted routine insecticide resistance tests using the WHO's standard susceptibility tests from 2013 to 2016. During the same period, the frequency of the target-site knockdown resistance (*kdr*) in the local vector population was also determined using polymerase chain reaction based *kdr* genotyping. Biochemical analysis for metabolic resistance was also conducted in 2015. Fludora Fusion was evaluated for 9 months on Bioko Island from 2016 to 2017, using the WHO's standard test procedure for determining residual effectiveness of insecticides on sprayed surfaces. The product is a formulated combination of clothianidin and deltamethrin (a pyrethroid). In 2016, the percentage mortality of the vectors to 0.05% deltamethrin was as low as 38%. The frequency of the West African form of knockdown resistance (*kdr-w*) in the vector population was as high as 80%, and metabolic resistance analysis indicated high upregulated

cytochrome P450s. However, the residual effectiveness of Fludora Fusion recorded mortalities above 80% after 72 hours for 8 months. Although both target-site knockdown resistance and metabolic resistance to pyrethroids were implicated in the local malaria vector population, Fludora Fusion was effective under field conditions in controlling the resistant vectors for a period of 8 months on wooden surfaces on Bioko Island.

### 1360

#### THE IMPACT OF INDOOR RESIDUAL SPRAYING (IRS) WITH PIRIMIPHOS-METHYL ON ENTOMOLOGICAL INDICES IN A MALARIA HYPERENDEMIC REGION OF WESTERN KENYA

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The Lake Victoria region of western Kenya remains one of the most important sources of malaria transmission nationally. IRS was last conducted in western Kenya with deltamethrin in 2012. Spraying was interrupted due to pyrethroid resistance and a lack of registered alternative insecticides. IRS with pirimiphos-methyl was reintroduced in Migori County in March 2017 with 212,049 structures sprayed. Fifteen months of baseline vector surveillance was conducted between December 2015 through February 2017 in 12 sentinel villages (6 designated for IRS and 6 unsprayed). Post-IRS surveillance will continue through September 2017. Monthly collections were performed by pyrethrum spray catch (PSC), indoor CDC light traps (CDC-LT) and window exit traps. During baseline a total of 135 houses were sampled by PSC, 170 by CDC-LT, and 70 exit traps in each sentinel village. Malaria vector species comprised of 85% *An. funestus* s.s., 13% *An. arabiensis* and 1% *An. gambiae* s.s. *An. funestus* was the dominant vector throughout, with two peaks of indoor resting density and biting rates associated with high rainfall between December to February and May to July. *An. funestus* biting began before 18:00 indoors and outdoors but peaked between 4:00 am and 6:00 am, with indoor biting continuing until 7:00 am. A total of 5,073 samples were analyzed for sporozoite ELISA with a 3.28% overall sporozoite rate for *An. funestus*. Both adult collected *An. funestus* and larval collected *An. arabiensis* were fully susceptible to pirimiphos-methyl and bendiocarb but strongly resistant to deltamethrin and permethrin. Both East (L1014S) and West (L1014F) *kdr* mutation was absent in *An. funestus* and occurred in *An. arabiensis* at low frequencies of <1%. The majority of *An. funestus* caught in exit traps were unfed or gravid with a greater proportion of blood-feds and half-gravids resting indoors. IRS with pirimiphos-methyl is expected to have a major impact against pyrethroid resistant, indoor resting *An. funestus* that currently dominate. Analysis of the effectiveness of pirimiphos-methyl IRS on entomological indicators of malaria transmission up to six months after spraying will be presented.

### 1361

#### DIFFERENTIAL IMPACTS OF INDOOR RESIDUAL SPRAYING ON THE CHARACTERISTICS OF MALARIA INFECTIONS IN A HIGH TRANSMISSION SETTING IN UGANDA

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Significant progress in malaria control has been realized over the last decade. However, progress in Uganda has been slow, and gains have been fragile. In Tororo, indoor residual spraying of insecticides (IRS) has been associated with a dramatic decline in the incidence of symptomatic malaria, but the impact of IRS on the prevalence of microscopic and sub-microscopic parasitemia has not been reported. Between August 2011 and May 2016, we followed a cohort of 364 children aged 0.5 - 10 years and 105 adults living in 117 households in Tororo. IRS with bendiocarb was initiated in December 2014 and has been conducted every 6 months thereafter. Routine blood smears were performed at least every three months; 7139 samples from children, 1713 samples from adults) were tested for microscopic parasitemia, and *P. falciparum*-specific loop-mediated isothermal amplification (LAMP) was performed on all smear negative samples to test for sub-microscopic parasitemia. Following the implementation of IRS, the incidence of malaria decreased from 3.25 to 0.63 episodes/person/year ( $p < 0.001$ ), the prevalence of microscopic parasitemia decreased from 32.1% to 17.3% in children (prevalence ratio (PR) = 0.60, 95% CI 0.56-0.66,  $p < 0.001$ ) but there was no significant change in adults (6.1% vs. 6.4%, PR=1.04, 95% CI 0.75-1.43,  $p=0.82$ ). The prevalence of any parasitemia (microscopic + sub-microscopic) decreased following implementation of IRS in both children (67.5% to 32.4%, PR=0.42, 95% CI 0.39-0.45,  $p < 0.001$ ) and adults (52.2% to 26.1%, PR=0.43, 95% CI 0.36-0.52,  $p < 0.001$ ). Our results suggest that, although IRS was associated with significant reductions in the incidence of malaria and prevalence of parasitemia, a substantial proportion of the population remained parasitemic, providing a continued reservoir for malaria transmission.

### 1362

#### POPULATION GENOMICS IN *PLASMODIUM VIVAX*: LEVELS OF GENETIC DIVERSITY IN AMERICA

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The Americas were the last continent to be colonized by humans carrying malaria parasites. Not unexpectedly, *Plasmodium falciparum* from the New World shows very little genetic diversity and meiotic recombination, compared with its African counterparts, and is clearly subdivided into local, highly divergent populations. However, limited available data have revealed an extensive genetic diversity in American populations of another major human malaria parasite, *P. vivax*. We characterize 9 genome sequences from a population from northwestern Brazil and compare these new data with published sequences from recently sampled clinical isolates from Brazil (n=11), Peru (n=23), Colombia (n=31), and

Mexico (n=19) to further explore genome-wide variation and divergence patterns in New World populations of *P. vivax*. The following main features of New World populations were revealed: (a) These parasites are as diverse (nucleotide diversity  $\pi$  between  $5.2 \times 10^{-4}$  and  $6.2 \times 10^{-4}$ ) as *P. falciparum* populations from sub-Saharan Africa and *P. vivax* populations from Southeast Asia (malaria transmission is substantially more intense); (b) they display several nonsynonymous nucleotide substitutions (some of them previously undescribed) in genes known or suspected to be involved in antimalarial drug resistance, such as *dhfr*, *dhps*, *mdr1*, and *mp1*, but not in the *crt-o* gene; and (c) *P. vivax* populations in the Americas are much less geographically substructured than local *P. falciparum* populations, with relatively little between-population genome-wide divergence.

### 1363

#### FINE-SCALE POPULATION GENETICS OF *PLASMODIUM FALCIPARUM* IN NORTHERN NAMIBIA

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Namibia has a national goal to eliminate malaria by 2020, however pockets of transmission and risks of importation still remain in Northern regions bordering Angola and Zambia. Molecular epidemiology can reveal important insights regarding contemporary and historical transmission and assess the impact of sustained control and elimination efforts. A total of 1696 samples from symptomatic malaria cases collected in 2016 from 23 clinics in 3 districts of the Kavango East region (Rundu (n=611), Nyangana (n=382), Andara (n=430)) and 6 clinics from the Zambezi region (n=273) were genotyped using 26 neutral microsatellite markers. 551 (32.5%) of the samples had monoclonal infections, of which 511 were unique haplotypes. Most infections in Kavango region contained multiple parasite genotypes (71%) with mean multiplicity of infection (MOI) of 2.4, high within-host diversity (mean Fws=0.7) and high genetic diversity (mean HE = 0.74), reflecting high rates of transmission and superinfection present during the regional outbreak which occurred at that time. Fine-scale temporal and spatial variation was observed in MOI consistent with the history of transmission intensity within this region. In contrast, Zambezi region had significantly lower polyclonal infection (49%), MOI (1.7), higher mean FWS score (0.86) and lower genetic diversity (0.69),  $p < 0.001$  vs. Kavango for all. In all districts, low but significant linkage disequilibrium (ISA: 0.01 - 0.04,  $p < 0.001$ ) and genetic differentiation between districts (Gst: 0.02-0.06,  $p < 0.001$ ) were observed. Genetic differentiation was strongly correlated with the distance between the districts ( $\rho = 0.95$ ,  $p = 0.003$ ), reflecting some population fragmentation due to isolation by distance along with spread of parasites between the districts via vector or human travel. The findings of this study suggest targeted elimination of malaria in one region would be difficult to achieve without a similar effort throughout Northern Namibia. Integrated analyses of genetic and human mobility data can give insight to accurately estimate routes of parasite importation and transmission dynamics in space and time.

### 1364

#### RELATING GENETIC SIGNATURES TO TRANSMISSION CONDITIONS RESPONSIBLE FOR THE EMERGENCE AND SPREAD OF MULTI-DRUG-RESISTANT PARASITES IN CAMBODIA

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Over the last decade, multiple independent emergences of artemisinin-resistant parasite strains have occurred in SE Asia. More recently the emergence and spread of parasites resistant to partner drugs in artemisinin combination therapies presents a challenge to the goal of elimination in the Greater Mekong Subregion. What are the characteristics of transmission networks in Cambodia that are responsible for the emergence and spread of ACT-resistant strains? Using a dynamical malaria model that tracks full parasite genomes, we have simulated a range of transmission topologies characteristic of malaria exposure in small forest-going populations. We relate the time series of genetic signatures observed in clinical samples — e.g. region of origin, drug-resistant markers, relatedness of parasite strains within and among infections — to infer the local intensity of transmission and connectivity between regions. We find that small effective populations, frequent founder effects, focal risk, long-range migration, and drug-usage patterns significantly affect the rate of selecting resistance in our simulations. Finally, we demonstrate the value of timely genetic surveillance to inform future treatment strategies in a world of widespread ACT failure.

### 1365

#### COMPARATIVE LONGITUDINAL POPULATION GENOMIC SURVEYS OF *PLASMODIUM FALCIPARUM* MALARIA PARASITES IN FRENCH GUIANA AND THAILAND

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Drug-induced selection pressures combined with changing epidemiological patterns can create strong signals in population genomic datasets, especially when the selected variants are new mutations and when they only originate once. Conventional tests for selection on contemporaneous sample collections are less powered to detect selection on pre-existing variants, or selection that acts on particular combinations of variants in different parts of the genome. Longitudinal population genomic surveys can complement these deficiencies and reveal the pattern and process of resistance evolution. Artemisinin combination therapies (ACTs) are exerting selective pressure on *P. falciparum* in northern South America, a region where *de novo* resistance to previous antimalarial drugs has repeatedly emerged in the past. We sequenced the genomes of a longitudinal collection of 243 *Plasmodium falciparum* parasite samples from French Guiana spanning 17 years to identify mutations that have changed in frequency due to natural selection or genetic drift. We identified and annotated single nucleotide polymorphisms (SNPs) from Illumina sequencing data and evaluated the functional enrichment and classes of SNP alleles that increased or decreased in frequency over time. We also intersected these results with a previous study we performed of

longitudinal population genomic variation in *P. falciparum* in Thailand, an epicenter of ACT resistance. In both locations we find evidence of changing allele frequencies in genes belonging to pathways previously implicated in artemisinin resistance (phosphatidylinositol phosphate regulation, proteasome/ubiquitination), as well as pathways that may represent an evolutionary response to drug pressure that does not explicitly confer drug resistance. We discuss the potential for this approach to detect early signs of ongoing adaptation and the implications of these findings for the mechanism of artemisinin resistance.

## 1366

### DUAL RNA SEQUENCING IDENTIFIES NOVEL HOST BIOMARKERS OF *PLASMODIUM* HEPATIC INFECTION

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While efforts to ultimately eradicate malaria expand, the liver-stage of *Plasmodium* has gained in importance. As the first major step in the human cycle of infection, the liver-stage of *Plasmodium* infection represents the critical window for prophylactic intervention. The parasite burden in hepatic development is also substantially lower than in later human stages and therefore represents a critical developmental bottleneck in the parasite lifecycle. Several recent studies, using newly developed genetic tools, have identified host cell factors and receptors, such as EphA2, which play a critical role in parasite exoerythrocytic development. In order to expand our understanding of liver-stage parasite biology, we report the findings of a dual RNA sequencing study to identify new host-parasite interactions via patterns of genetic dysregulation during hepatic infection. GFP-expressing *Plasmodium berghei* sporozoites were used to infect huh-7.5.1 liver cells, sorted via flow cytometry and combined host and parasite RNA was then extracted, sequenced and analyzed. We identified 3,142 host and 3,788 parasite genes with differential expression patterns during the *Plasmodium* exoerythrocytic lifecycle. These differentially expressed genes were validated through a variety of procedures, including comparison with a separate dual RNA-seq dataset from an independent human hepatocyte cell line (HC04), qPCR in multiple human cell lines as well as primary human hepatocytes and immunofluorescence in both *P. berghei* and *P. vivax*. Of all the differentially expressed genes identified, the most prominent was the host-factor *mucin13* (*muc13*). We observe that *muc13* is highly upregulated during late-stage parasite development in both *P. berghei* and *P. vivax*. In addition, genetic alteration of *muc13*, via either shRNA knockdown or CRISPR/Cas9 knockout, indicates that altered *muc13* levels affect parasite development. We believe *muc13* represents a novel host biomarker of parasite infection, the characterization of which will increase our understanding of both parasite detection and hepatic development and advancing malaria eradication efforts.

## 1367

### *PLASMODIUM VIVAX* WHOLE GENOME SEQUENCING TO ASSESS GENETIC RELATEDNESS OF POLYCLONAL RELAPSES IN CAMBODIA

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We previously performed targeted deep sequencing of *pvmsp1* in a Cambodian cohort, including 22 persons who developed 29 *Plasmodium vivax* recurrences in the absence of primaquine treatment. We found frequent multiclonal infection and recurrences with homologous strains suggestive of relapse. We are now pursuing whole genome sequencing of these relapses. For 10 isolates from 3 patients who each suffered multiple relapses containing homologous strains, *P. vivax* DNA was enriched by hybrid capture, sheared, then sequenced on an Illumina HiSeq2000. Reads were aligned to the Sal-1 reference genome and variants called using the GATK pipeline. Coverage  $\geq 5x$  was achieved across half the genome in 8/10 relapse isolates. We assessed genetic complexity and relatedness of the isolates in the context of 70 previously sequenced *vivax* genomes from the same region in northwestern Cambodia. The high complexity of infection found by deep sequencing was recapitulated on a genome-wide scale. 102,835 biallelic SNPs with 5x coverage across 80% of the 80 samples were used for an estimate of clonality based on the FWS statistic. All 10 relapse isolates were identified as polyclonal, with a median FWS of 0.60 (range 0.16-0.90). The FWS for each isolate decreased with successive episodes in 2/3 persons. Principal coordinates analysis of the 10 relapse genomes showed clustering by patient, indicating greater genetic relatedness among episodes within than between individuals. This genetic relatedness was recapitulated by STRUCTURE, which assigned isolates to three distinct "populations" corresponding to the three patients. Identity by descent analyses, with a framework to handle multiclonal infections, indicated a high degree of haplotype sharing among relapse samples within persons, consistent with our previous findings. All ten relapse samples aligned to the *pvmsp* VK210 clade. These analyses suggest that waves of reactivated hypnozoites within individuals are polyclonal and genetically related, with implications for distinguishing relapses from re-infections. Ongoing efforts are using whole genome amplification to sequence more relapses from the cohort.

## 1368

### ASSOCIATION BETWEEN DIFFERENT POLYMORPHISMS OF MTMR3 AND C1QTNF6 ON CHROMOSOME 22 AND SEVERE MALARIAL ANEMIA IN CHILDREN FROM WESTERN KENYA

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Severe malarial anemia [SMA, hemoglobin (hb)  $<5.0g/dL$ ] is the primary clinical manifestation of *Plasmodium falciparum* malaria in children ( $<5$  years). We used genome wide association studies and transcriptomics, to discover candidate genes associated with SMA. The selection strategy utilized polarized extremes of children ( $n=144$ ) with malaria stratified into low Hb (avg. Hb=4.1) and high Hb (avg. Hb=10.8) categories. For validation in the larger population ( $n=1,236$ ; age 3-36 mos.), we selected genetic variants based on an allelic distribution ( $>10\%$ ) for two genes: Myotubularin related protein 3 (MTMR3: -1207A/G, rs9614122 and 38935A/G, rs41158) and Complement C1q Tumor Necrosis Factor related protein 6 (C1QTNF6: -46 C/T, rs1001810 and 139 C/A, rs31015763). MTMR3 binds to phosphoinositide lipids and hydrolyze phosphatidylinositol phosphate, variation in MTMR3 is linked to lupus nephritis. C1QTNF6 increases phosphorylation of protein kinase B (PKB, AKT) and regulates proliferation, survival and angiogenesis. Global genomics revealed an increase in MTMR3 (2.65 fold,  $P=0.017$ ) and C1QTNF6 transcripts (1.30 fold,  $P<0.001$ ) in SMA. There was also copy number variation (CNV) gain for both genes. Logistic regression analysis controlling for confounding factors revealed that GG (38935) at MTMR3 was associated with susceptibility to SMA (OR=1.65; 95% CI: 1.10-2.46;  $P=0.015$ ). None of the MTMR3 haplotypes were associated with SMA. CT (-46) at C1QTNF6 was associated with susceptibility to SMA (OR=3.59; 95% CI: 2.07-6.22;  $P<0.001$ ). Haplotypic analyses showed that carriage of

CA (-46 C/139A) was associated with protection against SMA (OR=0.69; 95% CI: 0.46-1.03;  $P=0.068$ ), whereas TC (-46T/139C) was associated with susceptibility to SMA (OR=4.14; 95% CI: 1.25-13.67;  $P=0.020$ ). In addition, analysis of inefficient erythropoiesis [reticulocyte production index (RPI) <2.0] showed that C1QTNF6 CC139 genotype was protective against inefficient erythropoiesis (OR=0.49, 95%CI: 0.26-0.90,  $P=0.022$ ). These results demonstrate that variation in MTMR3 and C1QTNF6 alters transcription, susceptibility to SMA and erythropoiesis.

### 1369

#### HIV-EXPOSED BUT UNINFECTED INFANTS ARE AT INCREASED RISK FOR NEONATAL GBS DISEASE: SYSTEMATIC REVIEW AND META-ANALYSIS

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One million children die each year because of neonatal disease, *Streptococcus agalactiae* (group B *Streptococcus*, GBS) is the leading cause worldwide. Since 2010, evidence for an association between neonates born to HIV-infected mothers and GBS neonatal disease has been accumulating. We sought to assess to which extent HIV exposure of neonates is associated with GBS neonatal disease. We also assessed to which extent HIV infection in women is associated with maternal rectovaginal GBS carriage, the single most important risk factor for GBS neonatal disease. We searched MEDLINE, Embase, and Web of Science for studies assessing the association between neonatal GBS disease and HIV-status of the mother and studies that assessed the association between rectovaginal GBS colonization and HIV status in adult women, published up to 1 November 2015. We used the Newcastle-Ottawa Scale to assess quality of the studies and heterogeneity was assessed using the  $I^2$  statistic. A priori planned subanalyses were performed for the early-onset and the late-onset form of neonatal GBS disease separately. A total of 546 unduplicated citations were identified, 19 studies met the inclusion criteria. HIV-exposed neonates were more than twice as likely to have neonatal GBS disease compared to unexposed neonates (OR, 2.39;  $p=0.005$ ). HIV-exposed neonates were not at increased risk for early-onset neonatal disease (OR, 1.31;  $p=0.240$ ), but were 4.43 times more likely to have late-onset neonatal GBS disease (95% CI,  $p=0.001$ ). We built and will present a conceptual framework explaining these (discrepant) findings. There was no significant association between HIV infection status and rectovaginal GBS carriage (OR 1.09;  $p=0.55$ ), but these studies suffered from major limitations. Public health interventions preventing neonatal GBS disease are urgently needed for the increasing group of HIV-exposed neonates. Our conceptual framework identifies opportunities, such as different (breast) feeding strategies, for the clinical practice and global health policy to prevent disease. Well-designed studies should clarify the relation between HIV-status and GBS carriage.

### 1370

#### IMMUNOLOGICAL AND CLINICAL OUTCOMES OF HUMAN IMMUNODEFICIENCY VIRUS EXPOSED BUT UNINFECTED INFANTS COMPARED TO UNEXPOSED UNINFECTED INFANTS: A COHORT STUDY IN KISUMU, KENYA

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Approximately 1.5 million adults in Kenya are infected with HIV. Due to increased access to antiretroviral therapy and mother to child transmission interventions, the number of babies infected with HIV via vertical transmission is low resulting in a growing population: HIV exposed but uninfected (HEU) infants. While HEU infants are healthier than HIV positive infants, studies suggest that HEU infants/children experience increased morbidity, mortality, and hospitalizations compared to HIV unexposed uninfected (HUU) infants. We hypothesized that *in utero* exposure to HIV and/or HIV associated chronically activated maternal immune environment affects fetal immune development leading to immune dysfunction. We examined (1) the magnitude and duration of pro-inflammatory biomarkers in HEU vs. HUU infants using MagPix analysis, (2) the prevalence of *Plasmodium falciparum* malaria infection by PCR, (3) the prevalence and magnitude of antibodies against four vaccine antigens and fourteen different pre-erythrocytic and blood stage malaria antigens from birth to two years, and (4) the frequency and character of clinical events experienced by HEU vs. HUU infants. There were no significant differences in the levels of twelve different pro-inflammatory biomarkers in plasma of HEU vs. HUU infants at seven time points between birth (cord blood) and one year. Remaining analyses of laboratory studies are currently ongoing. Additionally, analyses of clinical events found that HUU infants experienced a greater number of sick visits as well as a greater number of severe disease diagnoses (severe malaria, severe pneumonia, gastroenteritis with severe dehydration) and hospital admissions compared to HEU infants. Possible explanations for these preliminary findings may include the prophylactic Bactrim given monthly up to 18 months, more frequent clinic visits, and more frequently replaced mosquito nets in the HEU infant cohort compared to the HUU cohort. Ongoing analyses will hopefully provide more complete information regarding the outcomes of HEU infants and help inform future recommendations and decisions concerning their care.

### 1371

#### USE OF BED NETS A SURROGATE MARKER FOR RISK BEHAVIOR TOWARDS HIV

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Despite huge global efforts, the incidence of HIV infection did not substantially decrease over the last years. Therefore, HIV prevention efforts need to more effectively identify and target individual risk groups. Information on bed net ownership and bed net use was collected during a population based cohort study in Southwest Tanzania, which investigated the occurrence of HIV, malaria and lymphatic filariasis (LF). A total of 2,189 participants from the Kyela District were included in the study in 2007, with 82% confirming the possession of a bed net. The prevalence of falciparum malaria was 7.1% in households without bed nets and, 4.1% in households with bed nets (RR 0.56, 95% CI 0.35 to 0.89,  $p=0.015$ ) and the prevalence of LF was 35.3% versus 23.6% (RR 0.76; 95% CI 0.64 to 0.91,  $p=0.003$ ) in the two groups respectively. HIV prevalence among the adult population was 21.0% in households without bed nets, but only 16.7% if a bed net was owned (RR 0.78; 95% CI 0.53 to 1.16,  $p=0.225$ ). For 1,360 individuals above the age of 14 years and during 3,819 person years (PY) of observation, 45 new HIV infections were observed. Adjusted for age, gender and socio economic status we found a significant association of bed net ownership and HIV incidence, with 2.82 new HIV infections/100 PY among those without bed net, compared to 0.97/100 PY among those with bed nets (RR 2.66; 95% CI 1.33 to 5.30,  $p=0.006$ ). When analyzing behavioral factors, we found significant differences in bed net ownership between different groups. Condom users were 5% (RR = 1.05, 95% CI 1.02 to 1.08,  $p=0.003$ ), circumcised individuals were 9%

(RR = 1.09 95%CI 1.05 to 1.14,  $p < 0.001$ ) more likely to own a bed net than non-users. Persons with more than one sex partners owned bed nets 8% less likely (RR 0.92 (0.87 to 0.97,  $p = 0.001$ ) with two sex partners and 16% less likely (RR = 0.84, 95% CI 0.77 to 0.92,  $p < 0.001$ ) with three sex partners. Religion or gender had no association with bed net ownership. Bed net ownership was associated with both, significantly lower HIV incidence and lowered behavioral HIV risk profile. These findings might be useful to identify persons at risk for HIV.

## 1372

### DEMOGRAPHIC AND CONTEXTUAL FACTORS ASSOCIATED WITH HIV MORTALITY IN AN AFRICAN COHORT IN EAST AFRICA AND NIGERIA

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Several studies have documented clinical factors as predictors of mortality among HIV. However, the contribution of contextual factors is not well documented. The African HIV cohort provides an opportunity to examine the potential influence of some of these. The African HIV Cohort Study prospectively enrolls adults at 11 PEPFAR-supported facilities in Uganda, Kenya, Tanzania and Nigeria. Data was collected on a variety of individual social factors at enrolment into the cohort and every 6 months thereafter. Mortality data from Jan 2013 to Feb 2017 was analyzed. Data from participants that had completed only a single visit was excluded unless status change data was available. Person time, mortality rates and site adjusted Cox proportional hazards models were fitted to evaluate the social and contextual factors associated with mortality. Data from 2172 HIV infected participants was used for analysis. Median age was 41 years (IQR: 34-48) and the majority were females (58.7%) and non-Catholic Christians (67.5%). During a median follow up period of 1.6 years (3796.07 PY), a total of 57 deaths were observed translating into a mortality rate of 15.02/1000 PY (95%CI: 11.58-19.47). Adjusted for site; age, gender, marital status, education and employment status did not predict mortality. Hazard for death was twice for Catholic Christian compared to non-Catholic Christian, HR 2.01(95%CI: 1.51-3.50,  $p = 0.014$ ). Hazard for death increased if a sibling or any immediate family member had been diagnosed with HIV, HR 5.6 (95%CI: 2.01-15.65,  $p = 0.001$ ) and HR 3.67(95%CI: 1.45-9.27,  $p = 0.006$ ) respectively, participant experienced domestic violence, HR4.9 (95%CI: 1.18-20.39,  $p = 0.029$ ), or homelessness/displacement, HR 7.62 (95%CI: 2.35-24.68,  $p = 0.001$ ) or use of traditional remedies for HIV/ other ailments, HR 3.66(95%CI: 1.75-7.68,  $p = 0.001$ ). Factors not associated with mortality included: distance from or time taken to get to health facility, travel costs, having a treatment supporter, alcohol consumption or cigarette smoking. Understanding contextual factors beyond achieving the goal of antiretroviral therapy is important in reducing HIV mortality.

## 1373

### RATE OF VIREMIA AND ITS PREDICTORS AMONG ADULT HIV INFECTED PATIENTS IN THE AFRICAN HIV COHORT

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Progress towards the UNAIDS 90-90-90 target requires regular monitoring of HIV treatment outcomes. We evaluated the rate of viremia and its associated factors in HIV infected participants in the African Cohort Study. The African Cohort Study prospectively enrolls adults at PEPFAR-supported facilities in Uganda, Kenya, Tanzania, and Nigeria. HIV management history and laboratory assessments are obtained at entry and every 6 months. Participants on antiretroviral therapy (ART) for >6 months were evaluated for viremia (HIV RNA >50 copies/ml) and change in CD4 count. A modified-Poisson generalized linear model was used to evaluate factors associated with viremia at the participant's most recent visit. As of June 2016, 1712 HIV-infected adults were eligible for this analysis across five recruitment locations: Kayunga, Uganda (16.4%); South Rift Valley, Kenya (39.4%); Kisumu, Kenya (17.0%); Mbeya, Tanzania (16.1%); Abuja and Lagos, Nigeria (11.0%). Viremia was observed in 315 participants (18.4%, 95% CI: 16.6% -20.2%); viral load >1,000 copies/ml in 171 individuals (10.0%, 95% CI: 8.6%-11.4%). Compared to Uganda, the risk of viremia at the most recent visit was statistically similar for the Kenyan sites, but was 2.56 and 3.42 fold higher for the Tanzanian and Nigerian sites respectively. In the multi-variable analysis, factors directly associated with viremia included any prior viremia (RR:1.54, 95% CI:1.01-2.37,  $p = 0.049$ ), viremia at the second most recent visit (RR:1.91, 95% CI: 1.24-2.92,  $p = 0.003$ ), being on second line ART (RR:1.49, 95% CI: 1.05-2.11,  $p = 0.027$ ) and having recently missed  $\geq 2$  days of ART by self-report (RR=1.90, 95% CI: 1.33-2.70). Increasing CD4 count at the most recent visit was inversely associated with viremia (RR: 0.51, 95% CI: 0.40-0.66,  $p < 0.001$ ). Results show that viremia in patients on ART in the African setting is common. CD4 count, past viremia, and ART adherence levels are predictors that should be closely monitored and can inform clinical practice. The association of second line therapy with viremia highlights the at-risk nature of this group and suggests a requirement for intensive monitoring and management.

## 1374

### CRYPTOCOCCAL ANTIGENEMIA IN HIV-INFECTED ADULTS IN THE AFRICAN COHORT STUDY

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In many low resource settings, patients with advanced immunosuppression continue to present late to antiretroviral therapy (ART) treatment programs with opportunistic infections. Cryptococcal meningitis is a co-infection associated with high risk of mortality before and after ART is started. The World Health Organization recommends routine Cryptococcus antigen(CrAg) screen in ART-Naïve adults followed by pre-emptive antifungal therapy if CrAg is positive for patients with a CD4 count less than 100 cells/mm<sup>3</sup>. The African Cohort Study(AFRICOS) prospectively enrolls HIV infected and HIV uninfected adults at 11 PEPFAR-supported facilities in Uganda, Kenya, Tanzania, and Nigeria. Serum CrAg was measured using latex agglutination(LA) at enrollment in HIV infected participants with CD4<200cells/ul irrespective of ART status. CrAg prevalence was estimated and assessed for associations with age, sex, CD4+ count, ART status and viral load using Student's t-test, chi2 test and a regression model as appropriate. From January 21, 2013 to March 1, 2017, 3,085 participants were enrolled in AFRICOS and 494 participants were eligible for CrAg screening, 97 had missing CrAg status. The seroprevalence of CrAg among the remaining 397 was 0.04%. The mean age for the CrAg positives was 42.0(SD11.2) compared to 39.2(SD9.9)

for the CrAg negatives. The proportion of males who were CrAg positive was 53.3%(8/15) versus 47.9%(183/397) who were CrAg negative. Sixty percent (9/15) of the CrAg positives were ART naive as opposed to 51.1%(195/382) who were negative. The mean CD4 count was 51 cells/mm<sup>3</sup> (SD50) for the CrAg positives while that of the negatives was 108 cells/mm<sup>3</sup> (SD 60). The mean log of viral load was 11.9copies/ml(SD2.1) for the CrAg positives versus 10.1(SD3.4) for the negatives. In the logistic regression model, CD4 count had a statistically significant influence on CrAg serostatus (P value=0.001) while viral load had borderline statistical significance (P value = 0.05). Age, gender and ART status had P values > 0.05. CrAg prevalence is low but CrAg screening is still warranted for all patients with CD4<200cells/ml irrespective of their ART status .

## 1375

### UNFAVORABLE TUBERCULOSIS OUTCOME ASSOCIATED WITH HIV, DRUG RESISTANCE, AND PREVIOUS TREATMENT IN INDONESIA

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Indonesia is a country with double burden of tuberculosis (TB) and HIV. The objective of this study is to determine factors associated with unfavourable TB treatment outcomes. A hospital-based TB Registry recorded data from 15 years old patients and older who were diagnosed with TB (ICD-10 code A15-A19). We analysed data collected from 1 January 2014 to 12 January 2016 in seven referral hospitals in Java and Bali Island. Data was analysed with SPSS ver. 22 and logistic regression was used to assess determinants of TB treatment outcomes. 2,051 TB patients were recorded. 1180 subjects (57.5%) were male and the median age was 38 years (IQR 27-50 years). 452 subjects (22.0%) had previous history of TB treatment. 1447 subjects (70.6%) had pulmonary TB, 580 subjects (28.3%) had extra pulmonary TB, 24 subjects (1.2%) had pulmonary and extra pulmonary TB. 216 subjects were HIV positive (10.5%) and 296 subjects had DM (14.4%). 248 subjects (12.1%) were resistant to at least one anti-TB drug by either X-pert or drug susceptibility test. Treatment outcome was unknown for 135 subjects (6.6%) and was unavailable for 1137 subjects (55.4%) who were still on treatment. Among 779 subjects with outcome, 358 (46.0%) were treatment success, 248 (31.8%) transferred out, 112 (14.4%) defaulted, 46 (5.9%) died, and 15 (1.9%) failed. After controlling for other factors, mortality was associated with resistance (P=0.000; aOR 53.1; 95% CI 9.7-290.0) and HIV (P=0.016; aOR 9.3; 95% CI 1.5-57.8), while unsuccessful TB treatment outcome was associated with resistance (P=0.001; aOR 8.2; 95% CI 2.4-28.2) and previous TB treatment (P=0.000; aOR 4.7; 95% CI 2.0-11.1). Engaging TB patients with their treatment remains a challenge in Indonesia, especially those with coinfections, comorbidities, and drug resistance. More effort should be made to identify drug resistant TB and HIV, as well as maintaining compliance to TB treatment.

## 1376

### SEROPREVALENCE OF CHIKUNGUNYA IN VIETNAM - EVIDENCE OF PAST BUT NOT PRESENT TRANSMISSION

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Arboviral infections have been a serious concern in tropical countries due to high-levels of transmission and complex transmission dynamics. Currently in Vietnam, there is a focus on dengue and Zika virus. As the global interest in chikungunya virus (CHIKV) is rising [1] an estimated 1.3 billion people are living in areas of potential transmission [2], the information about the CHIKV activity in Vietnam remains tenuous. Previously there have been only brief mentions of chikungunya cases in a few papers, mainly without solid evidence and data [2-5]. Furthermore,

those references were only about CHIKV in Vietnam a half decade ago, and there is little to no information about the current situation. The fact there have been recent outbreaks in the neighboring countries - Cambodia and Laos [6, 7] - leads us to pay more attention to chikungunya transmission in Vietnam. We performed a systematic review of CHIKV in Vietnam until present. We also conducted a seroprevalence survey using the chikungunya ELISA IgG test on serum samples from An Giang province (n = 91) and Ho Chi Minh city (n = 91) from 2015. The findings give us a certain evidence of past CHIKV activity: 25/182 seropositive (13.2%) overall samples, 9/91 seropositive (9.9%) with the age-adjusted value 10.44% in An Giang province, and 15/91 seropositive (16.5%), with the age-adjusted value 12.79% in Ho Chi Minh City. However, the age-stratified seroprevalence suggests that the last endemic ended about 30 years ago, and that there may be no current transmission activity in these places. The R0 for the past epidemics were calculated as R0 =1.12 and R0 =1.15 in An Giang and Ho Chi Minh City respectively. In conclusion, we find evidence of CHIKV outbreaks in the past, but little evidence of recent transmission. This is remarkable considering that the vectors of CHIKV have a high environmental suitability to Vietnam and are the same as dengue, which is known to have high transmission in Vietnam. Therefore, the possibility of future chikungunya transmissions remains, especially as we have shown here, when the proportion of the population that is susceptible is high.

## 1377

### CHARACTERIZATION OF SINDBIS VIRUS CIRCULATING IN KENYAN ECOSYSTEMS

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Sindbis virus (SINV) is one of the most widespread mosquito-borne viruses, with human outbreaks known to occur in Northern Europe and parts of Africa. Presently, little is known about Sindbis virus strains circulating in Kenya. We analyzed five SINV strains isolated in Kenya from mosquitoes trapped from diverse species and geographic locations between 2007 and 2013 to gain insight into their phenotypic and evolutionary aspects. In-vitro growth kinetic assays were determined by virus titration in Vero cells. Phylogenetic relationships and evolutionary inferences were performed using maximum likelihood and Bayesian statistical inference approaches. Genetic and adaptive molecular analyses were carried out targeting the virus envelope glycoproteins (E1, E2) and non-structural protein (nsP4) genes. In-vitro growth assays showed that one Kenyan SINV isolate had highest replication efficiency in culture. Phylogenetic analysis revealed that all the Kenyan SINV isolates belonged to genotype 1, clustering with strains originating mostly from northern Europe and other African countries. Alignment of protein sequences of the E1, E2 and nsP4 genes revealed a significant proportion of conserved sites among the Kenyan and foreign SINV strains. Evolutionary analysis predicted that the earliest introduction of SINV in Kenya may have occurred around the shores of Lake Naivasha 400 years ago. Evolutionary rate of SINV was estimated to be 6.89X10<sup>-5</sup> substitutions/site/year (95% highest posterior probability (HPD); 2.47X10<sup>-5</sup>-1.15X10<sup>-4</sup>). Natural selection analyses indicated that SINV E1, E2 and nsP4 protein encoding genes were predominantly evolving under negative selection. From 2007 to 2013, our results indicate that circulating SINV strains in Kenya were largely genetically similar to those detected in other African countries and northern Europe where human outbreaks have been reported. There is need to assess the public health impacts of SINV in Kenya and further surveillance is needed to establish the molecular diversity among SINV strains in the African region.

## 1378

**PREVALENCE OF CHRONIC JOINT PAIN FOLLOWING CHIKUNGUNYA INFECTION FROM A COLUMBIAN COHORT**

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Chikungunya (CHIK) fever is a viral illness spread by mosquitos that presents with fever, headache, muscle pain, rash and joint pain. Outbreaks have previously been restricted to Africa, Asia, and Europe, causing chronic arthritis lasting months to years in these areas. In 2013, CHIK virus was found for the first time in the Americas and has now infected over 800,000 people. The objective of this study was to determine the prevalence of chronic arthritis after CHIK infection in a Latin American cohort and define the clinical characteristics associated with chronic arthritis symptoms. 485 Colombian patients with serologically confirmed CHIK were included in the study. Patients received a baseline and a 20-month follow-up symptom questionnaire. Comparisons of the reported symptoms were analyzed using chi-square or the Kruskal-Wallis. The baseline characteristics of the patients include mean age 49±16 years, 80% female, 94% Mestizo ethnicity, and 78% with high school or less education. Common comorbidities seen in the study sample were hypertension, diabetes, lung disease and depression. 25% of the patients reported current joint pain at 20-month follow-up. The patients with persistent joint pain in weeks had increased duration of initial joint pain (45.3±39.5) compared to the no persistent joint pain group (10.5±25.1) with  $p < 0.0001$ . Of the 79 patients that missed work, 49 patients had persistent joint pain ( $p < 0.001$ ). Of the 46 patients that had symptoms impact their capacity to continue normal activity, 33 had chronic joint pain ( $p < 0.001$ ). Patients with persistent joint pain had increased disease activity of swollen joint count (0.5±1.0) and tender joint count (2.9±2.3) compared to the no persistent joint pain groups (0.06±0.3, 0.2±0.8, respectively) with  $p < 0.0001$ . CHIK caused significant debilitating chronic arthritis in 25% of the patients at 20-months post infection. The baseline cytokine profile of cases of CHIK arthritis in comparison to age and gender matched controls without persistent arthritis will be evaluated to further understand the development CHIK arthritis and the biomarkers that may predict persistent arthritis.

## 1379

**CHIKUNGUNYA - A RE-EMERGED TROPICAL DISEASE - DEVELOPMENT OF A NEW VACCINE**

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Chikungunya virus (CHIKV) is a mosquito-borne virus resulting in many patients in chronic and incapacitating arthralgia affecting all gender and age groups. Coinciding with an adaptation enabling unusually efficient transmission by *Aedes albopictus* mosquitoes, the virus re-emerged in 2004 and rapidly spread over Africa, Asia, the Americas and locally also in Europe since then. Hence, CHIKV is regarded as one of the most-likely re-emerged viruses to spread globally and morbidity due to this virus is considered a serious threat to global public health raising an urgent demand for efficient prophylaxis. However, at present there is no treatment or vaccine available. Facing the unmet medical need for a prophylactic intervention we initiated a program to develop a vaccine candidate (VLA1553) against CHIKV infections. Valneva's live-attenuated CHIKV vaccine candidate is based on the La Reunion strain

of the East Central South African genotype, is produced in Vero cells and is characterized by a 60 amino acid deletion in its nsP3 viral replicase complex gene leading to attenuation of the virus *in vivo*. The safety, immunogenicity as well as protective efficacy of the vaccine candidate was evaluated in mice and non-human primates. We demonstrate that our vaccine candidate is highly attenuated in animal models and causes no clinical manifestations typically associated with wt CHIKV infections in addition to strongly reduced viremia and cytokine levels. Moreover, VLA1553 is highly immunogenic, induces a strong and long-lasting neutralizing antibody response in animal models and protects against a high dose wt CHIKV challenge. Due to its safe and immunogenic potential we will enter into a blinded, randomized phase 1 first-in-human clinical study investigating the safety and immunogenicity of three dose levels administered intramuscularly in a CHIKV-naïve population as a single-shot immunization designed to elicit long-term immunological memory.

## 1380

**LOW FIDELITY ARBOVIRUS VACCINE STABILITY**

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While live-attenuated vaccines (LAVs) against RNA viruses are inexpensive and immunogenic, there is a risk of reversion or pseudoreversion due to the high error rate during RNA virus replication. There has been interest in increasing the safety of these LAVs by altering virus polymerase fidelity, which lowers virus virulence while theoretically increasing vaccine stability. We have created a low fidelity version (LF) of TC-83, a LAV for the arbovirus Venezuelan equine encephalitis virus. Mice vaccinated with LF TC-83 exhibit no morbidity post-vaccination, and result in complete protection post-challenge, as well as higher neutralizing antibody titers than the parental TC-83. To determine how decreasing fidelity affects the stability of the LF genotype and phenotype in a transmission cycle, TC-83 and LF TC-83 were passaged *in vitro* and *in vivo*. This is crucial to examine, because mice vaccinated with LF TC-83 produce viremias identical to wild-type vaccines, suggesting no difference in the probability of transmission to the mosquito vector. To examine how mutations accumulate and virulence changes during growth in a permissive host environment, TC-83 and LF TC-83 were passaged 10 times intracranially in infant mice. After passaging, virulence was assayed by subcutaneous injection into infant mice. Both TC-83 and LF TC-83 gained virulence during passaging. However, passaged LF TC-83 remained significantly less virulent than TC-83, which increased time to death from 10 to 5 days post-infection. Sequencing revealed no reversion of attenuating mutations in either virus, suggesting the appearance of pseudoreversions. To determine stability in a transmission cycle TC-83 and LF TC-83 were also passaged serially or alternating between two hosts *in vitro* using mosquito and mammalian cells. After 6 passages, TC-83 accumulated a greater number of SNPs than LF TC-83, but there were no reversions of the attenuating mutations for either virus. This all suggests that LF LAVs are still able to propagate through a transmission cycle, but are less likely to fully revert than wild-type LAVs making them a potential strategy for attenuating vaccines.

## 1381

**DENGUE AND CHIKUNGUNYA HUMAN TRANSMISSION IN WESTERN AND COASTAL KENYA: GEOGRAPHIC, CLIMACTIC, VECTORIAL AND SOCIODEMOGRAPHIC RISK FACTORS FOR EXPOSURE AND DISEASE**

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Recent dengue (DENV) and chikungunya (CHIKV) outbreaks have occurred in Kenya; however, the extent of their transmission and their public health impact have gone unmeasured. In order to understand the burden of exposure and resultant disease, and the continuum of risk created by climate, vector abundance, and human infection, both active and passive human surveillance, linked to vector and weather data, are needed. Two cohorts of children (Jan 2014- present) were enrolled at four Kenyan study sites (rural west, rural coast, urban west, urban coast): a healthy child cohort followed every six months to document asymptomatic CHIKV and/or DENV infections via IgG ELISA testing, and an acutely febrile child cohort followed to convalescence to document symptomatic disease via PCR and IgG ELISA testing. Questionnaire data were collected to describe demography, socioeconomic status (SES), and household environment. All life stages of *Aedes* spp. vectors were collected monthly in each site and immatures were reared to adulthood for species identification. Weather variables were collected both locally using HOBO loggers and remotely by satellite. Overall prevalence was 4.2-5.9% for DENV and 3.7-5.5% for CHIKV. For acutely ill participants, 0.7% (13/1844) seroconverted for CHIKV and 5.4% (97/1790) for DENV. CHIKV was more common in the west (4.9% vs. 1.7%). DENV was more common in rural sites (5.4% vs 3.6%). Among healthy cohorts (500 children per site), 11 seroconverted for CHIKV (0.6%) and 3 for DENV (0.1%). Seroconversion for CHIKV or DENV was associated with age, SES, mosquito exposure and avoidance behaviors, and hygiene and wealth indices. Infections were spatially clustered in all sites, indicating important ecological risks. Increased vector abundance and human transmission were noted during dry seasons, likely due to unsafe water storage. These data demonstrate ongoing transmission of DENV and CHIKV across diverse regions in Kenya and undocumented disease burden. Spatial and temporal heterogeneities in transmission patterns point to the potential of social and vector interventions to reduce risk of human DENV and CHIKV infection in Kenya.

### 1382

#### UTILIZING CERVIDS AS SENTINELS FOR EVALUATION OF EASTERN EQUINE ENCEPHALITIS EMERGENCE IN MAINE

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Despite a long history of eastern equine encephalitis virus (EEEV) detection in northeastern states such as Massachusetts, New York, Connecticut, and Rhode Island, EEEV has only recently been detected in the most north eastern states. Specifically, in Maine, the virus was first detected in migratory birds in 2001. Following individual veterinary cases reported from 2005-2008, an epizootic occurred 2009 that expanded the spatial extent of virus activity from two counties (York and Cumberland) to include three additional counties (Kennebec, Waldo, and Penobscot) in central Maine. In order to determine the distribution of EEEV in Maine, a collaboration among the CDC, Maine CDC, and Maine Medical Center Research Institute initiated an investigation into the geographic range of EEEV antibody seroprevalence using white-tailed deer (*Odocoileus virginianus*) and moose (*Alces americana*) as sentinels. Samples were collected from statewide tagging stations throughout the firearm season from 2009-2014. Collected serum samples were tested by plaque reduction neutralization assay (PRNT) for EEEV seropositivity. Initial findings from 2009-2011, which were previously reported, indicated a widespread prevalence of EEEV antibodies with positive samples identified from 10 out of 16 counties. Our supplemental findings from 2012 - 2014 indicate the

presence of EEEV in all 16 Maine counties. Combined cervid seropositivity rates were 6.7% (41/609), 8.9% (69/771), and 10.7% (51/473) for 2012, 2013, and 2014, respectively. Herein we report the additional findings from samples collected from 2012 -2014 and discuss the state of EEEV in Maine.

### 1383

#### EPITOPE EXPOSURE ON THE OUTER FACE OF THE CHIKUNGUNYA VIRUS ENVELOPE DETERMINES ANTIBODY NEUTRALIZING EFFICACY

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Chikungunya virus (CHIKV) is a re-emerging alphavirus that infects millions of people and causes a debilitating arthritic disease for which no specific treatment is available. Like many alphaviruses, the structural targets on CHIKV that elicit a protective humoral immune response in humans are poorly defined. We have used phage display against virus-like particles (VLPs) to isolate seven human monoclonal antibodies (MAbs) against the CHIKV envelope glycoproteins E2 and E1. One MAb, IM-CKV063, was highly neutralizing (50% inhibitory concentration, 7.4 ng/ml), demonstrated high-affinity binding (320 pM), and was capable of therapeutic and prophylactic protection in multiple animal models up to 24 h post-exposure. Epitope mapping using a comprehensive shotgun mutagenesis library of 910 E2/E1 alanine-scan mutants demonstrated that IM-CKV063 binds to an inter-subunit conformational epitope on domain A, a functionally important region of E2. Subsequent publications have expanded on this using cryo-EM analysis, and by demonstrating that IM-CKV063 blocks both virus entry and virus release steps. MAbs against the highly conserved fusion loop have not previously been reported but were also isolated in our studies. The fusion loop MAbs were broadly cross-reactive against diverse alphaviruses but were non-neutralizing. Fusion loop MAb reactivity was affected by temperature and reactivity conditions, suggesting that the fusion loop is hidden in infectious virions. Visualization of the binding sites of 15 different MAbs on the structure of E2/E1 revealed that all epitopes are located at the membrane-distal region of the E2/E1 spike. Interestingly, epitopes on the exposed topmost and outer surfaces of the E2/E1 trimer structure were neutralizing, whereas epitopes facing the interior of the trimer were not, providing a rationale for vaccine design and therapeutic MAb development using the intact CHIKV E2/E1 trimer.

### 1384

#### EL NIÑO AND DENGUE PREDICTION IN ECUADOR

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El Niño and its impact on local meteorological conditions potentially influences interannual variability in dengue fever transmission in southern coastal Ecuador. El Oro province is a key dengue surveillance site, due the high burden of dengue fever, co-circulation of all four dengue serotypes, proximity to an international border and major port, and the recent introduction of chikungunya and Zika viruses. In this study, we used climate forecasts to predict the evolution of the 2016 dengue season in the city of Machala, following one of the strongest El Niño events on record. We incorporated precipitation, temperature and Oceanic Niño

Index forecasts in a Bayesian hierarchical mixed model to predict dengue incidence. The model was initiated on 1 January 2016, producing monthly dengue forecasts until October 2016. We accounted for misreporting due to the introduction of chikungunya virus in 2015, by using active surveillance data to correct reported dengue case data. We then evaluated the forecast retrospectively with available epidemiological information. The predictions correctly forecast an early peak in dengue incidence in March 2016, with a 91% chance of exceeding the mean dengue incidence for the previous five years. Accounting for the proportion of chikungunya infections that had been incorrectly recorded as dengue cases in 2015 improved the prediction of the magnitude of dengue incidence in 2016. The main advantage of this dengue prediction framework is the use of long-lead seasonal climate and El Niño forecasts, which permits a prediction to be made at the start of the year for the entire dengue season. Combining active surveillance data with routine dengue reports improved not only model fit and performance, but also the accuracy of benchmark estimates based on historic seasonal averages. This study advances the state-of-the-art of climate services for the health, by demonstrating the potential value of incorporating climate information in the public health decision-making process in Ecuador.

### 1385

#### EFFICACY OF A DENGUE PURIFIED INACTIVATED VACCINE CANDIDATE IN MACAQUES REVEALS INSIGHTS ON ACCURATE CHARACTERIZATION OF POST-CHALLENGE VIRAL REPLICATION AND ON CORRELATES OF PROTECTION

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The need for improved dengue vaccine candidates remains since the only licensed vaccine shows variable efficacy depending on dengue virus (DENV) serotype and age. We previously reported that different formulations of adjuvanted dengue purified inactivated vaccine (DPIV) developed by the Walter Reed Army Institute of Research were immunogenic and efficacious against DENV replication in macaques. A GSK DPIV candidate, adjuvanted with either aluminum hydroxide or AS03 and based on attenuated viral strains, has now been tested in macaques. While all tested formulations induced neutralizing antibody responses against the four DENV serotypes, only partial efficacy at preventing replication of DENV1 and DENV2 challenge strains was observed. Post-challenge viral replication was monitored by measuring viremia and RNAemia using both freshly collected and frozen/thawed sera samples. We observed that freeze/thaw had moderate or no impact on viremia quantification in sera derived from naïve macaques at the time of the challenge (control animals), whereas, in DPIV-vaccinated macaques, the viremia was markedly reduced when measured using frozen/thawed sera compared to freshly collected sera. In contrast, RNAemia quantification was not impacted by freeze/thaw irrespective of the DENV immune status of the animals at the time of the challenge, and RNAemia profiles were highly similar to viremia profiles when this was determined using freshly collected sera. These results suggest that measuring DENV viremia using frozen/thawed samples may bias the results and lead to a possible over-estimation of vaccine efficacy. While viremia on frozen/thawed samples has been largely used in the past decades, RNAemia might be a preferred alternative to viremia quantification to accurately assess DENV replication. Finally, a statistically significant negative correlation was detected between the pre-challenge DENV-neutralizing antibody titers and post-challenge RNAemia levels, further supporting the role of neutralizing antibody responses in controlling DENV infection.

### 1386

#### DENGUE VIRUS SEROPREVALENCE IN MEXICO

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Dengue is the most important arboviral disease in the world. From an epidemiological perspective, several indicators have been used to determine the level of endemicity in different areas. Seroprevalence has been proposed as a marker of endemicity, but seroprevalence studies are scarce at community level and have been concentrated in geographic areas where dengue transmission is intense. We conducted a cross-sectional study with a stratified cluster random sampling design to measure the level of seroprevalence of antibodies to dengue virus (DENV) in México. The target population was school children from 6-17 years old from 22 of 32 states in Mexico. These 22 states were clustered in 4 regions: Pacific, South-Central, South-East and Low endemicity region. The Primary Unit Samples were schools randomly selected among those located in communities under 1,800 meters above sea level and with more than 10,000 inhabitants. A total of 2,548 subjects provided blood samples after informed consent signature to detect IgG antibodies in the serum through indirect ELISA test (Panbio E-DEN 01G). The overall seroprevalence of antibodies against dengue was 33.5% (CI95% 27.5-40.1). The highest regional seroprevalence was found in the South East, reaching 70.9% (CI95% 60.3-79.7), followed by South Central (44.5%; CI95% 33.0-56.5) and Pacific (38.8%; CI95% 25.3-54.4). Seroprevalence was significantly higher in older than younger children in South East region: 62.1% (CI95% 46.9-75.2) in children 6-8 years; 71.3% (CI95% 59.3-80.9) among those of 9-12 and 82.6% (CI95% 73.8-88.9) in 13-17 years-old. However, this pattern was not consistent in all regions. We did not find differences related to gender. According to the results, DENV seroprevalence in Mexico is heterogeneous not only at country level but even at regional and state level. Besides, as known, seroprevalence is linked to long term exposure and do not adequately reflects recent pattern of transmission, therefore we conclude to avoid the utilization of only one epidemiological indicator to define endemic regions.

### 1387

#### DEVELOPING AND OPERATIONALIZING NATIONAL-LEVEL EARLY WARNING AND RESPONSE SYSTEMS (EWARS) FOR DENGUE AND OTHER Aedes-BORNE ARBOVIRAL DISEASES

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Dengue and other *Aedes*-borne arboviral diseases are a growing global health threat. Country ability to identify an impending outbreak and deploy an early response is key to reducing the health, societal and economic burden of these diseases. However, outbreak alerts are not currently used to trigger early response. Developing, testing and adopting early warning and response systems (EWARS) will help countries mount effective outbreak response systems. To provide countries with a toolset for accurate temporal and geospatial prediction of outbreaks, WHO/TDR and its partners developed a model contingency plan for dengue surveillance, outbreak prediction/detection and response, supported by an operational guide - a computer-assisted programme to facilitate the practical use of the early warning tool at district level. These tools are being further developed towards including *Aedes*-borne diseases at-large through consultations with stakeholders and partners. A WHO/TDR

expert consultation examined the current opportunities and challenges of developing and operationalizing national-level, early warning and response systems (EWARS). It discussed experiences drawn from field-testing of the EWARS operational guide and the research needs to improve and evaluate this tool by country health systems. This presentation will summarize the recommendations emerged from this meeting and the future studies needed. Well-functioning EWARS can, in principle, improve population health by translating early signals into a set of explicit and geographically-targeted response measures, and direct the mobilization of appropriate resources in a timely fashion at the appropriate scale (i.e. local, regional, national). EWARS developed for dengue should be refined and adapted to country needs; they should also be further developed for other Aedes-borne arboviruses. Both generic and disease-specific EWARS are required for Aedes-borne arboviruses.

## 1388

### PATTERNS OF CELLULAR IMMUNITY AFTER INFECTION WITH A HUMAN CHALLENGE STRAIN

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A deletion variant of the DENV2 Tonga/74 strain lacking 30 nucleotides from its 3' untranslated region has previously been established for use in a controlled DENV human challenge model. To evaluate if this model is appropriate to derive correlates of protection for DENV vaccines based on cellular immunity, we wanted to compare how the cellular immune response to this challenge strain compares to the response induced by natural infection. To achieve this, we predicted HLA class I and class II restricted peptides from rDEN2Δ30 and used them, in an IFN-γ ELISPOT assay, to interrogate CD8+ and CD4+ T cell responses in healthy volunteers infected with rDEN2Δ30. At the level of CD8 responses, vigorous *ex vivo* responses were detected in approximately 80% of donors. These responses were similar in terms of magnitude and numbers of epitopes recognized to previously reported responses observed in PBMC from donors from DENV hyper-endemic regions. The similarity extended to the immunodominance hierarchy of the DENV nonstructural proteins NS3, NS5, and NS1 being dominant in both donor cohorts. At the CD4 level, responses were less vigorous compared to natural DENV infection, and were more focused on nonstructural proteins. The epitopes recognized following rDEN2Δ30 infection and natural infection were largely overlapping for both CD8 (100%) and CD4 (85%) responses. Finally, rDEN2Δ30 induced stronger CD8 responses compared to other more attenuated DENV isolates.

## 1389

### OPTIMIZATION OF THE PLAQUE REDUCTION NEUTRALIZATION TEST ON 96-WELL PLATES FOR DIVERSE DENGUE VIRUS 1-4 STRAINS

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The plaque reduction neutralization test (PRNT, also called the immunofocus reduction neutralization test, FRNT) is the most accepted approach for quantifying neutralizing-antibody responses to dengue virus (DENV) infection. While it is customary to perform this assay on 6- or 24-well plates to make plaque counting easier, we have adapted and optimized the PRNT for 96-well plates, which enables researchers to test more serum samples at a time and use less serum volume. We tested various assay parameters, including virus-serum incubation time, cell growth conditions, and plaque staining. By adjusting the experimental conditions of the assay, we obtained maximal neutralization titers, uniformity of plaque size and count, and consistency in staining intensity across all four DENV serotypes. To maximize PRNT50, we assessed multiple virus-incubation times of the serum mix (30, 60, 90, and 120min). We found that by 90min the PRNT50 doubled from the 30min titer for DENV-positive antisera, without increasing PRNT50 titers in DENV-negative antisera, indicating no false positives. To obtain a confluent monolayer of viable C6/36 cells, we adjusted the volume of resuspended cells (100μl or 200μl) used to prepare the 96-well plates, the incubator humidity (80% or 90%), and the number of days between plating cells and starting the neutralization assay (1-5 days). We observed significantly fewer plaques in wells at the edge of the plate as compared to the centers of each plate under conditions of low humidity, low volume, and more days until starting the neutralization assay; direct investigation of the cells revealed edge wells had fewer cells. Finally, to standardize the immunostaining process across DENV serotypes, we tested different concentrations of 2H2 1° (0.25-0.5μg/ml) and peroxidase conjugated 2° (0.25-0.33μg/ml) antibodies and found that some DENV strains could not be reliably stained with lower mAb concentrations. Together, our analyses of factors that influence PRNTs in 96-well plates reveal assay conditions that reliably produce high quality wells of plaques, reduce edge effects, and generate a cost efficient neutralization assay.

## 1390

### FLAVIVIRUS SEROPREVALENCE IN THE DEMOCRATIC REPUBLIC OF THE CONGO

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Flaviviruses such as Zika virus and dengue virus are known to be widespread in the Americas, but little is known about their current prevalence in African countries. Interestingly, recent reports indicate that Zika may have circulated unrecognized for years in certain parts of Africa and Southeast Asia, illustrating two key points: 1) Emerging pathogens with epidemic potential may be present in human populations prior to abrupt expansion, providing a window of opportunity for detection and prevention; and 2) infections causing nonspecific, acute febrile illness may be misdiagnosed, leading to suboptimal clinical management of patients and resource allocation. To address these gaps, we tested for antibodies to Zika virus, dengue virus and yellow fever virus in children under 5 from the Democratic Republic of the Congo using 978 dried blood spots collected during the 2013 Demographic and Health Survey. Sera was eluted from blood spots and stored at -80°C prior to screening with an IgG antigen capture ELISA. Preliminary results indicate a general flavivirus seroprevalence of approximately 3%, with many samples containing antibodies that react to antigens from multiple viruses, suggesting possible cross-reactivity or multiple exposures. Neutralization assays will clarify specific exposures. To complement this retrospective analysis, a prospective study is underway to estimate prevalence of flavivirus infection among patients presenting with febrile illness in Uganda. Results will support the development of better approaches for prevention, diagnostics and treatment of flavivirus infections in Africa.

## 1391

**SPACE-TIME INTERACTION OF DENGUE CASES IN AN AGENT-BASED MODEL**

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Dengue is a common mosquito-borne disease in tropical and subtropical regions. For a better understanding of the dynamic nature of dengue transmission, many studies have been conducted to find ecological and environmental factors that are associated with dengue virus (DENV) transmission. Due to limited range of mosquito movement, dengue cases are spatially and temporally clustered. This focality and space-time interaction have been examined through the analysis of reported cases with spatial statistics. Ecological analyses are most often based on the aggregated populations in enumeration units (e.g., census tract). Additionally, agent-based models (ABMs) have been employed by integrating domain knowledge of dengue to address research questions through simulations. For simulation-based research, validation is an important process that determines whether or not a model has sufficient accuracy to address research questions. Nonetheless, it is often neglected due to difficulties in obtaining data suitable for comparison with model outputs. To mitigate uncertainty about model specification, sensitivity analysis (SA) is often employed. SA helps researchers to understand model robustness or sensitivity of model outcomes to parameters of the model. In addition, pattern-oriented modelling (POM) has been emphasized for the purposes of designing, testing, and evaluating simulation models. Here, we employ a pattern oriented framework that uses both population level infection rates and local spatial pattern of infections to calibrate the model. The parameters with respect to mosquito population density, mosquito behavior, DENV introduction rates, and DENV transmission that reproduce realistic local clustering of dengue virus infection are often outside the range of previous models. Our spatial-statistics-based framework may be used for validation of individual-level simulation models of other infectious diseases and dynamic processes with observed space-time pattern data.

## 1392

**IMMUNOGENICITY OF THE CYD TETRAVALENT DENGUE VACCINE (CYD-TDV) USING A COMPRESSED SCHEDULE: RANDOMIZED PHASE II STUDY IN U.S. ADULTS**Judith Kirstein<sup>1</sup>, William Douglas<sup>2</sup>, Manoj Thakur<sup>3</sup>, Mark Boaz<sup>3</sup>, Thomas Papa<sup>4</sup>, Anna Skipetrova<sup>4</sup>, Eric Plennevaux<sup>4</sup>*<sup>1</sup>Advanced Clinical Research, West Jordan, UT, United States, <sup>2</sup>Benchmark Research, Sacramento, CA, United States, <sup>3</sup>Sanofi Pasteur, Swiftwater, PA, United States, <sup>4</sup>Sanofi Pasteur, Lyon, France*

The live attenuated tetravalent dengue vaccine (CYD-TDV) has been approved for use in endemic areas using a 3-dose schedule at 0, 6 and 12 months. An effective shorter schedule would have important implications for both travelers to endemic areas as well as public health programs in dengue endemic countries. We compared immune responses of 2 schedules of CYD-TDV in a non-endemic population. We also evaluated the impact of yellow fever (YF) co-administration. This phase II, open-label, multicentre study (NCT01488890) enrolled 390 healthy 18-45-year-olds in the USA with no prior dengue exposure. Participants were randomized (4:4:4:1) to 4 groups stratified by prior YF vaccination: Group 1, CYD-TDV standard 0-6-12 months schedule; Group 2, CYD-TDV compressed 0-2-6 months schedule; Group 3, CYD-TDV compressed schedule with YF co-administered (dose 1); Group 4, YF vaccination only. Neutralizing antibody (Ab) geometric mean titers (GMTs) and percentages of seropositive subjects (Ab titers  $\geq 10$  [1/dil]) were measured against each dengue serotype using a 50% plaque reduction neutralization test. On D28 after the 3rd CYD-TDV dose, there were no marked differences in GMTs or seropositivity rates between Groups 1 and 2. In each group respectively, 73.4% and 82.4% were dengue seropositive for  $\geq 3$  serotypes, with 50.0% and 42.6% seropositive against all 4 serotypes. There was no difference

between subjects who were flavivirus seropositive and seronegative at baseline with either schedule. At 6 months after the 3rd dose of CYD-TDV, in Groups 1 and 2, GMTs and dengue seropositivity decreased compared with D28 post-dose 3 for all serotypes, with the exception of a small increase in GMT for serotype 4 in Group 1. Dengue seropositivity remained above 70% for serotypes 2, 3 and 4 in Groups 1 and 2. Co-administration with YF did not affect Ab responses against dengue or impact vaccine safety after completion of the compressed schedule, compared to dengue or YF vaccination alone. Based on 6-month data post-dose 3, CYD-TDV given in a compressed schedule in a non-endemic setting can elicit similar Ab responses to the licensed CYD-TDV schedule.

## 1393

**SPATIOTEMPORAL EPIDEMIOLOGY OF DENGUE IN MYANMAR 2012-2016**Win Zaw<sup>1</sup>, Zaw Lin<sup>2</sup>, July Ko Ko<sup>1</sup>, Neriza M. Pantanilla<sup>3</sup>, Steeve Ebener<sup>3</sup>, Richard J. Maude<sup>1</sup>*<sup>1</sup>Mahidol-Oxford Tropical Medicine Research Unit, Bangkok, Thailand, <sup>2</sup>Vector Borne Disease Control, Department of Public Health, Ministry of Health, Nay Pyi Taw, Myanmar, <sup>3</sup>AeHIN GIS Lab, Manila, Philippines*

Dengue is a major public health burden in Myanmar. The National Strategic Plan for Dengue Prevention and Control aims by 2020 to reduce dengue morbidity by 25% and mortality by 50%; and by 2025 to reduce morbidity by 50% and mortality by 90% based on 2015 data. To support efforts to reach these goals it is important to have a detailed picture of the epidemiology of dengue in the country with which to plan control efforts. Health facility-level data on numbers of dengue cases from 2012 to 2016 were obtained from the Vector Borne Disease Control Unit, Department of Public Health, Myanmar. A detailed analysis of routine dengue haemorrhagic fever (DHF) incidence data over space and time was conducted to examine the spatial and temporal epidemiology of DHF. Detailed maps of incidence were prepared and trends in numbers of cases analysed and related to climate. DHF was found to be widespread across the country. The temporal pattern of dengue cases and fatalities was episodic with annual outbreaks and no clear longitudinal trend. There were around 40,000 reported cases and over 100 deaths in 2013 and 2015 with much lower numbers in 2012 and 2014 and 10,771 cases and 58 deaths in 2016. Case fatality rate was around 0.5% throughout. The peak season of dengue cases was from May to August in the wet season but in 2014 peak dengue season continued until November. In most years, the majority of cases were in urban areas whereas in 2015 rural areas reported more cases (>25000 compared to >15000 cases). To meet the national goals will require concerted control efforts across much of the country engaging populations in both urban and rural areas.

## 1394

**SPATIOTEMPORAL EPIDEMIOLOGY OF DENGUE IN THAILAND 2010-2016**Nattwut Ekapirat<sup>1</sup>, Darin Areechokchai<sup>2</sup>, Nipon Chinanonwait<sup>2</sup>, Steeve Ebener<sup>3</sup>, Richard J. Maude<sup>1</sup>*<sup>1</sup>Mahidol-Oxford Tropical Medicine Research Unit, Bangkok, Thailand, <sup>2</sup>Bureau of Vector Borne Disease, Department of Disease Control, Ministry of Public Health, Nonthaburi, Thailand, <sup>3</sup>AeHIN GIS Lab, Manila, Philippines*

Dengue virus infection is a major cause of major public health problems in many tropical countries including Thailand. In Thailand, dengue is widespread, particularly in urban areas, and numbers of cases peak during each rainy season between June and September. Dengue incidence varies greatly over space and time each year and it is essential to have a highly effective surveillance system to identify risk areas and detect spread of the infection for targeting of control measures to limit the size of outbreaks. By working in partnership with the Ministry of Public Health (MOPH), detailed routine surveillance data from whole of Thailand from 2010 to 2016 were analyzed and mapped at subdistrict level to identify the highest risk areas and determine trends in case numbers over time.

These incidence data were combined with detailed information from the Thai government on climate, population distribution and health service provision to produce maps of dengue risk and healthcare care with which to help guide service provision and control activities. In Thailand, dengue is highly seasonal with peaks in the wet season each year and predominance in urban areas particularly Chiang Mai and Bangkok. Numbers vary greatly year to year with large outbreaks in some years, including over 140,000 cases in 2015. This had fallen to 38,466 confirmed cases reported in 2016. The majority infected were aged 10 to 34 years (56%). Data analysis is ongoing including producing detailed annual maps of incidence and risk and time series analyses to help inform planning of disease control activities.

## 1395

### INTEGRATED IMMUNOGENICITY ANALYSIS OF A TETRAVALENT DENGUE VACCINE (CYD-TDV) UP TO 4 YEARS AFTER VACCINATION

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The efficacy of the tetravalent dengue vaccine CYD-TDV against all 4 dengue serotypes was demonstrated in 2 large pivotal phase III studies (pooled efficacy rates of 60.3% against virologically-confirmed dengue) conducting to WHO recommendation and licensure in several endemic countries for the prevention of dengue in individuals aged  $\geq 9$  years. An integrated summary of the immunogenicity of CYD-TDV was conducted in 5,780 participants aged  $\geq 9$  months to identify the parameters driving the neutralizing humoral immune response and to evaluate persistence over time. The immunogenicity profile of a 3-dose schedule of CYD-TDV (3 doses administered 6 months apart) was established across 16 phase II and III trials undertaken in endemic and non-endemic countries. Neutralizing antibody titres (Nab) in sera were determined at centralized laboratories using the 50% plaque reduction neutralization test (PRNT50) at baseline, 28 days post-dose 3 (PD3), and annually thereafter for up to 4 years PD3 in some studies. CYD-TDV elicits Nab responses against all dengue serotypes; geometric mean titres (GMTs) increased from baseline to PD3 regardless of baseline dengue status, region and age. A trend towards lower GMTs against serotype 1 compared to other serotypes was observed. Age, baseline dengue seropositivity - usually increasing with age - and region influenced GMTs: i) GMTs were usually higher in adults and adolescents compared to younger participants in endemic regions; ii) baseline dengue seropositive participants in general achieved higher PD3 GMTs for all serotypes than those who were seronegative regardless of the region; iii) participants in endemic countries achieved higher level of Nab than in non-endemic countries. Differences by age and region of endemicity persist above baseline levels in endemic countries. No effect of gender or prior exposure to other flaviviruses was observed. In the two pivotal studies, GMTs decreased initially during the first 2 years PD3 but appear to stabilize or slightly increase again in the third year which is consistent with other persistence studies conducted up to 5 years PD3.

## 1396

### SOCIO-ECOLOGICAL FACTORS AND PREVENTIVE ACTIONS ASSOCIATED WITH DENGUE INFECTIONS AT THE HOUSEHOLD-LEVEL IDENTIFIED IN A PROSPECTIVE DENGUE SURVEILLANCE STUDY IN MACHALA, ECUADOR

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In Ecuador, dengue fever and other febrile diseases transmitted by the *Aedes aegypti* mosquito are among the greatest public health concerns in urban coastal communities. Community- and household-level vector control is the principal means of controlling disease outbreaks. The purpose of this study was to assess the impact of dengue prevention knowledge, attitudes, and practices (KAPs), as well as socio-ecological factors, on the presence or absence of acute or recent dengue infections in the household. As part of dengue surveillance in Machala, Ecuador, we invited individuals with an acute dengue illness to participate, along with other members of the household and members of four neighboring households. We conducted diagnostic testing for dengue on all study participants, and we surveyed heads of households (HOHs) regarding KAPs. We compared KAPs and socio-ecological factors between households with (n=139) versus without (n=80) acute or recent dengue infections, using both bivariate and multivariate models. In bivariate analyses (but not multivariate modeling), the presence of dengue infections was positively associated with HOHs who were male, employed, and of younger age than households without recent or acute dengue infections ( $p < 0.05$ ). Dengue infection was not associated with knowledge or attitude, or with reported barriers to prevention activities. Dengue infections were associated with application of chemicals to standing water, and less use of indoor fumigation. Households with dengue infections were more likely to have a patio with more than 50% shade or to have adjacent abandoned property, and were less likely to have piped water inside of the house or have their trash picked up daily ( $p < 0.05$ ). We also present the results of multivariate modeling. Specific effective actions that can be considered to decrease the risk of dengue infections in the household include reduction of shade on the property, fumigating inside the home, and use of mosquito nets. Community-level interventions include clean-up of abandoned properties, daily trash pick-up, and reliable piped water inside houses.

## 1397

### DESIALYLATION OF PLATELETS CORRELATES WITH THROMBOCYTOPENIA IN ACUTE DENGUE

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Thrombocytopenia and platelet dysfunction are common in dengue and associated with complications such as bleeding and plasma leakage. The etiology of dengue-associated thrombocytopenia is multifactorial and includes increased platelet clearance. Two mechanisms that mediate platelet clearance are increased binding of the coagulation protein von Willebrand factor (VWF) to the platelet membrane and removal of sialic

acid (desialylation) from the platelet membrane. Using flow cytometry-based assays, we first showed in an observational study in Bandung, Indonesia, that circulating platelets of adult patients with acute dengue had bound more VWF to their membrane, which correlated inversely with platelet count ( $R = -0.65$ ;  $P < 0.001$ ). Next, we showed in a consecutive observational study in dengue patients in Jepara, Indonesia, that sialic acid was removed from the platelet membrane, which was also related to the severity of thrombocytopenia. Sialic acid on the platelet membrane is neuraminidase-labile, but dengue virus has no known neuraminidase activity, and plasma neuraminidase activity of patients was not increased. In a series of *ex vivo* laboratory experiments, we were able to demonstrate that the binding of VWF to platelets, by the addition of the VWF-activating protein ristocetin, leads to removal of sialic acid from platelets by translocation of platelet neuraminidase to the platelet surface. This VWF-induced removal of sialic acid from the platelet membrane could be prevented by the neuraminidase inhibitor oseltamivir. In summary, our findings suggest that the excessive binding of VWF to the membrane of platelets in acute dengue results in platelet neuraminidase-mediated removal of sialic acid. This process is likely to contribute to the dengue-induced thrombocytopenia and may be inhibited by oseltamivir.

### 1398

#### LEVERAGING STUDIES IN RETURNED U.S. TRAVELERS TO COMBAT EMERGING INFECTIOUS DISEASES

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Emerging infectious diseases affect the the health, economic productivity, and quality of life of billions throughout the world. Dengue, one of the most important arboviral infection, causes 400 million infections annually, resulting in 20,000 deaths. Emerging pathogens such as Ebola in 2014 and Zika in 2015 continue to present new challenges to the public health community. Thorough understanding of human immunity is key for developing vaccines and rapid diagnostic tests; however, challenges exist for high quality immunological studies in the resource-limited areas where these infections often occur. An attractive alternative approach is to study returned travelers exposed to tropical infections as we have done for over a decade for dengue and more recently for Zika. We recruit adult US travelers with confirmed infection or suspicion for exposure to arboviruses by word of mouth and targeted advertising campaigns among our university community and academic medical center. A short questionnaire is administered, 10 - 100 mL of peripheral blood are drawn, and plasma and PBMC are isolated and stored. As of April, 2017, we have enrolled 264 participants representing geographic regions of the US, Caribbean, Central America, South America, India, Sri Lanka, Europe, and Southeast Asia. To characterize the plasma, we screen via dengue or Zika IgG capture ELISA. Neutralization assays are run on the ELISA positive plasma to define the likely infecting flavivirus with greater specificity. This study has proved to be a valuable resource for various applications. We initially sought to determine the molecular determinants by which neutralizing antibodies interact with the four serotypes of dengue virus, finding that quaternary epitopes are targeted, and these are highly specific to the infecting serotype. We have recently used this tool to demonstrate that Zika type-specific antibodies are elicited in humans regardless of dengue exposure. Assessment of binding by Zika type-specific antibodies is a primary benchmark for our strategy to identify and develop recombinant antigens that may distinguish flavivirus infections in simple serologic assays.

### 1399

#### MOSQUITO-DELIVERY OF DENGUE VIRUS IN RHESUS MACAQUES DELAYED ONSET AND INCREASED PEAK VIREMIA COMPARED TO SUBCUTANEOUS INOCULATION

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Dengue virus (DENV) is transmitted by infectious mosquitoes via saliva containing antigenic proteins, with anti-hemostatic and immunomodulatory activities shown to promote arboviral infection in mouse models. In the present study, we examined the effect of varying routes of exposure in addition to mosquito factors on DENV infection in rhesus macaques in an effort to improve the DENV NHP challenge model. Prior to infection and assuming that pre-exposure to mosquito saliva impacts the host response to saliva components in the context of an infectious mosquito bite, all NHPs were sensitized to *Aedes aegypti* saliva by three weekly uninfected mosquito feedings. Four weeks later NHPs were then exposed to DENV-1 via subcutaneous (SC) inoculation of virus only, intradermal inoculation (ID) of virus only, ID inoculation of virus combined with salivary gland extract (SGE), or infectious mosquito feeding (IMF). NHPs were then followed for clinical signs, RNAemia, viremia, serum cytokines, hematology, clinical chemistries, and inoculation site histopathology. Although there were no overt signs of disease, the IMF group showed delayed onset of viremia of 2 days and up to 4-fold higher geometric mean peak viral loads compared to the SC group pre-specified as the control group. All groups demonstrated seroconversion with the highest neutralizing antibody titers against DENV-1 and high cross-reactive titers to DENV-2 and, to a lesser degree, DENV-3. A trend for lower cytokine levels in the ID+SGE and IMF groups compared to SC and ID groups was observed. More than one year after primary DENV-1 infection, NHPs in the SC and IMF groups were re-exposed by the same method to either DENV-1 (homologous challenge) or DENV-2 (heterologous challenge) to characterize viral replication patterns and host responses after a secondary infection. Analysis of the secondary infection is ongoing. Altogether, our current results showed that a more natural exposure method utilizing saliva-sensitized animals and infectious mosquitoes resulted in viral replication closer to what occurs in human, when compared with SC inoculation.

### 1400

#### ESTIMATING THE UNDERREPORTING OF DENGUE CASES, ARARAQUARA, BRAZIL, 2015

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Brazil is the country that reports the largest number of dengue cases in the world. Despite that, estimates on underreporting are also high. To estimate underreporting of dengue we have used the incidence data obtained from an ongoing cohort study and the cases reported to the Brazilian National Disease Reporting System (SINAN). A cohort study to assess the incidence of dengue among children and adolescents, from 2 to 16 years of age, in a previously recognized as a low endemic setting has started in August, 2014. A random sample of children and adolescents was selected from the population of Araraquara, a city in Central São Paulo State. Home visits were made to the families of selected children, to present the study and invite them to participate. The one who agreed

to participate signed an Informed Consent Form. An interview on socio-demographic characteristics was carried out and blood samples from the selected children were drawn for dengue baseline serology. Dengue IgG antibodies were tested by enzyme-linked immunosorbent assay (ELISA). Families are being contacted weekly for fever surveillance. Fever cases are submitted to dengue diagnosis tests (IgM, IgG, NS1 and PCR). The baseline seroprevalence of dengue IgG antibodies among the cohort participants was 15.3%. SINAN is the official web-based system for mandatory reporting diseases, run by the Brazilian Ministry of Health. City health departments are responsible for the implementation of the surveillance system. In the 2015 dengue epidemic season the cumulative incidence of symptomatic laboratory confirmed dengue among the cohort's participants was 8.3% (290/3.514). 8,408 confirmed autochthonous dengue cases were reported to SINAN in Araraquara in 2015, an incidence of 3.7%. The ratio of cohort/surveillance incidence was 2.2. It is smaller than previously reported for other settings in the country. The performance of local disease reporting services is probably related to the observed differences in underreporting estimates.

## 1401

### A PURIFIED INACTIVATED VIRION-BASED DENGUE VACCINE INDUCES NEUTRALIZING ANTIBODIES THAT TARGET QUATERNARY EPITOPES AND PROTECT FROM CHALLENGE IN RHESUS MACAQUES

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Dengue viruses (DENV1-4) are considered the most important emerging, human arboviruses with worldwide distribution in the tropics. Although there are live attenuated virus vaccine candidates in advanced clinical trials, and one has been approved to use in several countries, there is an urgent need to accelerate the development of second-generation vaccine strategies with less interference among the vaccine components while inducing antibodies (Abs) to relevant epitopes present on the whole virion. We tested a dengue vaccine based on a purified, inactivated virion (iDV) mixed with a novel alphavirus adjuvant (GVI3000), which consist of disarmed viruses that derive their activity from the replication of a truncated alphavirus RNA and *in vivo* mimic the earliest stages of a viral infection. The antigenic integrity of purified dengue virus antigens after inactivation by different protocols was determined using a panel of mouse and human monoclonal antibodies (MAbs) by ELISA. We confirmed the preservation of conformational epitopes in different domains of E protein, including recently characterized serotype specific, strongly neutralizing human MAbs that map to epitopes only preserved in the quaternary structure of the virion. Two doses of a tetravalent iDV mixture formulated with the GVI3000 adjuvant were administered to rhesus macaques six weeks apart. Neutralizing Abs to all 4 serotypes were induced and maintained in 15/16 animals for 18 weeks. The quality of the Abs induced after 2 immunizations and before challenge was determined by performing blockade of MAb binding assays and by depleting heterotypic Abs from immune serum samples, to estimate the contribution of serotype cross-reactive and type-specific Abs to neutralization. We found that iDV+GVI3000 immune sera blocked the binding of human MAb 1F4 to DENV1, and of human MAb 5J7 to DENV3. These type-specific MAbs bind to quaternary epitopes only present in virions and map to the ED/II hinge region in the respective serotype. These results suggest that inactivated DENV+GVI3000 induces Abs qualitatively similar to those found after natural infection.

## 1402

### POTENTIAL IMPACT OF DENGUE VACCINATION STRATEGIES WITH SEROTESTING IN VARIOUS ENDEMIC SETTINGS

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Dengue is a fast-spreading vector borne viral disease currently posing a risk for half of the world's population. CYD-TDV is currently licensed in 16 countries and was recommended in 2016 by the WHO for use in settings with high burden and in age groups with high level of seroprevalence. Phase III trials have shown that the efficacy of the vaccine varied based on prior dengue exposure. Although serotesting is neither available nor recommended, this difference triggered questions around the potential value of limiting vaccination to those with prior dengue exposure. This was investigated using an existing dynamic transmission model and we considered various levels of dengue transmission intensity (corresponding to a level of seropositivity at age 9 ranging from 10 to 90%). Using this model, we explored, both at the population and individual levels, how CYD-TDV vaccination would impact the population living in these settings, depending on whether serotesting is implemented or not to select people eligible for vaccination. We also assessed how this impact would be modified according to the performance of the test in terms of sensitivity and specificity. Our analysis indicates that serotesting generally leads to a reduced vaccination impact at the population level when compared to the same strategy without serotesting. In terms of efficiency (measured by the number of vaccinations and tests to be performed to prevent a dengue case), the value of serotesting is critically dependent on the level of transmission intensity: it is limited in high transmission settings but increased as the level of transmission intensity decreases. Performance was also found to be a critical factor: a lower sensitivity and specificity has always a negative impact in terms of efficiency. Our analysis did not address the feasibility aspects of using serotesting in large vaccination programs that are expected to raise significant challenges. In conclusion, our analysis highlights that the value of serotesting prior to dengue vaccination is critically dependent on the level of transmission of the setting for which vaccination is considered and on the performance of the test used.

## 1403

### A METHODOLOGICAL FRAMEWORK FOR ECONOMIC EVALUATION OF OPERATIONAL RESPONSE TO VECTOR-BORNE DISEASE FORECASTS

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Prediction information from infectious disease forecasting models are rarely used in response planning due to several factors, among which the most important is the inherent uncertainty associated with predicted disease events at different lead times. Incorporating prediction uncertainty in economic evaluations of early warning systems can be very informative for decision making among policy makers. In this study, we propose a methodological framework to assess the economic value of responding to early warnings of abnormal disease events. Using a probabilistic approach, we estimate the costs and the benefits of response at various lead times. We consider two scenarios where an early warning system provides (1) threshold based outbreak alerts or (2) actual disease forecasts. Adopting a health care provider perspective, the framework considers the costs of disease on the health system, the costs of response strategies

and the cost of running information management systems to arrive at total costs. Total benefits are measured as averted disease burden as a function of the effectiveness of response strategies at different lead times and are quantified as costs averted. We estimate the economic value as a net benefit which is calculated as total costs minus total benefits. We produce a probability density function for the net benefit using Monte Carlo simulations to factor in the uncertainty with predictions and the effectiveness of response strategies at each lead time and present the probability of a positive net benefit, which is a general signal to policy makers that the proposed response strategy is economically feasible. The proposed framework makes it explicit the trade-offs between forecast accuracy, response effectiveness, and costs in responding to infectious disease threats as a function of lead time and should guide sustainable integration of early warning systems into public health systems.

## 1404

### MODELLING THE REQUIREMENTS FOR SUCCESSFUL REACTIVE CASE DETECTION FOR DENGUE IN SINGAPORE

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Following a period of successful suppression of dengue in the 1980s and 1990s, Singapore has more recently experienced a resurgence triggered by repeated introductions from neighbouring endemic countries. While Singapore, arguably has a world leading dengue surveillance and control programme, the challenges of increased population density and an aetiology where dengue infected individuals are infectious before becoming symptomatic (and thus detectable) questions whether long term reactive case detection is a feasible strategy in this setting. Here we use geospatial data from 32,000 reported dengue cases 2013-2016 to characterise the spatial patterns of dengue spread using a mathematical model. This model was then used to test a range of hypothetical response and containment strategies with the ultimate goal of cessation of the outbreak. This revealed the increase in response time, control effectiveness or surveillance sensitivity that would be required to prevent future dengue outbreaks in Singapore. These findings and methods can be used by public health policymakers to design better strategies against emerging infectious diseases. These findings give insight into how to prioritise resources on activities that are most likely to stop an outbreak from spreading and can be reapplied to other vector-borne diseases such as chikungunya and Zika at a time when they are spreading globally.

## 1405

### DEVELOPMENT OF ENVELOPE-MODIFIED TETRAVALENT DENGUE VIRUS-LIKE PARTICLE VACCINE: IMPLICATION FOR FLAVIVIRUS VACCINE DESIGN

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The major challenge in dengue vaccine development has been the existence of antigenically distinct, but closely related four serotypes of dengue virus (DENV1-4). An ideal dengue vaccine should induce neutralizing antibody (NAbs) responses against all four serotypes simultaneously in a short period of time. Here we developed a novel tetravalent dengue vaccine utilizing virus-like particle (VLP) technology. VLPs are self-assembled particles consisted of viral structural proteins, which mimic the conformation of authentic native virus without viral genome. VLP vaccines offer several advantages in terms of safety and high immunogenicity. It has been reported that flavivirus VLPs including DENV VLP can be produced by co-expressing two viral proteins, pre-membrane and envelope (E). However, VLP yields were generally poor. Based on our previous finding in

alphavirus VLP development, we hypothesized that modification of the E conformation may increase the DENV VLP yield. We designed new DENV E by introducing mutations in the amino acids which may impact the conformational change of E. Among a total of 41 mutants, we found that a single amino acid change (F108A) in the fusion loop region increased the DENV1 VLP expression by 15-fold. By introducing F108A mutation and replacing the domain III of DENV2-4 E with the corresponding region of DENV1 E, we successfully created DENV2, 3, and 4 VLPs at a high yield. Tetravalent vaccination of DENV VLPs elicited high titer of NABs against all four serotypes. Geometric mean 50% focus reduction neutralization titers for DENV1-4 were 800-8,000. Antibody-dependent enhancement, which is considered to be the major mechanism underlying severe dengue, was not observed against any serotype. Notably, F108, the amino acid which we found to be critical for high-yield VLP production, is conserved among all flaviviruses. We also found that F108A mutation increased Zika virus VLP yield significantly. Our finding will not only facilitate vaccine development against DENV, but also against other structurally similar flaviviruses such as Zika or West Nile virus.

## 1406

### METABOLIC BIOSIGNATURES OF INFECTION: HOW DENGUE, CHIKUNGUNYA AND ZIKA VIRUSES DIFFERENTIALLY PERTURB HOST METABOLIC HOMEOSTASES

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Arboviruses such as dengue (DENV), chikungunya (CHIKV) and Zika (ZIKV) viruses place over three billion people at risk for infection. We have demonstrated that metabolic signatures in acute-phase clinical specimens have the potential to function as prognostic biomarkers of disease etiology, pathology and severity and could play a critical role in improving triage and clinical management of these viral diseases at an early stage. Specifically, we had previously reported that using a versatile metabolomics platform, we identified small molecule biomarkers (SMBs) that differentiate DENV, CHIKV and ZIKV infections from other febrile illnesses in acute-phase serum. These SMBs were also capable of predicting dengue disease severity. These studies used both reverse phase ultra-high performance liquid chromatography-mass spectrometry (RP-UPLC-MS) and hydrophilic interaction liquid chromatography (HILIC) LC-MS to characterize the serum metabolome of 535 patients from Nicaragua. These patients experienced different grades of dengue severity or infection with CHIKV or ZIKV and included cases with subsequently diagnosed dengue fever, dengue hemorrhagic fever, dengue shock syndrome, and confirmed chikungunya and Zika infections. We have now further identified biochemical pathways in which these SMBs have a role, and which may provide better insight into the metabolic signatures that are unique to infection with each of these viruses. We have validated and confirmed particular biochemical classes of metabolites and unique SMB bio-signatures that were previously identified as specific for dengue disease severity classifications and distinct from infections with CHIKV and ZIKV. Our SMB panel and pathway analyses have the potential to improve therapeutic options and early disease diagnoses and treatments to combat these devastating arboviral diseases.

### PRIOR YEAR'S TRANSMISSION INTENSITY INFORMS CURRENT RISK OF DENGUE VIRUS INFECTION IN THAI VILLAGES

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Dengue virus (DENV) infection generates broadly cross-reactive antibodies which may modulate the subsequent risk of infection and illness. For individuals, a transient period of cross-protection has been reported following infection. For populations, this transient, cross-protective, herd immunity following epidemics could manifest as oscillations in incidence in susceptible individuals. To study this, we used data derived from a longitudinal cohort study of DENV infection in schoolchildren in Kamphaeng Phet, Thailand, from 1998-2002. Active fever surveillance during epidemic periods and hemagglutination inhibition (HAI) testing of routine specimens identified dengue illnesses and seroconversions. Individuals with any DENV HAI titers  $\geq 320$  during the pre-season blood draw, reflecting possible recent exposure, were excluded from analysis. Hot spot analysis was performed using GPS coordinates for the child's home. Mixed effects logistic regression estimated the risk of DENV infection based upon the prior year's incidence rate for that child's village, controlling for age, mean age of the enrolled children in the village, and with random effects for individual and village. A total of 9579 person-years and 1216 dengue infections were eligible for analysis. Several communities demonstrated oscillations in transmission intensity, with significantly higher transmission years (hot spots) followed by significantly lower transmission years (cold spots), while others demonstrated more stable, mid-level transmission continuously across the five-year period. In regression analysis, the prior year's incidence in a village was significantly negatively correlated with current risk of infection for an individual; a 20% increase in prior incidence was associated with a 25% reduction in current risk. These findings indicate that the risk of DENV infection in Thai villages is associated with the intensity of prior epidemics and potentially driven by shifts in the degree of cross-protective herd immunity. These findings are limited by challenges in defining 'susceptibility' and 'herd immunity' and merit further investigation.

### TWO WAYS OR ONE: THE RELATIONSHIP OF ENDEMIC AND SYLVATIC DENGUE VIRUS

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Endemic viral lineages are believed to emerge from sylvatic lineages (hosted by wild non-human vectors). In arbovirus evolution, published work supports this hypothesis with a low amount of sequence data. In order to test this hypothesis we examined whether endemic strains evolved independently of sylvatic strains by examining the polarity of changes between endemic to sylvatic states based on a phylogenetic analysis. Using an original dataset published by Wang et al, 2001, which included 86 Dengue nucleotide sequences of the gene for the E protein of varying serotypes (Dengue 1-4) and epidemiological type (sylvatic vs endemic),

we expanded the dataset by adding 103 sequences with corresponding metadata of the gene for the E protein from NCBI's Genbank. We performed multiple sequence alignments with MAFFT under default parameters. A phylogenetic tree search under the maximum likelihood criterion for 100 replicates of RAxML was performed. The tree with the best likelihood score was visualized and sylvatic/endemic shifts were reconstructed in MESQUITE. The number of shifts between sylvatic and endemic strains was recorded and compared to the phylogeny constructed from the original dataset. By determining these patterns, targeted host control methods to mitigate the spread of these pathogens can be implemented for the different dengue serotypes and other flaviviruses with similar host biology.

### THE FIRST COMMUNITY-BASED INTERVENTION TO PREVENT DENGUE FEVER IN BURKINA FASO: AN IMPACT EVALUATION STUDY

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Dengue Fever (DF) has emerged as the most abundant vector-borne viral infection. Since 2013, DF outbreaks have been consistently reported during raining season in Burkina Faso (July-October). This study aims to evaluate the effectiveness of a community-based intervention implemented in August 2016 to prevent DF in a neighborhood of Ouagadougou, the capital city of Burkina Faso. The intervention was conducted in an area of the city (secteur 22) located at 1km around a public health care center. It had 3 components: i) support the community mobilisation and engagement; ii) develop trainings for the community leaders; iii) improve household knowledge's, attitude and practices (KAP) related to DF prevention. The intervention area has been compared with a control area (Juvenat) with the same socioeconomic characteristics, sanitation habits and 250 households have been randomly selected in both area for the study. The data collected before (October 2015) and after the intervention (October 2016) included 3 outcomes: i) the household respondents' KAP related to DF prevention; ii) the density of Aedes (DF vector) larvae and nymph in human-generated containers; iii) 2 household members (1 child and 1 adult) blood sample to test their exposure to Aedes bites (saliva) using blotting paper. We calculated a propensity score (PS) using each household characteristics and matched households from the intervention area with those from the control area using a stratification on the PS. Multilevel models with fixed effects were used to assess the effect of the intervention on the 3 outcomes. The households who received the intervention increased their knowledges on DF, the disease symptom, the number of mosquito nets and where less likely to assimilate DF to malaria; the Risk Ratio (RR) and 95% CI where respectively 1.13 (1.01-1.27); 1.44 (1.22-1.69); 1.30 (1.23-1.37); 0.7(0.58-0.84). The intervention reduced people exposure to DF vector bites but we did not find any impact on the density of vector larvae nor nymph in the households' domestic environment. Rigorous and well planned community-based interventions are feasible and can be effective to prevent DF.

### DETECTION OF DENGUE AND WEST NILE ANTIBODIES IN HUMANS IN CIUDAD JUAREZ, MEXICO

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Arboviruses are considered one of the most prevalent sources of disease in subtropical and tropical areas. Currently, there is some evidence of dengue

and West Nile viruses' transmission in U.S.-Mexican border communities. A serosurveillance study for dengue (DENV) and West Nile (WNV) viruses was performed in a human cohort in Ciudad Juarez, Mexico. Two blood samples were collected in June and November 2015 and tested for DENV or WNV IgG antibodies by ELISA (Enzyme-linked immunosorbent assay) and the specificity was confirmed by neutralization assays. The baseline seroprevalence rate was 12.8% (10/78) for DENV and 3.8% (3/78) for WNV, and the specific seroconversion rate for DENV (DENV-1 and/or DENV-2 serotypes) and WNV occurred in 8.2% (4/49) and 2% (1/49) respectively of those individuals with no antibodies in the first sample. With the seroconversion data, the overall seroprevalence was 19.7% (12/61) for DENV and 6.6% (4/61) for WNV, thus suggesting local transmission of DENV and WNV in Ciudad Juarez. Future epidemiological studies could help to understand the dynamic of these viruses in this U.S.-Mexican border city.

## 1411

### THE DYNAMICS OF DENGUE VIRUS INFECTION IN INDONESIA: OBSERVATIONS FROM A NATIONAL, MULTICENTER STUDY OF ACUTE FEBRILE ILLNESS AMONG HOSPITALIZED PATIENTS

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Recent estimates indicate that 3.9 billion people are at risk of dengue virus infection. In Southeast Asia, Indonesia shoulders the greatest burden of disease, with over 50,000 cases per year and a case fatality rate of 0.8%. Though the signs and symptoms of dengue fever are generally well-defined, the reduced diagnostic capacities of developing countries such as Indonesia can result in the misdiagnosis of cases. From 2013-2016, an observational study of patients hospitalized with an acute febrile illness was conducted at 8 top-referral hospitals across Indonesia to better understand the etiologies and diagnoses of infections. 1,486 patients enrolled in the study and provided acute and convalescent blood specimens for standard-of-care and study-specific diagnostic testing. Prior to hospitalization, 1212 (81.6%) subjects showed evidence of previous dengue virus infection by IgG ELISA. Acute dengue fever was confirmed in 428 (28.8%) subjects, 351 cases being secondary infections, making dengue virus the most common etiological agent in the study. Across study sites, the proportion of dengue fever cases ranged from 21.2% to 51.2%. A higher proportion of cases was observed in children, 226/623 (36.3%), compared to adults, 202/863 (23.4%). Using RT-PCR and sequencing, dengue virus serotypes were identified in 351/428 (82.0%) acute cases, the prevalence being 22.5% for DENV-1, 23.4% for DENV-2, 48.7% for DENV-3, and 5.4% for DENV-4. Clinically, the 428 lab-confirmed infections were initially diagnosed as dengue fever (141), dengue hemorrhagic fever grade 1 (176), grade 2 (55), grade 3 (3), grade 4 (5), typhoid fever (20), general viral infection (4), urinary tract infection (3), undifferentiated (6), and other illness (15). Dengue virus infection was not lab-confirmed in 71 subjects clinically diagnosed with dengue fever, suggesting an over-diagnosis of the illness in our study. One subject with confirmed DENV-3 infection died. The results of this study further strengthen our understanding of dengue fever in Indonesia and highlight the need for an accurate rapid diagnostic test to improve clinical case management.

## 1412

### ARTHROPOD EXOSOMES MEDIATE DENGUE INFECTION THROUGH A NOVEL EXOSOMAL MARKER

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The transmission strategies used by flaviviruses to exit arthropods and infect human host were envisioned as best approaches to develop transmission-blocking vaccines against vector molecules or determinants that facilitates pathogen transmission. Research in our laboratory has shown that mosquito-borne flaviviruses such as dengue and Zika viruses uses arthropod exosomes for transmission to human and other vertebrate cells. We have found that dengue and ZIKA viral RNA and proteins are transmitted from vector to the vertebrate host through exosomes. The exosomes containing dengue or ZIKA viral RNA and proteins were viable, secured and highly virulent in all tests such as re-infection kinetics, trans-migration assays and viral plaque formations suggesting arthropod exosomes as favorable modes of transmission. Our data also showed that arthropod derived exosomes facilitate infection of human cells that eventually produce exosomes loaded with viral RNA and proteins. We have identified a novel tetraspanin domain containing protein from mosquitoes that is involved in dengue viral RNA and protein transmission. Also, our studies highlight a direct interaction of the arthropod tetraspanin exosomal marker with dengue Envelope protein. Silencing of this novel arthropod exosomal marker, or treatments of antibody against this molecule or exosome release inhibitor (GW4869) lead to significantly reduced loads of viral RNA and E-protein in exosomes and in total cell lysates. Taking together, arthropod exosomes serve as secured vesicles for transmission of mosquito-borne flaviviral RNA and proteins that evade the human or vertebrate cells and blocking of novel arthropod exosomal marker blocks transmission.

## 1413

### A COMPARISON OF FOUR SEROLOGICAL METHODS AND TWO RT-PCR ASSAYS FOR DIAGNOSIS AND SURVEILLANCE OF ZIKA

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Zika virus (ZIKV) is a mosquito-borne flavivirus responsible for recent explosive epidemics in the Americas, and infection during pregnancy is linked to congenital birth defects. Diagnosis and surveillance of Zika have been challenging due to similar clinical manifestations and extensive antibody cross-reactivity with endemic flaviviruses, such as dengue virus (DENV). Here, we evaluated four serological and two real-time RT-PCR methods in paired acute (day 2-4) and convalescent (day 14-16) samples from our 13-year on-going Pediatric Dengue Cohort Study in Nicaragua, to which Zika was added in January 2016. 264 paired samples were tested from 110 Zika cases confirmed by rRT-PCR - 44 with documented history of previous DENV infection (DENV-immune) and 66 DENV-naïve; 79 RT-PCR-confirmed dengue cases; and 75 non-Zika, non-dengue other febrile illnesses. Two IgM capture ELISAs were evaluated: a standardized in-house assay (CNDR MAC-ELISA) and the NIAID-BE1 MAC-ELISA, based on CDC's initial ZIKV MAC-ELISA test. In preliminary analysis, the sensitivity and specificity of the CNDR MAC-ELISA was 96.3% (95%CI 90.8-99.0) and 90.8% (85.1-94.9), respectively, while the NIAID-BE1 MAC-ELISA had a

specificity of 87.9% (80.1-93.4) and sensitivity of 85.5% (78.9-90.7). The CNDR MAC-ELISA displayed equal sensitivity in DENV-naïve and DENV-immune Zika cases, whereas the NIAID-BEI MAC-ELISA demonstrated lower sensitivity in DENV-immune cases. An Inhibition ELISA method for detecting total Zika antibody was standardized, resulting in a sensitivity and specificity of 67.3% (57.5-76) and 62% (54.4-70.4), respectively. The NS1 blockade-of-binding (BOB) ELISA that measures anti-ZIKV NS1 antibodies was also evaluated, yielding 86.2% (77.1-91.3) and 92.8% (87.5-96.4) sensitivity and specificity, respectively. Finally, two rRT-PCR methods that detect ZIKV, DENV, and chikungunya virus were compared: the ZCD and the Triplex assays. The high sensitivity and specificity of the CNDR MAC-ELISA and the BOB ELISA implicate these assays as solutions to the urgent need for serological diagnostic and surveillance tools for countries affected by Zika.

## 1414

### DIFFERENTIATING ZIKA AND DENGUE VIRUS INFECTIONS WITH A LINEAR PEPTIDE ARRAY

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Identifying women at risk of congenital Zika virus (ZIKV) infection is complicated by the lack of sensitive, specific, and scalable diagnostic tools. Conventional serologic assays, which are necessary to identify at-risk pregnancies in women who have already cleared viremia, cannot distinguish between ZIKV and closely related, co-endemic flaviviruses such as Dengue virus (DENV). In order to develop ZIKV-specific serologic assays, ZIKV-specific epitopes that do not cross react with DENV-specific epitopes must be identified. We utilized rhesus macaque serum from pre-ZIKV challenge and post-ZIKV challenge timepoints to identify linear B cell epitopes unique to ZIKV. We designed a high density peptide array containing peptides representing a library of multiple Flavivirus polyproteins and measured the reactivity of pre-ZIKV challenge and post-ZIKV challenge rhesus macaque sera to the peptides. We identified two linear epitopes in the ZIKV envelope glycoprotein region that demonstrate reactivity with post-ZIKV challenge sera in three macaques challenged with Asian lineage ZIKV. The pre-ZIKV challenge sera from these animals did not react with these linear peptides. Importantly, this ZIKV-immune sera did not cross react with the corresponding peptides in the envelope glycoprotein of DENV serotypes 1, 2, 3 or 4. Studies evaluating the peptide array with DENV immune sera and the development of enzyme-linked immunosorbent assays with these peptides are underway. These unique linear epitopes may be utilized to develop sensitive and specific diagnostic serologic assays for ZIKV infection.

## 1415

### ASSESSMENT OF SEXUAL TRANSMISSION POTENTIAL OF SPONDWENI SEROGROUP VIRUSES

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Spondweni virus (SPONV) and Zika virus (ZIKV) are mosquito-borne flaviviruses that are classified in the Spondweni serogroup. Although primarily transmitted by mosquitoes, human-to-human sexual transmission of ZIKV has been documented with the Asian genotype during the ongoing outbreak in the Americas and an African ZIKV genotype was implicated with the first reported case of ZIKV sexual transmission. In order to assess the potential for sexual transmission of SPONV, the most closely related virus to ZIKV, and to assess the efficiency of sexual transmission by Asian and African genotype ZIKVs, testicular tropism and the presence of virus in seminal fluids was assessed in inoculated interferon alpha/beta and gamma receptor deficient mice (AG129). Male mice were inoculated

subcutaneously (s.c.) with SPONV (South Africa, 1955), ZIKV-PRVABC59 (Asian-Puerto Rico, 2015), ZIKV-P6-740 (Asian-Malaysia, 1966), or ZIKV-DakAr 41524 (African-Senegal, 1984) viruses. To determine the potential for sexual transmission, seminal fluids were collected from the uteri of mated female CD1 mice and the presence of infectious virus in these ejaculates determined by plaque assay. Only 3-8% of ejaculates from SPONV-inoculated males were positive for infectious virus, despite titers in the testes that were comparable to those of PRVABC59 (mean 7-7.2 log<sub>10</sub> PFU/g tissue). In contrast, virus was detected in 83% of the ejaculates from PRVABC59-inoculated mice. Significantly fewer (25%) ejaculates from P6-740-inoculated mice had detectable infectious virus. Due to the rapid mortality of the s.c inoculated males with the DakAr strain, the potential for sexual transmission could not be assessed in this model. Overall, infection with the PRVABC59 strain resulted in a significantly higher sexual transmission potential in this mouse model compared to the other two ZIKV strains or SPONV. Application of this model for future single-cell analysis using flow cytometry will delineate host cell populations that harbor virus and grant viral access to, and persistence in, immune privileged sites protected by the testes-blood barrier.

## 1416

### LONG TERM ASYMPTOMATIC DETECTION OF VIRAL RNA IN URINE AND SALIVA FOLLOWING ACUTE ZIKA INFECTION IN NICARAGUA 2016 - 2017

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Case studies of travelers returned from Zika-endemic areas have documented likely sexual transmission of Zika virus (ZIKV), and a longitudinal study in Puerto Rico described persistently positive PCR for ZIKV in different body fluids with rare recovery of infectious virus from semen or serum. To further define the timing of risk for non-vector-borne transmission, we monitored the presence of ZIKV RNA in blood, saliva, urine, and vaginal fluid or semen following acute infection in León, Nicaragua. To date, we have enrolled 12 cases and obtained specimens at 7, 14, 21, 28, 60, and 90 days post symptom onset. The mean age of the cases was 21 years of age (range 10 - 54 years), 50% were female with 4 of them being of reproductive age, 1 was pregnant (32 weeks at enrollment). In total, 108 of 238 (45%) specimens tested ZIKV-positive by real time PCR. The frequency of ZIKV detection by specimen type was as follows: 51 (70%) of 73 urine, 39 (50%) of 78 saliva, 11 (14%) of 79 whole blood and 7 (88%) of 8 vaginal fluid. Of note, 6 (50%) of 12 cases showed ongoing asymptomatic shedding of ZIKV RNA in saliva and urine through 90 days. In general, ZIKV was more consistently detected in urine at later time points than in other fluids. ZIKV RNA was intermittently detected in whole blood, out to 90 days in one case, indicating that a single negative result does not rule out future shedding and potential ongoing risk for transmission. Three of the 4 women of reproductive age shed the virus in vaginal fluids for 21, 28 and 90 days, respectively. This study extends previous knowledge about asymptomatic shedding of ZIKV and highlights the potential risk for asymptomatic ZIKV transmission via fluids from genitourinary organs or by blood transfusion.

## 1417

### A NOVEL MOLECULAR ASSAY FOR THE DETECTION OF ZIKA RNA IN WHOLE BLOOD AND URINE SAMPLES

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The recent ZIKA virus (ZIKV) outbreak in the Americas has been shown to cause a spectrum of neurologic complications including microcephaly. Greater access to testing is needed to improve clinical outcomes and aid

epidemiological surveillance in these impacted regions. We developed a novel molecular assay that includes reverse-transcription PCR (RT-PCR) pre-amplification followed by isothermal amplification/detection steps. The assay was used for the detection of ZIKV RNA in whole blood and urine specimens. The assay incorporated MS2 bacteriophage as an internal positive control for all sample preparation, RNA extraction, amplification and detection steps. Magnetic bead-based RNA extraction was used on the raw samples. Following the multiplexed RT-PCR pre-amplification step, the amplified products were split into two separate wells for isothermal amplification and detection of ZIKV and MS2, respectively. The isothermal amplification method utilized nested primer pairs enabling both amplification and detection. These primer pairs contained pairwise complementary 5' ends that resulted in amplicons containing 5' overhangs. During the isothermal amplification, these overhangs facilitated the generation of concatemers, which are detected with a dsDNA-specific fluorescent dye. Limits of detection (LoD) of 400 RNA copies/mL for whole blood and 200 copies/mL for urine were achieved for the ZIKV PRVABC59 strain. The assay detected multiple ZIKV strains including the recent Puerto Rican PRVABC59 strain and two African strains DakArdD 41662 and MR766. No cross-reactivity with eight closely related viruses and pathogens that produce ZIKA-like symptoms at clinically relevant concentrations was observed. A high load (>106 copies/mL) of the same pathogens did not inhibit detection of ZIKV present at 2xLoD. The ZIKV assay showed reliable results with small-volume (75uL-100uL) whole blood and urine specimens. This assay is currently being integrated onto an automated sample-to-answer diagnostic platform which will increase the access to ZIKV testing at point of care settings.

## 1418

### POTENT AND BROADLY NEUTRALIZING DOMAIN II ANTIBODIES INDUCED DURING ACUTE ZIKA VIRUS INFECTION OF A PREVIOUSLY DENGUE-EXPOSED INDIVIDUAL

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Zika virus (ZIKV) is a mosquito-borne flavivirus associated with fetal microcephaly and serious neurological complications. To date, the majority of ZIKV infections have occurred in endemic regions for the related dengue virus (DENV). A number of anti-DENV antibodies have been demonstrated to cross-react with ZIKV, both in terms of neutralizing antibodies (NAbs) and antibody dependent enhancement (ADE). In DENV-naïve individuals neutralizing responses are generally ZIKV-specific, while in DENV-experienced individuals ZIKV infection leads to potent anamnestic anti-DENV responses. It is unknown what constitutes these responses, in particular whether it consists of broad NAbs or a mix of more specific NAbs. Characterization of plasmablasts using Immune Repertoire Capture technology (IRC™) was performed on PBMCs from an individual with acute ZIKV infection and previous exposure to DENV. A large plasmablast expansion was observed. Analysis of heavy- and light-chain (VD)J sequence usage revealed evolution of clonal families of antibodies in response to infection. Two distinct lineages emerged representing 70% of total Ig sequences. 18 mAbs representing the two major lineages, together with five smaller families, were screened for neutralization and ADE between DENV and ZIKV. All but two of the smaller lineages were neutralizing, including potent mAbs with ZIKV NT50's <0.01 µg/ml. No highly type-specific mAbs were observed, rather a diverse pattern of neutralization, even within an individual lineage. Among 5 mAbs representing one major lineage, some were most potent against DENV1, others against DENV4, and others against ZIKV, with ratios of D1/ZIKV titers varying from 0.1 - 8. Shotgun mutagenesis epitope mapping demonstrated epitopes for two of these broadly neutralizing mAb lineages lay within domain II of E, close to

the fusion loop. Overall, this work suggests that NAb responses to ZIKV in flavivirus-experienced individuals are extensively shaped by previous DENV exposure. We will continue to study longitudinal samples in DENV-naïve and experienced individuals in order to determine the extent of diversification and selection

## 1419

### HUMAN PRIMARY CELL IMMUNE RESPONSES TO FLAVIVIRUSES ARE MODULATED BY BOTH THE VIRAL SPECIES AND THE AGE OF THE DONOR

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The emerging Zika virus epidemic across the Americas has resulted in many public health challenges, particularly because of its ability to cause severe congenital defects and Guillain-Barre Syndrome. Circulating in regions endemic to Dengue infections, Zika is also transmitted by infected *Aedes* mosquitoes, leading to plausible exposure to both viruses. In an *in vitro* model of primary monocytes enriched from either human adult peripheral blood or cord blood, we infected cells with DENV or ZIKV in the presence or absence of monoclonal antibodies that permit infection via Fc receptor-mediated entry. Cells and supernatants were collected longitudinally to evaluate the kinetics of the immunological responses to DENV and ZIKV infection. While little-to-no infection occurred without antibody treatment, we observed distinct age correlates of infection, with cells of fetal origin demonstrating greater susceptibility to infection in the presence of antibody. Further, immune responses to ZIKV were far less robust compared to DENV, with the fetal responses even further attenuated compared to adult cells. The low, or even absent, primary cell immune response to ZIKV mirrors the mild nature of clinical symptoms following ZIKV infection. Specific understanding of the immune parameters involved in response to Fc receptor-mediated ZIKV entry and how the virus is modulating the innate host response to infection will provide valuable direction for the development of vaccines and therapeutics, specifically for the fetal population that is at such risk for adverse effects following ZIKV infection.

## 1420

### ZIKA VIRUS INFECTION IN HUMAN SERTOLI CELLS INDUCES ROBUST ANTIVIRAL DEFENSE RESPONSE AND COMPROMISES BLOOD-TESTES BARRIER INTEGRITY

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Recent outbreaks of Zika virus (ZIKV), a neglected mosquito-borne flavivirus, have identified sexual transmission as a new route of disease spread, not reported for other flaviviruses. Confirmed reports of ZIKV in human seminal fluid for months after the clearance of viremia suggest the ability of ZIKV to establish persistent infection in the seminiferous tubules, an immune privileged site in the testis protected by the blood-testis barrier, also called the Sertoli cell barrier (SCB). However, cellular targets of ZIKV in human testis and mechanisms by which the virus enters seminiferous tubules remain unclear. We demonstrate that primary human SCs were highly susceptible to ZIKV as compared to the closely related dengue virus and induced expression of IFN-α, key cytokines and cell-adhesion molecules (VCAM-1 and ICAM-1). Further, using an *in vitro* SCB model, we show ZIKV was released on the adluminal side of the SCB model with higher efficiency than the blood-brain barrier. ZIKV-infected SCs exhibited enhanced adhesion of leukocytes that correlated with decrease in the SCB integrity. ZIKV infection did not affect the expression of tight and adherens junction proteins such as ZO-1, claudin and JAM-A, however exposure of SCs with inflammatory mediators derived from ZIKV-infected macrophages led to the degradation of ZO-1 protein that correlated with increased SCB permeability. Taken together, our data suggest that infection of SCs

may be one of the crucial steps by which ZIKV gains access to the site of spermatozoa development and identify SCs as a therapeutic target to clear testicular infections. The SCB model opens up opportunities to assess interactions of SCs with other testicular cells and test the ability of anti-ZIKV drugs to cross the barrier.

## 1421

### SEARCHING FOR ZIKA VIRUS IN INDIA

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In 1952, Zika neutralizing antibodies were reported from patients in India. As of early 2015 Zika outbreaks have been reported from >65 countries. With ever increasing international travel and the presence of both the mosquito vector and susceptible individuals, India is at high risk for Zika reintroduction and transmission. Manipal University Center for Virus Research (MCVR) conducts Zika virus surveillance in India, and reports to the state and national levels weekly. In 2014, MCVR, as part of Global Health Security (GHS), established prospective hospital-based surveillance for acute febrile illness (AFI) in India. This platform now comprises 29 sentinel hospitals across 10 Indian states. All specimens are sent to MCVR via cold chain (maintained at 2-80 C) daily. Beginning in 2016, all samples are screened for Zika virus. Archived samples collected and stored before January 2016 have also been tested for Zika. Uniplex rRT-PCR testing is performed on serum and urine of all cases and a subset of 2000 samples were tested by Triplex RT-PCR Assay. Sera were also tested for dengue by IgM ELISA / NS1Ag / PCR and for Chikungunya by IgM ELISA / PCR. We tested 17,050 AFI cases and all were negative for Zika [Karnataka (0/4538), Kerala (0/1788), Goa (0/1789), Assam (0/2020), Gujarat (0/573), Maharashtra (0/792), Jharkhand (0/793), Tripura (0/1032), Tamil Nadu (0/2778), and Odisha (0/947)]. Patients ranged between 1 to 65 years (median 26 years); 53% were males. 887/17,050 (5%) serum samples were dengue-positive and 162/17,050 (1%) were Chikungunya-positive. Although India is endemic for dengue and chikungunya, has a history of Zika presence, and the vector and ecological conditions are conducive for Zika transmission, our comprehensive surveillance has found no evidence of Zika virus activity in the country. This extensive AFI surveillance network, and the larger GHS platform, will be essential for early detection of Zika introduction and other emerging pathogens in India.

## 1422

### PROLONGED RNA SHEDDING OF ZIKA VIRUS (ZIKV) AND CHIKUNGUNYA VIRUS (CHIKV) DURING ZIKV AND CHIK CO-INFECTION IN DIFFERENT COMPARTMENTS

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Virus detection in body fluids may persist for several weeks or months in ZIKV mono-infections (ZIKVMono). Prolonged RNA shedding in some compartments have implications for non-vector-transmission, diagnosis and probably, pathogenesis. In areas of arbovirus co-transmission, the occurrence of co-infections is not uncommon, but virus dynamics in ZIKV and CHIKV in co-infection (Coinf) is poorly established. The aim of the present study is to determine the duration of ZIKV and CHIKV RNA shedding in different body fluids of ZIKV/CHIKV co-infected

individuals. ZIKV and CHIKV serum, urine, tissue-sample, synovial (SF) and cerebrospinal fluid (CSF) sample collection were obtained from 49 individuals attending a tertiary-care hospital (HUCFF/UFRJ, Rio de Janeiro, Brazil). RT-PCR for detection of ZIKV and CHIKV RNA was performed, and reactive samples underwent viral isolation. In serum, the medians (MD) for the time of the last detection of ZIKV RNA in ZIKVMono were 4.5 days (95% confidence intervals [CI] 2 - 9) and 95th percentiles (95th %iles), 54 days. In Coinf, the median was 25 days (95% CI: 7-41) and 95th %iles, 284 days. In urine, MD was 9 days (95% CI: 4 - 30) and, 95th %iles, 31 day, in ZIKVMono, while in Coinf, MD= 30.5 days (95% CI: 10 - 46) and 95th %iles, 259.9 days. Prolonged viremia and viruria in ZIKVMono and Coinf groups differed significantly ( $p < 0.05$ ). MDs were 13 days (95% CI: 5 - 32) and 14.5 days (95% CI: 6 - 59) in serum from CHIKV mono infection (CHIKVMono) and Coinf, respectively. In mono and coinfection, the latest detection of CHIKV RNA was 120 days. Duration of CHIKV detection in the urine of CHIK Mono and Coinf were similar (MD=8.5 days (95% CI: 0 - 32) vs. 9 (95% CI: 0 - 14) and maximum of 120 days in both groups. ZIKV RNA shedding in SF was detected at 4 days post onset (dpo) in ZIKV Mono and 61dpo, in Coinf. Also, CHIKV RNA was detectable at 120dpo and 76 dpo in CHIKV Mono and Coinf, respectively. Maximum time of detection in CSF was 34 dpo. Prolonged times of ZIKV RNA clearance in different compartments occur during ZIKV/CHIKV co-infections. In contrast, duration of CHIKV RNA shedding remains the same in mono and co-infections.

## 1423

### LACK OF EVIDENCE OF ANTECEDENT ZIKA VIRUS INFECTION IN GUILLAIN-BARRE SYNDROME PATIENTS HOSPITALIZED IN A REFERRAL HOSPITAL IN SOUTH INDIA, 2014 - 2016

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Zika virus (ZIKV) infection has been associated with increased incidence of Guillain-Barré syndrome (GBS). In the context of global travel and the presence of both the mosquito vector and susceptible individuals, India is at risk for ZIKV transmission. We conducted a retrospective evaluation for ZIKV infection among patients admitted with GBS to the National Institute of Mental Health And Neuro Sciences (NIMHANS) hospital, a large referral neurological tertiary care center in south India. A case was defined as a patient admitted to NIMHANS from June 2014 to December 2016 with GBS, defined according to National Institute of Neurological Disorders and Stroke criteria. All GBS patients received standard neurological exam; serum and cerebrospinal fluid was obtained. Stored serum specimens were tested for ZIKV RNA, dengue RNA and chikungunya RNA (CHIKV) using the CDC Triplex real time polymerase chain reaction assay (RT-PCR). Of 222 GBS cases, the mean age was 36 years (range 2 - 76 years) and 151 (68%) were male; 98 (44%) of cases occurred during the monsoon season. The mean duration between onset of illness to hospitalization was 8 days (range 1 - 30 days). Of 222 patients, 202 (91%) had upper limb weakness, 39 (18%) had impaired joint position sense, and 20 (9%) had difficulty breathing. The mean Hughes disability scale score during hospitalization was  $3.6 \pm 0.8$ . Of 222 patients, 70 (32%) reported symptoms of an antecedent illness [fever (35, 50%), diarrhea (22, 31%), respiratory symptoms (10, 14%)] before neurological symptom onset. All tested serum specimens were negative for ZIKV and CHIKV; one sample was positive for dengue. Although India is endemic for dengue and CHIKV, and ecological conditions are conducive to possible Zika transmission, our retrospective analysis of hospitalized GBS patients found no evidence of antecedent ZIKV infection. Evaluation of sera collected late in the course of illness may have limited the sensitivity of RT-PCR testing. Continued

prospective evaluation for ZIKV among GBS patients, which includes testing of both serum and urine collected during acute illness, is warranted to support early detection of ZIKV in India.

## 1424

### SCREENING OF RECOMBINANT ZIKA VIRUS PROTEINS AS ANTIGENS TO DEVELOP AN ELISA FOR THE SERODIAGNOSIS OF ZIKA VIRUS INFECTION

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The severe clinical sequelae following Zika virus (ZIKV) infection warrants development of a sensitive diagnostic assay. Due to shorter duration of ZIKV viremia in infected patients, a serological assay plays an essential role in Zika diagnosis. Unfortunately, clinical interpretation of the current antibody detection assays for ZIKV is challenging due to significant level of cross-reactivity observed among ZIKV patient antibodies with other flaviviruses. With a goal of developing an IgM ELISA with high sensitivity and minimal cross reactivity, we extensively screened ZIKV viral proteins (NS1, NS5, Envelope, and VLP) to be used as antigens to bind ZIKV antibodies. We sought to minimize cross reactivity by incorporating a cocktail of flaviviral antigens as competitors in the ZIKV specific detection conjugate. Assay performance was evaluated for positive percent agreement (PPA) using panels of serial bleeds collected from 50 symptomatic subjects from a ZIKV endemic region that tested positive for Zika RNA, and for negative percent agreement (NPA) with a separate set of 50 donors that exhibited febrile symptoms. Samples from patients positive for IgM against Dengue, West Nile and Chikungunya viruses as well as IgM positive samples from eight other viruses including specimens confirmed positive for Malaria infection were also examined. The Inbios ZIKV Detect™ IgM capture ELISA was used as the comparator method. Among the antigens and combinations tested the highest specificity was observed with the Antigen format 1 with 100% NPA and 3% cross reactivity primarily against malarial infection and CHIKUN samples. No cross reactivity was detected against DENV and WNV positive IgM samples for this format. The relatively lower PPA (89%) obtained with this antigen format was maximized to 96% by replacing antigen format 1 with another antigen combination. Antigen format 2, however, showed relatively lower specificity due to the cross reactivity to some other viruses in the flavivirus family. Our results indicate that with careful selection of ZIKV antigens it is possible to attenuate cross reactivity and maintain good sensitivity for the Zika IgM assay.

## 1425

### PUBLIC HEALTH AT THE FOREFRONT: BUILDING LOCAL HEALTH DEPARTMENT CAPACITY TO IMPROVE ZIKA PREGNANCY AND BIRTH DEFECTS SURVEILLANCE AND REFERRAL TO SERVICE IN UNITED STATES (US)

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Local health departments are on the front lines meeting the needs of pregnant women and children affected by Zika across the US communities. Through partnerships with national clinical and community organizations and state and local health departments, the US Centers for Disease Control and Prevention (CDC) has increased the capacity of local health departments to conduct Zika pregnancy and birth defects surveillance by providing field support and tools and resources. Representatives from CDC, local health departments, and maternal and child health care organizations will interactively describe their challenges, solutions and lessons learned in working to improve Zika surveillance and referral to service for affected pregnant women and children. We will select several local health department representatives that received CDC field support to share their stories at the session to illustrate how local partners, clinicians, state, federal, vector, and community organizations came together in

focusing on building communities response to fight the Zika outbreak. The session will describe approaches, tools, and resources local health departments, ranging from data collection, to public health education and clinical outreach. The participants will end the session by discussing how lessons learned and models of targeted local capacity development can be scaled to a national level during the Zika outbreak and future infectious disease emergencies.

## 1426

### PREVALENCE OF PREVIOUS AND RECENT INFECTIONS BY ZIKA VIRUS, DENGUE VIRUS AND CHIKUNGUNYA VIRUS IN PREGNANT WOMEN AND SURVEILLANCE FOR CONGENITAL ZIKA INFECTIONS IN SALVADOR, BRAZIL

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An unprecedented outbreak of Zika virus (ZIKV) happened in Brazil in 2015. Approximately 18,372 cases were notified in Salvador, Bahia, northeastern Brazil. Since February 2016, we started an active hospital surveillance for congenital Zika virus infection and a cross-sectional study in a reference maternity hospital to evaluate the prevalence of previous and recent infections by ZIKV, Dengue virus and Chikungunya virus in pregnant women as well as the prevalence of congenital infections by ZIKV in newborns. Until now, we enrolled 224 women and 223 newborns. The mean age of women was 25 years and 146 (61%) reported an exanthematous illness during pregnancy. The majority of cases occurred between February and April 2016. Most babies were born in a vaginal delivery (60%), with gestational age of 37.4 weeks. 53% of the newborns were male. 48 (21.5%) of the newborns were reported as microcephaly, of these, 16 (33%) were admitted in a neonatal intensive care unit and 4 (8.3%) died. 133 women were tested by ELISA IgG for Zika, Dengue and Chikungunya by the time of the delivery. The majority of them (94%) were Dengue IgG positive, 94 (71%) Zika IgG positive and 39 (28,6%) Chikungunya IgG positive. The Zika IgM ELISA in samples from mothers and newborns are in progress. ZIKV RT-PCR was done in 114 newborns samples (umbilical cord blood and urine), with 17 (15%) positive results, of these 5 were from microcephalic babies but 12 were normocephalic babies. The RT-PCR positivity was 19.2% in microcephalic newborns and 13.6% in normocephalic newborns. Thus, we suggest that microcephaly should be considered the severe form of congenital Zika infection and that less severe presentations of this congenital infection should be evaluate. We intend to further characterize the clinical manifestations associated with congenital ZIKV infection in a prospective follow-up study of these babies with congenital infections by ZIKV (with and without microcephaly) to describe clinical manifestations, complications and natural history of the disease.

## 1427

### GROWTH OUTCOMES OF NEWBORNS BORN TO WOMEN BORN TO WOMEN WITH POSITIVE ZIKA SCREENS DURING PREGNANCY, HIMA SAN PABLO BAYAMÓN CASE SERIES

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Since the Zika Virus epidemic outbreak in the Americas in 2015, several studies have confirmed a causal link between infection during pregnancy and a range of congenital anomalies. In response to the global public health emergency, mandatory active surveillance during pregnancy was established in Puerto Rico. Both symptomatic and asymptomatic exposures in pregnancy are documented. There is a growing body of evidence supporting that the risk of intrauterine Zika infection related to central nervous system development persist throughout pregnancy. However, we have identified a gap in the evidence regarding disproportionate growth parameters and sex discrepancies in the susceptibility to adverse outcomes. A consecutive case series was established through examination of hospital registry of live births from August 2016 through March 2017. Sample selection was based on exposure to maternal Zika infection during pregnancy following the Puerto Rico Department of Health reporting criteria. Growth measures including head circumference (HC, cm), Length (cm) and weight (kg) extracted from the hospital registry was evaluated using the World Health Organization's Nutritional Survey platform to determine the sex adjusted Z scores. The criteria for microcephalus as defined by the Department of Health was defined by a HC Z score < -3. Sex specific prevalence was also evaluated. We identified 82 infants (56.1% female) with history of maternal Zika exposure as confirmed by laboratory testing. The prevalence of premature birth was 8.5%, and the mean Z score for HC was  $-0.29 \pm 1.34$ , Length  $-0.22 \pm 1.56$ , and weight  $-0.35 \pm 1.17$ . The prevalence of microcephalus for the entire series was 3.7%, while the sex specific prevalence for females was 6.7%. This reports adds to the evidence confirming the central nervous system effects of intrauterine Zika exposure. A sex disparity for microcephaly was observed, which requires further study.

## 1428

### BREAST MILK AND ZIKA VIRUS INFECTION IN PREGNANCY, THAILAND 2016 - 2017

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Zika virus (ZIKV) infection in pregnancy causes a serious birth defect of fetus and newborn. Further more, the newborn may be at risk of infection by ZIKV-contaminated in breast milk. We evaluated the ZIKV presenting in the breast milk among postpartum mothers who infected in different gestational age (GA) with aimed to describe the incidence and characteristic of pregnancy. The national guideline for ZIKV testing in breast milk was established since February 2016. The criteria for testing by real-time RT-PCR in the breast milk (volume 7-10 mL) was any postpartum woman who had history of 1) prolong ZIKV viremia duration > 1 month or 2) ZIKV infection before delivery < 1 month or 3) ZIKV infection in postpartum. Molecular sequencing was used for confirmation the virus. Newborns were required to test ZIKV-PCR in their blood and urine including IgM within 3 days after delivery. Total 27 postpartum women were met criteria for breast milk testing. Number of case in category 1, 2 and 3 was 16, 10 and 1 respectively. Six cases (22%) were positive ZIKV-PCR in breast milk. Molecular sequencing revealed Asian lineage. Among 6 positive-postpartum women were aged range from 16 to 32 years old (median 26.5) and 4 of them (67%) were symptomatic. All of them were infected during 36 - 39 weeks of GA (60%,  $p = 0.04$ ). The duration from detection till delivery was 1 - 2 weeks. The 6 babies's birth weight was 2,770 - 3,640 grams (median 3,110) with normal head size (range 32 - 34 centimeters). Six newborns were negative ZIKV-PCR and IgM. So far,

none of them was reported abnormality and still be follow up for at least 2 years. The 6 babies were temporally suspended breast feeding till test negative in breast milk. In conclusion, the risk of ZIKV presenting in the breast milk is clearly demonstrated in pregnant who infected in very late third trimester and was regardless of clinical presentation. The prolong viremia in first and second trimester was not positive in the milk might due to milk production starting in perinatal period. We strongly advice to test the ZIKV in breast milk among postpartum who was ZIKV infection in third trimester particularly above 36 weeks of GA.

## 1429

### FUNCTIONAL DIFFERENCES AND HOST ANTIVIRAL RESPONSES TO NICARAGUAN ZIKA VIRUS AND PROTOTYPE STRAINS REVEALED IN FIRST-TRIMESTER VILLUS EXPLANTS

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Zika virus (ZIKV), a member of the *Flavivirus* family, is responsible for the recent Zika pandemic in the Americas. Women infected during pregnancy have prolonged viremia and increased risk of infecting their babies, which can result in devastating birth defects designated Congenital Zika Syndrome. Why the American ZIKV strains disseminate to the human placenta during gestation is unknown. We reported that both American and prototype African ZIKV infect numerous primary cells from human placentas and fetal membranes, and viral envelope (E) and nonstructural protein 3 (NS3) are expressed and infectious progeny produced (Tabata, *et al.* Cell Host & Microbe, 2016). Here, we studied ZIKV replication in a functional model of first-trimester, anchoring villus explants and compared Nicaraguan (Nica1, Nica 2) strains with a highly-passaged, prototype Ugandan strain (MR766). Infection was observed in *foci* of mitotic cytotrophoblasts (CTBs) proximal to villus cores in columns of proliferating cells and invasive CTBs and in Hofbauer cells nearby and new villus sprouts. CTBs in Nica ZIKV-infected villi migrated from distal cell columns, invaded extracellular matrix and formed anchoring villi. In contrast, migration of prototype-infected CTBs was limited, higher titers of progeny were made, and fragmentation occurred, suggesting cell death. Given that ZIKV infection varied among placentas, we considered host cell factors might suppress infection. Accordingly, we discovered uninfected villi secrete cytokines that contribute to a precipitous reduction of ZIKV titers. Robust production of IFN- $\beta$  in explants infected in the presence of conditioned medium from uninfected explants was observed, and NS3 protein was reduced in CTBs. Our results identified mitotic CTBs and Hofbauer cells as primary targets of infection in anchoring villi and detected production of cytokines, including IFN- $\beta$ , that naturally reduce ZIKV replication. Our results suggest CTBs and Hofbauer cells could sustain persistent infection under conditions that limit antiviral responses, and that early treatment might reduce the viral load and transplacental transmission.

## 1430

### POTENT ACTIVITY OF THE BROAD SPECTRUM INHIBITOR FAVIPRAVIR ON IN VITRO USUTU VIRUS REPLICATION AND IN A MOUSE INFECTION MODEL

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Usutu virus (USUV) is an emerging flavivirus in Europe, and member of the Japanese encephalitis serocomplex. Furthermore, *Culex pipiens* is the main vector of the virus. However, *Aedes albopictus* can be involved as well. This virus mainly affects birds, however, it has also been found in rodents, bats and horses. In addition, a few cases of human infection have also been reported, showing that USUV has the capacity of adapt to new

hosts. Currently, there are no antiviral drugs available for the treatment or prevention of USUV infections. Favipiravir, also known as T-705, is a broad-spectrum antiviral agent of RNA viruses. This molecule has been approved in Japan for treatment of influenza virus infections. We report here for the first time on the selective antiviral activity of favipiravir on USUV replication in tissue culture with an EC50 of  $90 \pm 26.1 \mu\text{M}$ . Moreover, we established a panel of *in vitro* assays to allow the identification of USUV inhibitors and demonstrate that favipiravir efficiently inhibits viral replication. Infection of AG129 mice (which lack alpha/beta IFN and gamma IFN receptor genes) with USUV showed disease symptoms after 2 days of infection. Besides, high levels of viral RNA were detected in the serum, spleen and brain, of USUV-infected mice. Additionally, first proof-of-concept of the anti-USUV effect of favipiravir is demonstrated *in vivo*. In the AG129 mice oral administration of favipiravir (300 mg/kg/day) reduced viremia and it delays the time to disease progression, which also validates this small animal model to assess the *in vivo* efficacy of novel USUV inhibitors.

## 1431

### SERODIAGNOSIS OF FLAVIVIRUS INFECTIONS AMONG THE BAKA PYGMY POPULATIONS IN CAMEROON USING AN IN-HOUSE MAC-ELISA

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Although there are several types of life cycles, many arboviruses have basically a sylvatic cycle. Pygmies are known to be the first inhabitants of the forest. In Cameroon, little is known about arboviruses prevalence among these populations. Since 2004, Centre Pasteur of Cameroon (CPC) is the reference center for the yellow fever case-based surveillance. In 2013, CPC launched the first sentinel surveillance project of arboviruses. All these monitoring activities however do not involve the Pygmy populations, who seldom visit hospitals and whose lifestyle increases the risk of exposure to arboviruses. The aim of this study was to assess the endemicity of yellow fever virus (YFV), dengue virus (DENV) and West Nile virus (WNV) in the Baka Pygmy population of Cameroon. A cross-sectional study was carried out in different pygmy camps in Cameroon between 2005 and 2010. Serum samples from volunteers were collected and processed. A total of 242 samples obtained from the Baka pygmies were included in this study. These sera were tested for the detection of Immunoglobulin M antibodies (IgM) to three most common *Flaviviruses* such as YFV, DENV and WNV; using in-house M Antibody Capture Enzyme Link ImmunoSorbent Assay (MAC-ELISA) technique adapted from CDC (Center for Disease Control and Prevention, Atlanta, USA) and World Health Organization (WHO) protocols. Antigens used were either produced by the CPC arbovirus laboratory (DENV and WNV antigens) or provided by WHO (YFV antigens). Of the 242 serum samples tested, 9 (4%) were IgM positive for *Flavivirus* antibodies, synonym of recent or ongoing arbovirus infections. IgM ELISA was positive in 8 (3.3%) and 1 (0.4%) samples respectively for YFV and WNV. One sample was IgM positive for both YFV and DENV suggesting *Flavivirus*-induced antibody cross-reactivity. *Flaviviruses* are prevalent in pygmy camps in Cameroon and remind us to pay attention to these populations when implementing public health activities. Therefore, further clinical and virological surveillance of arbovirus infections among Pygmies should be considered, and could lead to their isolation and characterization.

## 1432

### MODELING THE SPREAD OF MOSQUITO-BORNE DISEASE IN THE NORTHERN GREAT PLAINS OF THE U.S

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Despite advances in the control of infectious diseases, mosquito-borne diseases still pose an enormous threat to humans around the globe. The virus has complex transmission dynamics, which will require efficacious tools for disease mitigation. Not surprisingly, statistical models have become powerful tools that are particularly helpful in understanding factors contributing to increase the incidence. However, recent work is often limited by the simple assumption that WNV cases increase with increasing mosquito numbers, which ignores the fact that outbreaks can take place even when mosquito abundance is low. Our research focus here is the West Nile Virus (WNV), which has been endemic in the U.S for past 15 years. The study objective is to design statistical approaches capable of identifying and determining the relative importance of factors controlling the prevalence of WNV in humans across the Northern Great Plains. Our modeling is unique in its focus on both mosquito numbers and human disease cases independently. In effect, we developed two statistical models - one tracking mosquito abundance and the other simulating numbers of human disease cases. Our findings demonstrated that incorporating water body information improved the simulation of mosquito abundances. River flooding, driven by weather hundreds of miles away, turned out to be more important than variability in the area of lakes due to rainfall. Moreover, we found transmission rate and mosquito numbers to be the key controlling factors for human disease cases. The model showed that disease cases were not abundant when the transmission rate was low, even if mosquito numbers were relatively high. This was the first study to show the importance of individually assessing the dynamics of mosquito abundances and disease cases. This work provides statistically rigorous tools for prediction of WNV risk. The findings and conceptual framework of this statistical approach could potentially be applied to prediction analysis of other mosquito-borne diseases.

## 1433

### INCREASED ANTIBODY DIVERSITY GENERATED BY ADJUVANTS CORRELATES WITH PROTECTION IN RECOMBINANT PROTEIN-BASED FLAVIVIRAL VACCINES

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Flavivirus infection is the leading cause of death due to mosquito-borne viruses worldwide. Clinical development efforts for this virus family include promising vaccine candidates for dengue virus (DENV), Zika virus (ZIKV), West Nile Virus (WNV), tick-borne encephalitis (TBE), and yellow fever (YF, 2nd generation). We have previously described the preclinical development of an adjuvanted subunit vaccine for WNV that is capable of generating complete and durable protection after a single immunization. The protection observed with our vaccine required a formulated TLR-agonist adjuvant. In order to better understand the mechanism of adjuvant enhanced protection, we have examined the effect of adjuvant formulation on antibody diversity following immunization in preclinical models. Using a novel, deep sequencing-based methodology, we find that protective adjuvant formulations induce a more diverse antibody response characterized by expanded V-gene usage and increased sequence diversity. In addition, we have examined the role of the bioactive adjuvant components and find that TLR agonists in combination with saponin molecules result in the highest antibody diversity following immunization.

We have subsequently extended these findings to similar vaccines for ZIKV, suggesting that induction of a diverse antibody response may generally correlate with preclinical protection. In addition, these studies identify adjuvant formulations which may generally enhance antibody diversity when combined with flaviviral antigens.

## 1434

### USE OF QUANTITATIVE REAL-TIME POLYMERASE CHAIN REACTION TO IDENTIFY NOROVIRUS DIARRHEA IN INDIAN CHILDREN IN THE FIRST 3 YEARS OF LIFE: A REANALYSIS OF A BIRTH COHORT STUDY

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Noroviruses are an important cause of acute gastroenteritis in children. However, limited data is available on norovirus diarrhea in community settings in Asia. In our study, norovirus gastroenteritis was evaluated in 1856 diarrheal episodes in a birth cohort of 373 children followed up to the age of 3 years in a semi-urban slum in Vellore, India, from 2002 to 2006. We used quantitative multiplex real-time PCR (qPCR) to detect norovirus GI and GII in 1711 (92.2%, 1711/1856) available stool samples from the 1856 diarrheal episodes and compared it with previous results where conventional PCR had been used to test all the 1856 diarrheal samples for GI and GII. A Ct value cut-off of 35 for GI and 37 for GII was used for the qPCR assay. qPCR detected noroviruses in 334 episodes (19.5%, 334/1711), of which 60 episodes (3.5%, 60/1711) were norovirus GI, 269 (15.7%) norovirus GII, while 5 (0.3%) were mixed infections with both norovirus GI and GII. In comparison, conventional PCR was able to detect noroviruses in 207 (11.2%, 207/1856) episodes, of which 49 (2.6%) were norovirus GI, 150 (8.1%) norovirus GII, and 8 (0.4%) were mixed infections with both norovirus GI and GII. The use of qPCR improved the detection of noroviruses in diarrheal samples from 11.2% (conventional PCR) to 19.5% and indicated a high burden of diarrheal disease associated with this pathogen in developing countries.

## 1435

### POPULATION STRUCTURE AND TRANSMISSION DYNAMICS OF NOROVIRUS IN A PERUVIAN BIRTH COHORT

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The population structure and transmission dynamics of norovirus (NoV) epidemics in tropical regions like Peru remains unclear. Redressing this knowledge gap has relevance for NoV vaccine design and public health response. We therefore undertook a fine-scale phylogenetic analysis leveraging molecular data collected as part of NoV surveillance birth cohort in a Lima shanty-town. RT-PCR NoV positive fecal specimens were collected longitudinally from 291 symptomatic and asymptomatic immunocompetent infants and children in Las Pampas de San Juan de Miraflores, Lima Peru over 2007-2011 and then sequenced targeting the 5' 330 nt region of the capsid gene VP1. GI (n = 84) and GII (n = 458) sequences were aligned with background data from public databases. Maximum likelihood phylogenies were inferred using RAXML with nucleotide substitution models selected by JModelTest2. The age structure of NoV epidemics was estimated by performing an equality-of-medians test of ages from well-supported transmission clusters, and within-host persistence times of NoV strains were determined. The majority of known GI and GII genotypes were detected in the birth cohort, with co-circulation of up to 25 variants per year, including within-genotype variants and

a novel divergent lineage. There was evidence of multi-year subtype persistence within the cohort. Within-host strain persistence times was as high as 47 days (median 12 days, IQR 7-28 days). There was weak evidence that NoV transmissions occurred between participants of similar ages ( $p = 0.09$ ), and national and continental spatial structure was noted across Peru and other Latin American countries. In conclusion, the ecology of NoV in a Peruvian birth cohort is characterized by broad diversity, including a possible novel genotype. NoV epidemics may have a fine-scale age structure suggestive of an age-dependent host susceptibility to particular NoV subtypes. The upper range of gut NoV strain shedding time is high and further supports the concept of a pediatric enteric "virome".

## 1436

### PRELIMINARY REPORT OF A STUDY ON EFFECTIVENESS, SAFETY AND ACCEPTABILITY OF CERVICAL CANCER SCREENING USING VISUAL INSPECTION WITH ACETIC ACID AND COLD COAGULATION BASED SINGLE VISIT APPROACH IN YAT SAUK TOWNSHIP IN SHAN STATE, MYANMAR

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Cervical cancer is the second most frequent woman cancer in Myanmar and screen coverage was lower than one percent in Myanmar (WHO, 2014). Cryotherapy for treatment of pre-malignant lesion has been found not feasible for women in rural area. Being a preventable cancer, the organized screening program using simple, practical and cost effective technology is necessary to improve coverage. The aim of this study is to evaluate the effectiveness, safety and acceptability of visual inspection with acetic acid (VIA) and cold coagulation (CC) based single-visit approach in cervical cancer prevention (CCP). A descriptive community-based action research was conducted from June 2016 to March 2017. Well-trained central CCP mobile team from Women and Children Hospital, Taungyi and medical officers from Yat Sauk township, visited to Yat Sauk Township, Shan State fortnightly during the weekends and mass screening was conducted using VIA and CC based single-visit approach. During 9 visits, 1867 married women of 30-49 years aged group were screened and screen coverage was 2.66%. Test was positive in 48 women and screen-positive rate was 2.57%. All VIA positive women were eligible to CC and all agreed to have treatment on the same visit after proper counseling and treatment rate was 100%. On one-month follow up visit, watery vaginal discharge for 2 to 3 weeks was the only symptom reported by all except two women. One needed antibiotics for infection and another one needed reassurance for symptom of burning sensation at SPA. On one-year follow up visit, VIA will be repeated to check persistence after CC and will assess women's satisfaction on CCS program. CC is a safe alternative way of treatment for VIA positive women of CCS program for areas where cryotherapy is not feasible because of difficulty to have medical grade carbon-dioxide. We need to wait one year follow up visit to assess the effectiveness of CC. During screening visits, VIA training was given to 42 local basic health staff to sustain CCS.

## 1437

**AN OUTBREAK OF FEBRILE SYNDROMES IN THE NORTH OF PERU: EMERGING AND REEMERGING ARBOVIRUSES**

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Arboviruses are one of the most common causes of acute febrile illness and an emerging health problem in South America. In Peru, the number of Dengue cases have double in the last year; however, less than 50% of acute febrile illness are laboratory confirmed leading to an underdiagnoses of other important arboviruses. This study was undertaken to assess the prevalence of Dengue (DENV), Oropuche (OROV), Chikungunya (CHIKV) and Zika ZIKV in patients with acute febrile illness from Cajamarca, Peru. Samples were obtained from patients with acute febrile illness during an outbreak of febrile syndromes in the North of Peru in November 2016.

A total of 95 specimens were collected and assessed for the presence of DENV, OROV, CHIKV, and ZIKV via RT-PCR. DENV virus RNA was detected in 29.47% (28/95) was the most prevalent arboviruses, CHIKV 3.16% (3/95), ZIKV 6.32% (6/95) and OROV with 0% cases. Among all patients, the most common symptoms accompanying DENV positives were: fever 82.15% (23/28), headache 35.1% (10/28), retrocular pain 21.43% (6/28), myalgias 17.86% (5/28) and arthralgias 14.29% (4/28). In the case of ZIKV the most common symptoms is fever 83.33% (5/6) and headache 33% (2/6) cases. For CHXV the most common symptoms is fever, headache and arthralgias with 66.67% (2/3) for each case. DENV-3 was the most predominant in 14.29% (4/28); DENV-2 was found only 2 sample and 7.14% (2/28). No DENV-1 or DENV-4 serotypes were observed. Twenty-two cases 78.57% (22/28) couldn't be characterized for any serotype. In conclusion, DENV are the most common arboviruses in Cajamarca. However, there is a need to characterize the unidentified serotypes, in order to determine if there is a new variant or a new serotype circulating. To enhance arbovirus surveillance is crucial to understand the role of these pathogens in Peru. PCR represents a reliable test for arboviral surveillance and should be considered as the preferred method for laboratory confirmation in Peru.

## 1438

**SEROPREVALENCE OF EBOLA VIRUS AMONG HEALTH CARE WORKERS IN YAMBUKU HEALTH ZONE, DEMOCRATIC REPUBLIC OF CONGO**

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Ebola virus disease (EVD) is caused by a zoonotic filovirus infection that can be highly lethal in humans. Since the first outbreak in Yambuku in 1976, a total of seven confirmed EVD outbreaks have occurred in the Democratic Republic of Congo (DRC). Health care workers (HCW) are at particularly high risk of EVD infection given the high titers of virus in bodily fluids and lack of compliance with universal precautions to prevent exposure either due to lack of knowledge, training or equipment. We conducted a study in Yambuku, to determine the prevalence of Ebola antibodies in HCWs. Field collection occurred in April 2016. Interviews and blood specimens

were collected from all consenting individuals. Serum samples from 250 HCWs based in 8 health facilities in Yambuku health zone were screened for Ebola virus zaire (EBOV) GP Ig using Human Anti-Zaire Ebola Virus Glycoprotein (GP) IgG ELISA Assay kits (Alpha diagnostic International, Inc.) in Kinshasa, DRC. Among 250 health care workers, 36% (n=90) were seropositive for EBOV GP IgG. Notably, 30.8% (n=41) of subjects born before the 1976 outbreak were seropositive, while among those born after 1977, 43.3% (n=49) were seropositive. Also, we found that HCWs with direct contact with patients were 34.4% (n=113) seropositive, indirect contact, 39.1% (n=18), and limited contact 43.8% (n=7). Our findings suggest that despite the absence of a reported outbreak since 1976, many HCWs are still potentially exposed to EBOV in Yambuku. Additionally, the general population may also be continually be exposed to EBOV. Further research is needed to determine if virus is still circulating in the area and could pose a risk for an outbreak.

## 1439

**SEROPREVALENCE OF POLIOVIRUS ANTIBODIES SURVEY IN MALI, GUINEA AND CÔTE D'IVOIRE**

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Since the launch of Global Polio Eradication Initiative in 1988, great strides have been made towards the goal of polio eradication. The latest Wild Poliovirus was detected in West Africa in 2011, however, acute Flaccid Paralysis surveillance in West Africa has not been consistent. There is concern that population immunity to polioviruses may be low, especially in the Ebola affected countries; the potential population immunity gap favors spread of wild poliovirus if imported from endemic zones and favors emergence and transmission of vaccine derived polioviruses. Worldwide Polio program needs to understand underlying population immunity estimates to guide future programmatic actions. We conducted a cross sectional study to quantify the level of serological protection against poliovirus serotypes 1,2 and 3 in children aged 6-11 months and 36-48 months. From June 2016 to January 2017, 1059 subjects were enrolled in Mali, Guinea and Côte d'Ivoire based on the list obtained from the census data at each site and each age group. 526 children were in the age group 6-11 months and 533 in the 36-48 months. The neutralization assay was performed at the CDC laboratory (Atlanta) to determine the seropositivity for antibodies defined as reciprocal titer greater or equal to 8. The three West African Countries achieved high population immunity against PV1 and PV3 however PV2 seroprevalence was low in the young age group in Côte d'Ivoire and Guinea. Children born after the tOPV to bOPV switch has lower seroprevalence for PV2 than those born before the switch. Age, Country, vaccination history and nutritional status were predictive of seronegativity. Acute malnutrition was observed in 13% of children in Côte d'Ivoire, 15% in Mali and 45% in Guinea, chronic malnutrition was observed in 15% of children in Guinea, 19% in Côte d'Ivoire and 24% in Mali. In conclusion, there continues to be a high degree of immunity to PV1 and PV3 strains in the population. Decreased immunity to PV2 could have implications for disease and vaccine.

### SEROLOGICAL SURVEY TO MONITOR POPULATION IMMUNITY TO MEASLES AND RUBELLA VIRUSES AFTER A NATIONAL MEASLES-RUBELLA VACCINATION CAMPAIGN IN ZAMBIA

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Household surveys are routinely conducted after vaccination campaigns to estimate coverage; however, they remain an indirect measure of population immunity. Serological surveys directly measure population immunity and can be used to identify pockets of susceptibility and inform targeted vaccination strategies. In September 2016, Zambia conducted a national measles-rubella vaccination campaign for children 9 months to 14 years of age. Following the campaign, the Ministry of Health conducted a nationwide post-campaign evaluation survey (PCES) to assess vaccination coverage. We nested a serological survey within the PCES in Southern Province to estimate population immunity to measles and rubella viruses and evaluate the feasibility of nesting a serosurvey within a household survey. The four serological survey teams consisted of two field coordinators, four supervisors and 16 data collectors and were paired with a PCES team in the field. The serosurvey team administered a brief questionnaire and obtained dried blood spots by fingerprick from all members in a household selected for the PCES. Fifteen of the 26 clusters selected for the PCES were included in the serosurvey and 148 households were enrolled. Of the 978 residents of enrolled households, 801 were home at the time of the survey and 698 (89.5%) agreed to participate. Of these, 419 (60%) were <15 years old and eligible for the campaign. The median age of participants was 11 years [IQR 6-26] and 47% were male. Three percent of participants refused blood collection, resulting in DBS specimens from 679 participants. The DBS are being tested for anti-measles and anti-rubella virus IgG by enzyme immunoassay and seroprevalence results will be presented. This study demonstrated that fingerprick blood samples for serology can be collected during household surveys, although operational challenges were encountered. Social mobilization activities may have reduced the refusal rate to lower than anticipated. These findings will inform future studies and programs that aim to integrate serology into household surveys to assess age-specific population immunity.

### MAPPING ANTIBODY EPITOPES ON THE EBOLA VIRUS ENVELOPE PROTEIN BY SHOTGUN MUTAGENESIS

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To characterize the detailed immune response to Ebola virus (EBOV), we have mapped the epitopes for over 100 monoclonal antibodies (MAbs) that target the EBOV surface glycoprotein, GP. We used Shotgun Mutagenesis to create two comprehensive alanine scan libraries arrayed in 384 well plates, one comprising 641 single Ala mutations in full-length EBOV GP, and a second library for GP lacking the mucin-like domain. GP variants were expressed in human cells and assayed for reactivity with MAbs, using high-throughput flow cytometry to identify GP residues required for the binding of each MAb. As described in recent publications, a broad variety of MAbs have been mapped, including the ZMapp therapeutic MAb cocktail; MAbs from human survivors, including 6 MAbs

from a human survivor of Bundibugyo ebolavirus infection; a MAb, cross-reactive with other ebolavirus species, which binds to the GP head region and blocks the interaction of GP with its endosomal receptor Niemann-Pick C1; and MAbs whose synergistic combination transformed a non-neutralizing MAb into a potent neutralizer. The epitope maps obtained for these different types of MAbs have expanded our understanding of how the immune system recognizes EBOV GP, and correlating MAb epitopes with their neutralizing capabilities is being used to develop anti-EBOV therapeutics and vaccines. This includes identifying mutations that increase the exposure of neutralizing epitopes, which can impact the design of future anti-ebolavirus vaccine strategies. For insights into the requirements for EBOV infectivity we also performed infectivity assays with the full EBOV GP mutation library using a lentivirus pseudotype system. To identify uncharacterized EBOV cellular receptors we similarly assayed wild-type GP infectivity in non-permissive cells expressing our membrane proteome array (MPA) of over 4,500 unique human membrane proteins individually expressed in eukaryotic cells. This has identified a number of candidate membrane proteins that enable EBOV infectivity.

### HOUSEHOLD LEVEL MEASLES VACCINATION COVERAGE AND ASSOCIATED HISTORY OF MEASLES DISEASE AMONG CHILDREN 9-59 MONTHS IN THE DEMOCRATIC REPUBLIC OF CONGO

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Global suboptimal measles vaccination coverage ensures that measles remains an important public health concern. The Democratic Republic of Congo (DRC) demonstrates heterogeneous measles vaccination coverage, and quantifying the risk of measles for children within households at varying vaccination coverage levels is necessary to evaluate the overall effects of immunization programs. We assessed measles disease history among children participating in the 2013-2014 DRC Demographic and Health Survey (DHS). Our study sample consisted of 6,597 children aged 9-59 months whose mothers were selected for household interview. Measles vaccination status was obtained either via maternal report or vaccination card. A hierarchical Bayesian model was used to examine effects at the household, cluster, and provincial levels. An index child was selected from each household, and households were categorized by number of siblings vaccinated (excluding the index child) out of total number of siblings, forming three main categories: no vaccination coverage, heterogeneous coverage, and full (100%) coverage for measles vaccination). In households with completely unvaccinated siblings, 13% of unvaccinated and 10% of vaccinated index children reported measles. In households with a mixture of vaccinated and unvaccinated siblings, 12% of unvaccinated and 6% of vaccinated index children reported measles. In households with fully vaccinated siblings, 8% of unvaccinated and 10% of vaccinated index children reported measles. Further adjusted analyses were completed to quantify measles risk to vaccinated and unvaccinated children within households, and sensitivity analysis limited to vaccination report via dated card was performed. This study demonstrates the importance of quantifying the impact of household vaccination coverage among vaccinated and unvaccinated children 9-59 months in DRC and suggests the need for further research to aid strategic vaccination program policy development.

## 1443

**FORECASTING AND ASSESSMENT OF AUTOCHTHONOUS YELLOW FEVER OUTBREAK IN BRAZIL**Dhananjai M. Rao<sup>1</sup>, Carmen Asburn<sup>2</sup>, Paul D. Stamper<sup>2</sup><sup>1</sup>Miami University, Oxford, OH, United States, <sup>2</sup>MRI Global, Gaithersburg, MD, United States

As of March 2017, two states of Espírito Santo (ES) and Minas Gerais (MG) in Brazil have accounted for over 561 confirmed and 416 suspected cases of Yellow Fever (YF). The number and rate of cases is growing at an alarming rate, particularly when compared to prior outbreaks. With the vaccine stockpile depleted from the mid-2016 Angola outbreak in West Africa, forecasting epidemic progression is necessary to enable judicious use of vaccines to contain the outbreak. Accordingly, we propose the use of a novel computational epidemiology system called EpiRadar for forecasting and analysis of YF progression to inform health policy to contain the outbreak. EpiRadar was used to conduct analysis of the YF outbreak using a detailed temporospatial epidemic model of ES and MG states, involving: humans and three jungle mosquito species (*H. Janthinomys*, *H. Leucocephala*, and *S. Chloropterus*). Machine-learning methods based on a genetic algorithm have been used to calibrate parameters to fit the model to current outbreak in ES and MG. The calibration also accounts for ~55% of infections being asymptomatic and ~33% have mild or subclinical symptoms (estimates from past outbreaks) -- that is ~88% of infected individuals typically do not seek medical help and thus are not included in outbreak reports. Leave one out cross validation has been used to ensure that the model is not overfitted. Stochastic simulations into the future have been used to generate forecasts. Calibration data indicates that 45% ( $\pm 5\%$ ) of the population is immune due to prior outbreaks or vaccinations. Our forecasts show that, if current trends continue, then by July we predict the reported severe cases to grow to 4000 to 6000 cases, with 6000 to 10,000 infective individuals in ES and MG area alone. Generalized Sensitivity Analysis (GSA) shows that mosquito population plays a greater role than bite rate suggesting that controlling mosquito population is important. Analysis on policy and comparisons of our model predictions to incoming data is ongoing. Additionally, the influence of non-human primates to the force-of-infection is underway.

## 1444

**IDENTIFICATION OF CONSERVED MOTIFS IN VIRUSES BELONGING TO GUAMA SEROGROUP (ORTHOBUNYAVIRUS, BUNYAVIRIDAE)**Valéria L. Carvalho, Márcio R. Nunes, Daniele B. Medeiros, Sandro P. Silva, Clayton P. Lima, Jedson F. Cardoso, João L. Vianez Júnior, Davi T. Inada, Sueli G. Rodrigues, Pedro F. Vasconcelos  
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The Guama serogroup belongs to the *Orthobunyavirus* genus, *Bunyaviridae* family, composed of tripartite, single-stranded, and negative-sense RNA genomes, namely, large (L), medium (M), and small (S) genomes. The Guama serogroup includes viruses isolated in the Brazilian Amazon such as *Guama virus* (GMAV), *Catu virus* (CATUV), *Bimiti virus* (BIMV), *Mirim virus*, *Moju virus* (MOJUV) *Ananindeua virus* (ANUV), *Timboteua virus*, as well as others. This study aimed to identify orthobunyavirus conserved motifs in the aminoacidic sequences of the GMAV, CATUV, BIMV, ANUV and MOJUV. These viruses were sequenced, and we got complete sequences of the S-RNA (GMAV, CATUV, BIMV, ANUV, MOJUV), M-RNA and L-RNA (GMAV, BIMV, MOJUV) segment of them. We recognized in the viruses of the Guama serogroup the six conserved motifs in the gene N described by Saeed *et al.* (2001) and a seventh motif group specific (positions 212 to 237). Conserved residues observed in orthobunyaviruses related to packaging of the ribonucleoprotein were identified in the GMAV, CATUV, BIMV, ANUV and MOJUV alignment (positions P129, G135, I/L162, I235); there was a change in the residue Y158/I. Residues conserved associated to the

RNA synthesis were detected in the Guama serogroup viruses: positions F22, F148, L164, F180, L181, K183, Y189, W197, W217, F229 with the change of residue Y176F, and the residues R45, R99 and K55 related to binding with RNA were found in these viruses. In the NSm protein (GMAV, BIMV, MOJUV) was observed the motif GDFTNxCNSC (position 423 to 432; x: K→F) found in the Bunyamwera and California groups. The cleavage site between NSm/Gc correspond to the residue A475. A transmembrane region with potential to anchor the membrane in the Gc protein was identified (1.411 to 1.431 aa.). The cleavage sites to the trypsin were predicted: LRPI (MOJUV), THAV (BIMV) and SYVL (GMAV). The fusion peptide in the glycoprotein Gc was identified: GMAV (1062 to 1078), BIMV (1065 to 1081), MOJUV (1064 to 1080). In the gene L of GMAV, BIMV, MOJUV, we detected four conserved regions (I, II, III, IV) described to others orthobunyaviruses, including the motifs Pré-A, A, B, C, D, E in the region III.

## 1445

**ANALYTICAL PERFORMANCE OF THE FILMARRAY® GLOBAL FEVER PANEL**Jared R. Helm<sup>1</sup>, Corike Toxopeus<sup>1</sup>, Natalie Batty<sup>1</sup>, Olivia Davidson<sup>1</sup>, Brandon Marble<sup>1</sup>, Bryan T. Gnade<sup>2</sup>, Stefan Fernandez<sup>2</sup>, Cynthia Phillips<sup>1</sup><sup>1</sup>BioFire Defense, Salt Lake City, UT, United States, <sup>2</sup>U.S. Army Medical Materiel Development Activity, Fort Detrick, MD, United States

Acute Febrile Illness (AFI) can be caused by a large number of pathogens that include bacteria, viruses and parasites. BioFire Defense is developing the Global Fever (GF) Panel to be used on the FilmArray System in collaboration with the Department of Defense and NIAIDb. The FilmArray is an *in vitro* diagnostic test platform that combines nucleic acid purification and nested multiplex PCR for the simultaneous identification of many infectious agents in under an hour using a closed, sample-to-answer system. The FilmArray GF Panel detects and identifies nucleic acid from Chikungunya virus, CCHF virus, dengue virus (serotypes 1-4), Ebolavirus, Lassa virus, Marburgvirus, West Nile virus, Yellow fever virus, Zika virus, *Bacillus anthracis*, *Francisella tularensis*, *Leptospira* spp., *Salmonella enterica* serovar Typhi and Paratyphi A, *Yersinia pestis*, *Leishmania donovani* complex, and *Plasmodium* spp. in venous blood specimens from individuals with signs and/or symptoms of AFI or recent AFI and with known or suspected exposure to target pathogens. Estimated LoD studies demonstrate clinically relevant detection levels and exclusivity testing shows high specificity. For example, LoD levels for the following organisms: dengue virus (serotypes 1-4) at 7-150 copies/mL, Marburgvirus at 2-10 copies/mL, Zika virus at 2 copies/mL, *Leishmania* at 22 parasites/mL, *Plasmodium* at 180 parasites/mL, *Bacillus anthracis* at 3 CFU/mL, and *Yersinia pestis* at 3 CFU/mL. Off-panel exclusivity studies assessing specificity with closely related organisms or organisms that may be found in whole blood show no significant cross-reactivity. A multiplex FilmArray panel could aid in rapid and actionable AFI diagnosis. (a. MCS-JPEO and USAMMDA Contract No. W911QY-13-D-0080, under the NGDS program b. NIAID Contract No. HHSN27220160002C, "Advanced Development of Multiplex Diagnostic Platforms for Infectious Diseases (Global Fever Panel)")

## 1446

**DETECTION OF MLB ASTROVIRUS IN A PEDIATRIC HOSPITAL AT LIMA-PERÚ**Macarena Vittet<sup>1</sup>, Giuliana Oyola<sup>1</sup>, Gerardo Sanchez<sup>1</sup>, Mayra Ochoa<sup>1</sup>, Fabiola Colquechagua-Aliaga<sup>2</sup>, Dante Figueroa-Quintanilla<sup>2</sup>, Holger Mayta<sup>1</sup>, Mayuko Saito<sup>3</sup>, Sarah-Blythe Ballard<sup>4</sup>, Robert Gilman<sup>4</sup><sup>1</sup>Infectious Diseases Research Laboratory, Department of Cellular and Molecular Sciences, Universidad Peruana Cayetano Heredia, Lima, Peru, <sup>2</sup>Unidad de Rehidratación Oral, Instituto Nacional de Salud del Niño, Lima, Peru, <sup>3</sup>Tohoku University Graduate School of Medicine, Sendai, Japan,

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Human Astroviruses (HastV) are one of the major viral agents of acute gastroenteritis in young children, recently, a new genogroup has been reported as MLB. This new genogroup has been found in stool samples from patients with diarrhea. However, there is a lack of understanding if this genogroup is related to acute gastroenteritis or other diseases. We evaluated the burden of HastV associated to diarrhea. We collected stool samples from a case-control study from the hospital "Instituto Nacional de Salud del Niño" in Lima, Perú, samples were collected from October 2013 to May 2016. Cases were children with gastroenteritis symptoms who met the WHO definitions. Controls were children without gastroenteritis symptoms at the time of enrollment or in the past 30 days. Classic HastV detection was performed by RT-q PCR, all positives samples were amplified by conventional PCR and send for sequencing. MLB Astrovirus detection was performed by RT-PCR with consensus primers targeting a partial region from RdRp gene. All positive samples were amplified with another PCR with specific primers for MLB and amplification products were send for sequencing. A total of 1117 samples from diarrhea cases and 482 from controls were analyzed. Prevalence of classic HastV among cases was 5.81% (65/1117) and 2.07% (10/482) among controls (OR=2.92). HastV was detected mostly in children aged 12-24 months. The most common genotype was HastV1. Severity analysis was performed using Vesikari score. Preliminary data on MLB detection involved a total of 399 samples (133 diarrhea and 266 non- diarrhea). A prevalence of 1.72% was found (3/174).

## 1447

### MIDGUT MICROBIOTA COMPOSITION FROM FIELD COLLECTED AND EMERGED MOSQUITOES *ANOPHELES ALBIMANUS* FROM COLOMBIA

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Recent work has revealed an important role of specific mosquito midgut microbiota in reducing *Plasmodium* parasite development, and those bacteria constitute valuable candidates for a biocontrol approach. Mosquitoes acquire their microbiota from the environment and transfer it trans-stage; however, little is known about the factors influencing the bacterial community composition of Neotropical *Anopheles* mosquitoes. Therefore, this study characterized the midgut microbiota of the Colombian main malaria vector *An. albimanus*. Mosquitoes analyzed were field adults and emerged from larvae collected in a locality of the Colombian Pacific Coast. Their midgut microbiota was analyzed by 16S rRNA gene Illumina Mi-Seq. At the genus level, higher bacterial richness was detected in mosquitoes from the field compared to emerged mosquitoes. *Bacillus* spp. predominated in field collected mosquitoes and *Acinetobacter* spp. in emerged mosquitoes. An increased abundance of bacterial genera detected in field collected mosquitoes suggests that bacterial communities are more influenced by the mosquito environment than by the breeding sites where their larvae develop. This result provides the basis for the design of new and effective vector interventions to control malaria transmission.

## 1448

### SPATIAL TOOLS FOR OPTIMIZING TSETSE CONTROL IN GAMBIAN SLEEPING SICKNESS FOCI

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Human African trypanosomiasis (HAT), commonly known as sleeping sickness, is a neglected tropical disease affecting populations in sub-Saharan Africa, with Gambian HAT (gHAT) being the most common form of the disease. Whilst control of gHAT is predominantly achieved through case detection, tsetse control using small insecticide treated targets ('tiny targets') has also been shown to reduce transmission risk in gHAT foci in Uganda, Guinea, Chad, and Côte d'Ivoire. At present, tiny targets are being deployed along rivers located within high transmission risk areas. Once deployed, the progress of the intervention is monitored using a network of tsetse traps to assess the abundance of tsetse in and around the area over time. As the tiny target programme continues to geographically scale up, it is becoming increasingly challenging to manage these monitoring activities. The goal of this project was therefore to develop and implement spatial tools for optimising this process. The aims of the project were two-fold: (1) to analyse the current costs (both financial and time) of the current monitoring programme in north west Uganda, and establish a method of determining optimal monitoring sites, and (2) to develop an electronic system for collecting, collating and visualising monitoring data. To address (1), remotely sensed, and field data were collected for the study area, and used to undertake a land cover classification analysis. These data, in addition to information on the costs associated with travelling between monitoring sites, were used to undertake a cost-distance analysis and subsequently produce an accessibility map. This map was then combined with information on the spatial distribution of tsetse to derive a monitoring site selection strategy. To address (2), an electronic system was developed which involved both a smartphone app for collecting daily monitoring data, and a web browser which displayed a map of the resulting data in real-time. This electronic system was trialled in Uganda in June 2017, and a series of interviews were conducted with both the trap monitors and the control programme supervisor to assess its utility and scalability.

## 1449

### DEVELOPMENT OF MOLECULAR METHODS FOR THE DETECTION AND QUANTIFICATION OF PHLEBOTOMINE SAND FLY LARVAL DNA IN SOIL

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Sand flies (Diptera: Psychodidae: Phlebotominae) are a group of haematophagous insects of great medical concern. They are known vectors of several parasites including *Leishmania* protozoans, which are responsible for 20,000-40,000 human deaths per year globally. The ecology, geographical distribution and vectorial competence of Phlebotomine sand flies has been the subject of significant research during the last decades, resulting in the description of more than 900 different species including proven or suspected vectors. Currently, available sand fly control techniques are focused to a significant extent on controlling adult stages, due to the wide knowledge gap regarding the ecology of sand fly larvae. Better understanding of larval ecology will result in designing more efficient and targeted control strategies against major *Leishmania* vectors. The majority of previous studies investigating sand fly breeding sites were based on emergence trapping techniques of adult sand flies with limited information in relation to abiotic and organic matter parameters of the soil or the preferred depth for breeding. Here we developed a novel real-time PCR technique aiming to detect, identify and quantify sand

fly DNA directly from soil. The method was optimized under laboratory conditions for the identification of two major leishmaniasis vectors of the Mediterranean region, *Phlebotomus tobbi* and *P. papatasi*. Since the majority of DNA extraction kits from soil samples focus on bacterial DNA, 3 different protocols were tested in order to design the most appropriate one for targeting sand fly DNA. The developed method is appropriate for detecting sand fly DNA from different layers of soil avoiding the need for mechanical recovery of larvae or trapping emerging adults. This new approach can be used to build on our currently available knowledge on larval ecology by adding accurate information on the breeding site preferences of sand flies.

## 1450

### ENTOMOLOGICAL STUDY ON A RECENT MALARIA OUTBREAK IN ANKILILOAKA, A SEMI-ARID AREA IN THE SOUTHWESTERN REGION OF MADAGASCAR

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The southwestern region of the Island is characterized by a sub arid climate. However, since December 2015, Ankiloaka which parts of the region is subjected to a multiple recrudescence of human malaria. A basic entomological data collection was carried out in March and April 2016 in two villages of Ankiloaka to study the malaria vector species composition, their behaviors, their density and their infectivity. Study of mosquito larvae breeding sites was conducted according to the WHO method. Adult mosquito sampling was performed using human landing catches, indoor pyrethrum spray catches and mouth aspirator for outdoor mosquitoes. Collected mosquitoes were morphologically identified. The standard PCR method was used to identify the sibling species of the complex *An. gambiae*. Overall, 153 *Anopheles* larvae breeding sites were found in Ankiloaka: rice fields (67%) were the most abundant and permanent breeding site identified. Seven *Anopheles* species were found. All *An.gambiae* s.l. was identified as *An. arabiensis*. Three malaria vector species: *Anopheles funestus*, *An. arabiensis*, *An. mascarensis* were found. The relative density of *An. funestus* was very high. Overall, 270 *Anopheles* vectors were collected. In the first village, *An. funestus*, *An. arabiensis*, *An. mascarensis* represent respectively 70.31%, 26.56%, 3.12%. In the second village, they represent respectively 83.78%, 15.31% and 0.9%. *An. funestus* is the most abundant vector; its aggressive density indoor was high and the aggressive density outdoors of *An. arabiensis* was important. The basic entomological data collected during this study revealed that permanent irrigated rice fields were the main mosquito breeding sites in Ankiloaka. They may maintain the high density of malaria vectors in Ankiloaka area. *An. funestus* and *An. arabiensis* were the main malaria parasite vectors in Ankiloaka, and possibly implicated to the malaria outbreak occurring there in 2015. All data collected during the study were essential for the malaria vector control in the southwestern region of Madagascar and used as baseline for the next entomological survey.

## 1451

### GENERATING LAB-REARED MOSQUITOES WITH FIELD-RELEVANT MICROBIOMES

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The microbiome of mosquitoes has been demonstrated to affect many important biological phenotypes such as development, fitness, and vector competence for numerous pathogens. Studies with mosquitoes invariably do not control for microbiome effects, and the relevance of laboratory-acquired microbiomes to mosquito biological parameters in the field is unclear. This study was undertaken to determine if mosquitoes could be

reared in the laboratory with field-relevant microbiomes. *Culex territans* and *Anopheles punctipennis* larvae were harvested from multiple sites in Central Pennsylvania and raised in one of three conditions: standard lab conditions, natural pond water supplemented with sterile nutrients, or natural pond water with no supplementation. Pupae/emerging adults were collected from the same sites. Microbiomes were characterized via MiSeq sequencing of the V4 region of the 16S rRNA gene. Results from this study will guide efforts to recreate naturalistic mosquito microbiomes in the lab for experimental studies, as well as helping to understand factors influencing mosquito microbiome composition in the field.

## 1452

### EVALUATING GRAVID AEDES TRAPS AND PROKOPACK ASPIRATORS FOR AEDES ALBOPICTUS SURVEILLANCE IN TWO NEIGHBORHOODS OF ATLANTA, GEORGIA

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The invasive, container-dwelling mosquito species *Aedes albopictus* spread rapidly from its introduction to Texas in 1985 and is now well established in most of the Southeast, South Central, and Mid-Atlantic United States. Accurate surveillance for *Ae. Albopictus* is important for monitoring the potential introduction of arboviruses. Although several methods are available, they vary in their cost, personnel involvement and requirement of specialized equipment. The Biogents Sentinel trap is commonly used for passive adult *Aedes spp.* collections, however its reliance on electricity and chemical lures as well as cost limit their widespread adoption. The Gravid Aedes Trap has emerged as a low-cost option for passive *Ae. spp.* sampling that does not require batteries or chemical attractants, but its use in the United States has been limited. We conducted surveillance for *Ae. Albopictus* in two neighborhoods in Atlanta, Georgia, one with low *Ae. Albopictus* abundance (Grant Park) and one with high *Ae. Albopictus* (Shallowford), with a matched-pair design comparing GATs with aspiration of adult mosquitoes resting outdoors. The backyards of 55 houses (26 in Grant Park, 29 in Shallowford) were aspirated for 10 minutes using a Prokopack and one GAT placed for 4 days from mid-August to mid-September 2016. A total of 191 adult mosquitoes including 122 female *Ae. Albopictus* were collected by GATs and 163 adult mosquitoes including 54 female *Ae. Albopictus* by aspiration. The percentage of infested houses detected by a GAT was 82% for Grant Park and 85% for Shallowford, while the percentage of infested houses detected by a Prokopack was 45% for Grant Park and 50% for Shallowford. The odds of detecting female *Ae. Albopictus* in Shallowford was 3.25 times higher with a GAT than a Prokopack, but the odds of detection with a GAT vs Prokopack did not differ significantly in Grant Park. Our study demonstrates successful collection of *Ae. Albopictus* using GATs compared to Prokopack aspirators for neighborhood-level surveillance and has implications for future *Ae. Albopictus* surveillance in the United States.

## 1453

### ASSESSMENT OF POTENTIAL SAND FLY VECTORS IN LEISHMANIASIS AND BARTONELLOSIS ENDEMIC AREAS IN THE ECUADORIAN SIDE OF THE ECUADOR-PERU BORDER

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Phlebotomine sand flies (Diptera: Psychodidae) are vectors of leishmaniasis in coastal, Andean and Amazonian regions of South America. These species also are vectors of human bartonellosis or Carrion's disease in

Ecuador and Peru. Leishmaniasis and human bartonellosis are known to be endemic along the Ecuador-Peru border with hundreds of cases of both diseases reported every year in northern Peru, whereas there have been few leishmaniasis cases and no bartonellosis cases reported from the Ecuadorian side in the last few years. Moreover, information on sand flies transmitting these pathogens in this region is lacking. This study aimed to characterize the sand fly fauna and identify potential leishmaniasis and bartonellosis vectors in two provinces of Ecuador close to the Peruvian border: El Oro (southwestern Pacific coast) and Zamora-Chinchipec (southeastern Amazonian region). Sand flies were collected in November and December 2016 using standard CDC light traps, Mosquito Magnet trap and Protected Human Bait. A total of 221 specimens belonging to different *Lutzomyia* species were identified: 107 (48%) from El Oro and 114 (52%) from Zamora-Chinchipec. Standard CDC light traps collected the highest number of sand flies in El Oro (83%), whereas Protected Human Bait was the most effective in Zamora-Chinchipec (78%). In El Oro, the species collected were *Lutzomyia trapidoi* (33%), *Lu. gomezi* (30%), *Lu. (Pressatia) spp.* (23%), *Lu. abonnenci* (5%), *Lu. hartmanni* (2%), *Lu. amazonensis* (2%), *Lu. aragaoi* (1%), *Lu. undulata* (1%), *Lu. shannoni* (1%), *Lu. serrana* (1%), and *Lu. barretoii majuscula* (1%). In Zamora-Chinchipec, only three species were recorded: *Lu. robusta* (95%), *Lu. maranonensis* (4%), and *Lu. castanea* (1%). *Lutzomyia trapidoi* and *Lu. gomezi* are highly anthropophilic and thus considered potential vectors in El Oro, whereas *Lu. robusta* and *Lu. maranonensis* are likely to play a role in pathogen transmission in Zamora-Chinchipec. Assessment of *Leishmania* spp. and *Bartonella* spp. infection rates in captured sand flies will further define the role of these species as potential vectors in this region.

## 1454

### THE RURAL-URBAN GRADIENT OF HOUSE INFESTATION WITH *TRITOMA INFESTANS* IN AN ENDEMIC MUNICIPALITY OF THE ARGENTINE CHACO REGION

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The Gran Chaco ecoregion is one of the main hotspots of neglected tropical diseases in the Americas, including Chagas disease. The occurrence of the major vector *Triatoma infestans* has historically been linked to poor rural housing, but (peri)urban infestations have been increasingly reported over recent decades. We conducted a cross-sectional survey of house infestation with *T. infestans* in all rural, periurban and urban sections of Avia Terai, an endemic municipality in the Argentine Chaco, as part of a 3-year intervention program combining insecticide spraying with educational aspects and community mobilization. The district had been sprayed with pyrethroid insecticides by Chagas vector control technicians 2-5 years before our survey. The baseline prevalence of house infestation decreased along a rural-to-urban gradient ranging from 43% (among 275 rural houses inspected), 16% (367 periurban houses) to 14% (402 urban houses), as determined by manual searches with a dislodging aerosol conducted over October 2015-March 2016. Bug colonies were found in nearly all infested houses. Most infestations occurred in peridomestic structures housing chickens and other domestic animals. Domestic infestations displayed a similar rural-to-urban gradient from 9%, 4% to 2%. Urban foci were spread through the town, and periurban infestations largely differed among neighborhoods (range, 0-59%). The village-specific prevalence rates of house infestation recorded in rural areas before control interventions (2011-2013) and in 2015 were similar. These results attest the fast recovery of triatomine populations after traditional insecticide spraying campaigns that did not include sustained vector surveillance and control afterwards. The large indices of

house infestation found in periurban and urban habitats of traditionally endemic regions reveal the need of revising current vector control practices in affected areas of the Gran Chaco.

## 1455

### CHALLENGES IN MEASURING AND ANALYZING VECTOR CONTROL INTERVENTIONS: INDICATORS, BASELINES AND DEFINITIONS

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Implementation of Indoor residual spraying (IRS) for vector control is undergoing significant shifts as insecticide resistance has emerged for some insecticides and other insecticides are coming onto the marketplace, driving a need for rotation and layering of products. In addition, insecticide treated bednets (ITNs) and other vector control tools are similarly undergoing transformations that could add complexity to malaria impact evaluations. At the same time, advances in satellite technology, rapid reporting, and routine surveillance system have allowed for more comprehensive assessment of the impact of these tools and their combinations in near real time. However, the ability to cross-analyze vector control interventions across different countries, implementing partners, and implementation strategies is hampered by a multiplicity of measurement methods and indicator definitions. As new vector control tools are implemented, and current tools are expanded, the need for robust and translatable definitions, indicators and enumeration strategies becomes ever more necessary. This paper focuses first on the challenges of definitions for standard indicators such as coverage, populations at risk, and populations protected. It shows how a small change in definition can impact the interpretation and analysis of the effectiveness of vector control programs. This paper also addresses how differences in definitions for baselines, including coverage and targeting metrics, affect the interpretation of impact. Finally, examples of enumeration strategies, to accurately define households/structures and populations at risk are presented. Standardization is especially important for programs that seek to understand cost-effectiveness of vector control interventions across various countries and epidemiological settings without reliance on costly trials. A unified, or translatable, set of definitions, indicators, and enumeration methodologies is critical at this stage to adequately assess the impact of vector control tools and their combinations in real settings as part of a robust monitoring and evaluation system.

## 1456

### ENVIRONMENTAL RISK FACTORS OF TUNGIASIS IN HAITI: A NEGLECTED DISEASE

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Tungiasis, a skin disease caused by the penetration of sand fleas (jiggers), is a neglected tropical disease endemic to impoverished regions of the Americas, especially Haiti. Unfortunately, there has been little surveillance for this condition, despite prevalence rates as high as 30% in a cross-sectional study of four distinct geographical locations in Haiti. Complicating the matter further, Haiti has lost 97% of its forests since 1987 creating an environment that is hospitable to sand flea infestation, which may increase the risk of tungiasis. Other studies have identified environmental risk factors for tungiasis including elevation above 2000 meters, sandy soil (which can result from deforestation), and poverty. This study aims to identify potential high risk regions of tungiasis transmission based on environmental factors, including remote sensing data focusing

on normalized vegetation difference index (NDVI), elevation, and population density. Through spatial analysis of these variables, we hope to identify high risk regions that can guide future surveillance efforts investigating the burden of tungiasis in Haiti.

## 1457

### THE STEROID HORMONE 20-HYDROXYECDYSONE (20E) TRANSCRIPTIONALLY REGULATES THE MIDGUT OF *ANOPHELES GAMBIAE* AND *Aedes Aegypti* TO PROMOTE BACTERIAL EXPANSION

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*Anopheles gambiae* and *Aedes aegypti* mosquitoes serve as important vectors for human pathogens. As the first major tissue barrier bloodmeal-acquired pathogens encounter in the mosquito, understanding the different signals that regulate cells of the midgut epithelium during a bloodmeal is critical for identifying novel mechanisms that may be important during infection. We found that injection of 20-hydroxyecdysone (20E), a steroid hormone that is essential for vitellogenesis, induces expansion of commensal bacteria in the absence of a bloodmeal in both *An. gambiae* and *Ae. aegypti* as measured by colony-forming-units and 16S qPCR. Bacterial commensals have been shown to be important in shaping midgut epithelial responses to both *Plasmodium* and Dengue virus (DENV) infections. During a bloodmeal, the commensal population undergoes dramatic quantitative and population level changes. A bloodmeal results in an overall increase in the number of commensals, with the peak of expansion occurring between 18 and 30hr post-bloodmeal (PBM) and corresponding to a loss in community diversity. While the dramatic increase in nutrients from a bloodmeal enables robust bacterial proliferation, we hypothesize that the expansion also requires a tolerating signal from the midgut epithelium. On average, the commensal expansion post-20E injection is 60% of the expansion post-bloodfeeding. In addition to quantification, we characterized the microbial communities expanding in the midgut after 20E injection and bloodfeeding. To elucidate the molecular mechanism responsible for commensal expansion we performed RNA-Seq in both of these mosquitoes to identify early(5hr)- and late(18hr)- midgut-specific transcriptional targets of 20E and comparative analysis of differences between the two species is ongoing. We hypothesize that a subset of targets found in both mosquitoes are directly responsible for a tolerating signal that allows the bacteria to expand post-bloodmeal and may simultaneously increase susceptibility of the midgut epithelium to pathogen infection.

## 1458

### RETENTION OF DUPLICATED LIGHT AND VISUAL RECEPTORS IN MOSQUITO LINEAGES BY POSITIVE SELECTION AND DIFFERENTIAL EXPRESSION

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Opsins are light sensitive receptors associated with visual processes. Insects typically possess opsins that are stimulated by ultraviolet, short and long wavelength (LW) radiation. Six putative LW-sensitive opsins predicted in the yellow fever mosquito, *Aedes aegypti* and malaria mosquito, *Anopheles gambiae*, and eight in the southern house mosquito, *Culex quinquefasciatus*, suggest gene expansion in the Family Culicidae (mosquitoes) relative to other insects. Here we report the first detailed molecular and evolutionary analyses of LW opsins in three mosquito vectors, with a goal to understanding the molecular basis of opsin-mediated visual processes that could be exploited for mosquito control. The transmembrane domains of the mosquito LW opsins share between

60 to 100% amino acid identity. Time of divergence estimates suggest that the mosquito LW opsins originated from 18 or 19 duplication events between 166.9/197.5 to 1.07/0.94 million years ago (MY) and that these likely occurred following the predicted divergence of the lineages Anophelinae and Culicinae 145–226 MY. Fitmodel analyses identified nine amino acid residues in the LW opsins that may be under positive selection. Of these, eight amino acids occur in the N and C termini and are shared among all three species, and one residue in TMIII was unique to culicine species. Alignment of 5' non-coding regions revealed potential Conserved Non-coding Sequences (CNS) and transcription factor binding sites (TFBS) in seven pairs of LW opsin paralogs. Our analyses suggest opsin gene duplication and residues possibly associated with spectral tuning of LW-sensitive photoreceptors. We explore two mechanisms – positive selection and differential expression mediated by regulatory units in CNS – that may have contributed to the retention of LW opsin genes in Culicinae and Anophelinae. We discuss the evolution of mosquito LW opsins in the context of major Earth events and possible adaptation of mosquitoes to LW-dominated photo environments, and implications for mosquito control strategies based on disrupting vision-mediated behaviors.

## 1459

### THE MIDGUT ESCAPE BARRIER FOR CHIKUNGUNYA VIRUS IN *Aedes Aegypti* IS ASSOCIATED WITH PROTEINASE ACTIVITY

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Chikungunya virus (*Togaviridae*; *Alphavirus*; CHIKV) is an emerging mosquito-borne virus, which is transmitted to humans by *Aedes aegypti* and *Ae. albopictus*. Following oral acquisition from a vertebrate host, the virus containing bloodmeal is deposited in the midgut lumen. Virus enters the epithelial cells and replicates in them before disseminating from the midgut to secondary tissues including the salivary glands. Once the latter are infected the virus is transmitted to another vertebrate host. The viral exit mechanism from the mosquito midgut, the midgut escape barrier (MEB), is poorly understood although it is an important determinant of vector competence. We hypothesize that bloodmeal intake leading to midgut tissue expansion causes structural changes in the midgut surrounding basal lamina (BL). These structural changes, which are caused by enzymatic processes involving proteinases, make the BL permissive for virions to pass through. Collagen IV, an essential component of the BL, was less abundant in midguts of females at 12-36 h post-bloodmeal. This correlated with a high collagenase activity in midguts and high virus dissemination rates. To reveal candidate genes involved in the process, we conducted a comparative transcriptomic analysis of midgut samples from mosquitoes, which had received a saline meal (SM) or a protein meal (PM) containing CHIKV as substitutes for a CHIKV containing bloodmeal. Twenty three genes encoding trypsin, metalloproteinases, and serine-type endopeptidases were significantly upregulated in midguts of mosquitoes at day 1 following SM or PM ingestion. Two of the genes were *Ae. aegypti* late trypsin (*AeLT*) and serine collagenase 1 precursor (*AeSP1*), the former of which showed strong metalloproteinase activity *in vitro*. Similarly, matrix metalloproteinases (MMPs) *AeMMP1* and *AeMMP2* showed strong metalloproteinase activity *in vitro*, which was inhibited by the *Ae. aegypti* tissue inhibitor of metalloproteinases (*AeTIMP*). Our results support the conclusion that BL degradation due to proteinase activity during meal digestion may be the mechanism behind CHIKV dissemination from the midgut in *Ae. aegypti*.

## 1460

**DETERMINING THE EXPRESSION PROFILE OF SPERMATOGENESIS GENE HOMOLOGUES THROUGHOUT ALL DEVELOPMENTAL STAGES OF ANOPHELES ALBIMANUS, MAIN MALARIA VECTOR IN CENTRAL AMERICA**

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Central America is currently working towards malaria elimination. Despite the mass distribution of insecticide treated nets, malaria persists in focalized areas of Guatemala. This has important social and economic consequences for the elimination target, and sustainable and specific methods for mosquito control will be required to reach the regional malaria elimination target. The sterile male technique was successful in the 1970's at controlling *Anopheles albimanus*, the main vector in the region. We propose that sterile males may be produced by silencing genes vital for spermatogenesis. A loss of function of *boule (bol)* and zero population growth (*zpg*) genes in mosquitos generates sterile adults. In the present study, we quantified *bol* and *zpg* expression profiles by real time PCR, to determinate in which life stage of *An. albimanus* these genes will have a higher expression. For that, we extracted RNA from all life stages, using a standardized amount of 30mg of tissue per pool. We generated cDNA from larval, pupal and adult stages, with females and males analyzed separately. Finally, we measured mRNA expression levels for each stage by qPCR. The results showed that *bol* and *zpg* have high expression rates in male adult stages, with three- and 30- fold the initial gene expression observed in first instar larvae, respectively. Interestingly, we also detected expression of both genes in female adults, which could suggest that these genes have other functions on females. According to these results, we propose that continuously feeding dsRNA to larvae for silencing these genes when they begin to be expressed, at first instar larvae, would have a high impact on adult male fertility. If that is the case, using dsRNA to silence one of these genes could lead to adult sterile males that could be used as a complementary strategy for malaria elimination.

## 1461

**IDENTIFICATION OF METABOLIC CHOKE POINTS FOR CONTROLLING DENGUE VIRUS TYPE 2 INFECTION IN THE MIDGUT OF AEADES AEGYPTI MOSQUITOES**

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Dengue viruses (DENV) are mosquito-borne viruses that cause almost 400 million infections and 10,000 deaths in humans annually. The biochemical environment within both human and mosquito hosts is known to play a critical role in determining the outcome of virus infection. Previous studies have found alterations in lipid profiles and rearrangement of the membrane architecture of host cells to support viral genome replication, protein translation and particle assembly during DENV type 2 infections. In this study, we adopted an untargeted metabolomics approach to explore temporal alterations of the lipid environment in the midgut of *Aedes aegypti* mosquitoes during DENV infection. Using high-resolution liquid chromatography-mass spectrometry, we observed a shift of several lipid classes during the time course of infection. Phosphoglycerolipids, major components of cellular membranes, temporally increased coincident with viral replication dynamics. The abundance of certain bioactive sphingolipids was also elevated early post infection. To identify the role of these lipids in defining the outcome of virus infection, we knocked down the expression or inhibited the function of key enzymes in select pathways using double stranded RNA and chemical inhibitors. We observed a reduction of DENV

replication and particle production in both mosquito cells and the midgut of *Aedes* mosquitoes. Specifically, inhibition of *de novo* fatty acyl-CoA synthesis, key intermediates of lipid metabolic pathways, and enzymes along the *de novo* sphingolipid and sphingosine synthesis pathways were detrimental to the virus. Similar effects were also observed in human cells upon DENV infection indicating a conserved requirement of these host resources during the viral life cycle. In summary, we have carried out the first study interrogating the biochemical environment required for DENV replication in the mosquito and identified the role of sphingolipids in the complex interaction of virus and host. Our data suggested that these metabolic control points could be targeted for intervention of virus transmission by the mosquito vector.

## 1462

**THE STRUCTURE AND FUNCTION OF ALBICIN: A NEW WORLD ANOPHELINE MOSQUITO SALIVARY PROTEIN THAT INHIBITS OF THE ALTERNATIVE PATHWAY OF THE HUMAN COMPLEMENT**

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Albicin is a 13.4 kDa member of the SG7 protein family expressed in the salivary glands of the central American malaria vector *Anopheles albimanus*. Albicin inhibits the human alternative complement pathway, by stabilizing the C3 convertase (C3bBb). More specifically, the inhibitor-bound convertase is unable to cleave C3, which in turn prevents the amplification of the complement response. Here we present the crystal structure of albicin along with analysis of the physical properties of its complex with C3bBb. Using site-directed mutagenesis in conjunction with surface plasmon resonance and size exclusion chromatography, we identify key residues, which stabilize the albicin bound convertase complex. The composition and thermodynamic properties of the bound complex were examined using analytical ultracentrifugation. While the structural homolog of albicin, expressed in the salivary glands of *Anopheles darlingi*, also inhibits the C3 convertase, the related SG7 proteins in *Anopheles gambiae* and *Anopheles stephensi* do not exhibit anti-complement activity. A better understanding of structure of albicin and its complex with C3bBb may facilitate the identification of the function of other SG7 protein family members.

## 1463

**STEROID HORMONE SIGNALING IN ANOPHELES GAMBIAE MOSQUITOES AFFECTS THE SPOROGONIC CYCLE OF PLASMODIUM FALCIPARUM PARASITES**

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After an infectious blood meal, oogenesis in *Anopheles gambiae* females is spatially and temporally linked with *Plasmodium falciparum* development. Oogenesis is initiated following blood feeding by a peak of the insect steroid hormone 20-hydroxyecdysone (20E). This 20E signal induces vast transcriptional and metabolic responses in multiple female tissues to promote blood meal digestion and the incorporation of nutrients into developing eggs. While these dramatic 20E-regulated changes occur, *Plasmodium falciparum* parasites also attempt to establish infection in the mosquito. Here we reveal an unexpected interplay between the pathways regulated by 20E and parasite development in *An. gambiae*. We show that impairing 20E signaling via depletion of the 20E nuclear hormone receptor, Ecdysone Receptor (EcR), differentially affects female reproductive processes and multiple stages of *P. falciparum* development. Females impaired in 20E signaling develop significantly fewer eggs, a phenotype at least partially mediated by the reduced expression of known 20E-induced yolk protein precursors. These EcR-depleted females also

harbor fewer *P. falciparum* oocysts. Strikingly, however, in EcR-silenced females, *P. falciparum* parasites develop faster and become infectious sooner, consistently producing more salivary glands sporozoites at earlier time points after an infectious blood meal. The mechanisms driving these contrasting effects on parasite development are still being determined, nonetheless this first demonstration that 20E signaling impacts the rate of *P. falciparum* development has marked implications for our understanding of the molecular mechanisms critical for disease transmission. Overall, these data demonstrate that steroid hormone signaling plays a complex and previously unappreciated role in regulating the sporogonic cycle of *Plasmodium* parasites.

## 1464

### MULTIPLE TISSUE MICRORNA TRANSCRIPTOME-WIDE ANALYSIS IN THE MALARIA VECTOR, *ANOPHELES GAMBIAE* S.S.

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A key component of regulating gene expression is a small class of RNA molecules termed microRNAs (miRNAs). Most miRNAs are differentially expressed in tissues, while a small number are solely expressed in specific tissues. Recent studies illustrate the significance of miRNAs in regulating blood feeding and immunity in mosquitoes. As a first step towards our goal to identify miRNAs that regulate tissue-specific functions, twelve small RNA libraries consisting of four different tissue types with three biological replicates were constructed and analyzed. Tissues analyzed were midgut, fat body, ovaries, and remaining tissues, the latter group includes the head and thorax of the mosquito. Small RNA libraries were prepped using Illumina TruSeq Small RNA Prep Kit. Library size selection was done by Pippin Prep to ensure proper selection of miRNAs. Small RNA libraries were sequenced by TruSeq Sequence-By Synthesis v3 chemistry. Raw reads were analyzed by CLC genomics software. Our preliminary analysis found approximately 75% of reads for the fat body libraries matched current known miRNAs. However, other tissue libraries found a higher percentage of non-annotated reads. Preliminary analysis found multiple instances of miRNAs enriched in specific tissues. *miR-1174*, *miR-1175*, and *miR-12* were enriched in the midgut. *miR-92b* and *miR-989* were enriched in ovaries. *miR-957*, *miR-133*, and *miR-210* were enriched in our 'remaining tissue'. Our data agrees with previous results of tissue-specific miRNA expression. Further validation by real time PCR expression analysis agreed with our small RNA library analysis. Lastly, we tested the effects of blood feeding on the expression levels of candidate miRNAs with interesting results. Overall, understanding expression patterns of miRNAs in multiple tissues demonstrates their complexity and starts the groundwork for determining their relevance in disease transmission in the malaria vector, *Anopheles gambiae*. Lastly, defining candidate tissue-specific miRNAs is of importance as these miRNAs likely play key roles in maintaining tissue complexity.

## 1465

### GENOMIC AND PHYSIOLOGIC CHARACTERIZATION OF *SERRATIA MARCESCENS* ISOLATED FROM THE GUT OF *ANOPHELES STEPHENSI*

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Bacteria of the genus *Serratia* are common commensals in the *Anopheles* mosquito midgut, and show great potential to repress *Plasmodium* development. However, the molecular mechanisms involved in interactions between commensal *Serratia* and mosquito hosts as well as other associated symbionts remain largely unknown. Our study demonstrated that *Serratia marcescens* strain ano1 and the bacterium *Elizabethkingia anophelis* isolated from *Anopheles stephensi* stably persisted in larval and adult mosquitoes (98.5% infection rate) while *Serratia fonticola* isolated from *Aedes* had significantly lower infection rate (32.5%) in *Anopheles*,

supporting the hypothesis that *S. marcescens* is a stable commensal in *A. stephensi* gut. Nevertheless, *S. marcescens* caused up to 67% death rates in larval or adult mosquito within 4 days when inoculated into mosquito hemolymph, indicating that *S. marcescens* is also an opportunistic pathogen in *Anopheles stephensi* when it bypasses the gut. To explore these phenotypic and genetic attributes, we sequenced and annotated genomes from two *A. stephensi*-associated *S. marcescens* and compared them to those forming symbiotic relationships to other mosquitoes, insects, nematodes and plants. *S. marcescens* ano1 and ano2 were quite distinct from *Serratia* sp. previously isolated from *Anopheles gambiae*. *S. marcescens* ano1 was resistant to numerous antibiotics, and showed high survival in the mosquito gut niche. It produced alpha-hemolysin(s), possibly contributing to lysis of ingested erythrocytes. *S. marcescens* ano1 and ano2 had predicted virulence factors that are possibly involved in attacking parasites and/or could cause opportunistic infection for mosquito hosts. *S. marcescens* ano1 and ano2 possessed multiple mechanisms for antagonism against other microorganisms, including the production of bacteriocins and multi-antibiotic resistant determinants. Genes contributing to potential anti-malaria activity including serralysins, hemolysins and chitinases only found in some *Serratia* species.

## 1466

### A HETERODIMER OF AALRIM1 AND AAAPL1 IS REQUIRED FOR *AEDES AEGYPTI* IMMUNE REACTIONS TARGETING DIVERSE PATHOGENS

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*Aedes aegypti* is responsible for transmission of several arboviruses as well as filarial nematodes and *Plasmodium* parasites. For transmission to occur, pathogens must overcome the mosquito immune system. Therefore, we are interested dissecting molecular immune mechanisms required for limiting infection with the goal of developing novel strategies to block disease transmission. We have taken a comparative approach to analyze *Ae. aegypti* complement components given that this pathway provides robust protection against infection by diverse pathogens in *Anopheles gambiae*. We found that, like their orthologs in *An. gambiae*, two Leucine-Rich Repeat Immune Proteins, AaLRIM1 and AaAPL1, circulate in the hemolymph of *Ae. aegypti* in a disulfide-linked complex. RNAi silencing of either AaLRIM1 or AaAPL1 abrogates the complex. These genes, as well as several other putative *Ae. aegypti* complement components, are upregulated following bacterial challenge, infection by a filarial nematode, and activation of the REL1 signaling pathway. Furthermore, silencing AaLRIM1 or AaAPL1 renders mosquitoes highly susceptible to bacterial infection. We are currently addressing the role the AaLRIM1/AaAPL1 complex plays in complement activation, what immune effector functions it triggers, how the *Ae. aegypti* complement pathway is regulated, and whether it functions to limit arboviral infection.

## 1467

### FABULOUS SIGNALING: THE IMPACT OF THE TOLL PATHWAY ON MOSQUITO-PATHOGEN INTERACTIONS

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The Toll pathway is a central regulator of immunity in insects and controls innate immune reactions that limit a wide range of infections. Manual annotation and phylogenetic analysis of the entire Toll-like receptor (TLR) gene repertoire in 20 sequenced mosquito species reveal expansions of the TOLL1 and TOLL5 clade, hypothesized to contain the immune-functioning TLRs in mosquitoes. Our data indicate that this gene family has a larger gene repertoire than previously described with clade-specific expansions within distinct anopheline lineages. To probe this pathway, we utilized a fungal infection model using the entomopathogen, *Beauveria bassiana*. We show dose-dependent modulation of *B. bassiana* within

the malaria vector, *Anopheles gambiae*. The cuticle of 2-4 day old females was exposed by direct contact to an oil suspension of spores [ $1.24 \times 10^9$  spores/mL] applied to filter paper substrate. Infection with *B. bassiana* decreased median survival of *An. gambiae* by  $10 \pm 1.5$  days compared to controls (Unpaired t-test,  $P=0.003$ ). Quantification of fungal genome equivalents by qPCR revealed logarithmic growth over six days, culminating in a conidial genome count of  $2.3 \times 10^5 \pm 0.6 \times 10^5$  at six days post infection, coinciding with an incidence of high mortality. The course of infection is modulated by the Toll pathway; knockdown of the downstream transcription factor *REL1* and the pathway inhibitor *Cactus* by RNAi show decreased and increased survivorship to fungal infection, respectively. Importantly, these knockdown phenotypes were strongly influenced by dose, with highest fitness gains (using survival as proxy) observed in low dose infections. Together with published data, your findings confirm *B. bassiana* as a natural infection model to probe the role of the Toll pathway in mosquito humoral immunity. Importantly, these data suggest that increased Toll pathway signaling may provide a resistance mechanism to *B. bassiana* infections dependent on dose and coverage if used as a mosquito bioinsecticide. Partial funding provided by the National Institutes of Health through R01-AI095842.

## 1468

### PYRETHROIDS MAINTAIN REPELLENT EFFECT ON Aedes Aegypti MOSQUITOS WITH KNOWN RESISTANCE

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Pyrethroid-treated clothing is commonly worn for protection against mosquitoes; pyrethroids are both insecticides and repellents. Pyrethroid resistance has become increasingly common in *Aedes aegypti*, the vector of dengue, Zika, and other arboviruses. Specific mutations to the voltage-gated sodium channel lead to knockdown resistance (kdr), but it is not clear whether they are associated with alterations in repellency. We measured mutations in kdr in *Aedes aegypti* from New Orleans, LA (pyrethroid-sensitive) and San Juan, PR (resistant) and measured both lethality and repellency for clothing impregnated with pyrethroids using arm-in-cage tests. PCR and Sanger sequencing were used to detect mutations at loci known to be associated with pyrethroid resistance (1016 and 1534). 7/19 New Orleans mosquitos and 10/13 San Juan mosquitos had 1016 kdr mutations (OR 5.71; 95%CI 0.95, 41.23); 9/19 New Orleans mosquitos and 6/9 San Juan mosquitos had 1534 kdr mutations (OR 2.22; 95%CI 0.33, 17.45). Arm-in-cage trials of 100 sensitive or resistant *Aedes aegypti* females were performed for 10 minutes to bare arm or an arm clothed in military camouflage impregnated with deltamethrin, permethrin, or etofenprox. Trials were repeated 4-5 times on different days. Number of landings, number of blood meals, and immediate and 24-hour mortality were recorded. Mortality was extremely low in all trials. Compared to bare arm, mosquitos demonstrated a 16%-65% reduction in landings on pyrethroid-treated cloth. Etofenprox had less repellent activity than deltamethrin and permethrin. Resistant (San Juan) mosquitos showed a greater reduction in landings on etofenprox and permethrin than wild-type mosquitos. Our data show that kdr mutations are associated with pyrethroid resistance but are likely not the only contributors. Pyrethroids appear to maintain repellent effect against resistant mosquitos. This suggests that even in places where pyrethroid resistance is widespread, these insecticides still have a role for use as repellents on clothing.

## 1469

### IMPLICATIONS OF REDUCED SUSCEPTIBILITY TO INSECTICIDES IN MALARIA VECTORS IN AN AREA WITH HIGH ITN COVERAGE

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In sub-Saharan Africa, insecticide treated net (ITN) coverage has greatly improved in the last 10 years. Despite this, Bungoma East Sub County in Western Kenya has recorded little reduction in malaria prevalence. Biological factors in the mosquito vectors, especially shift in biting times and increased tolerance towards the insecticides used in malaria control may contribute to persistent malaria burden. The present study was designed to determine the susceptibility status of malaria vectors and ITN coverage related to ability of vectors to obtain a blood meal in Bungoma East sub-county. Larvae were sampled from breeding sites in 4 villages and transferred to an insectary. Mosquito larvae were raised in their own breeding water and fed with yeast. 3-5 day-old adult female anopheles were then exposed to 0.05% deltamethrin and 0.75% permethrin according to the standard WHO test procedure. In addition, WHO cone assays were conducted on pieces cut from new Olyset long-lasting ITN. In order to assess the ITN usage and coverage, households around the sampled villages were asked about bednet ownership. In addition, the study enrolled 9 households and measured ITN use longitudinally. Indoor resting adult mosquitoes were collected from these homes, sorted by genus, and graded according to abdominal status. The 1-hr knocked-down rate varied considerably between the 4 villages, from 50-90% for permethrin and 60-90% for deltamethrin. 24-hour mortality in all villages was less than 90% threshold set by the WHO. Permethrin mortality was lowest in K village (66%) and highest in M village (88%), whereas deltamethrin mortality was lowest in L (36%) and highest in J (88%). We observed very low mortality in WHO cone assays (0-16.7%). The longitudinal study showed that 100% of sleeping spaces had an ITN and daily individual usage exceeded 97% over 10 weeks. Despite this, almost 50% of the total female mosquitoes collected indoors were blood fed or half gravid. When compared to the low levels of insecticide susceptibility, particularly in the cone assays, we cannot rule out an effect of resistance on vector feeding in our study area.

## 1470

### INVESTIGATING ENDECTOCIDE USE IN LIVESTOCK AS A TOOL TO HELP ELIMINATE RESIDUAL MALARIA IN CENTRAL AMERICA

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In Central America, malaria has been reduced using indoor insecticide spraying and insecticide-treated bed nets. But these tactics may not eradicate malaria because they rely on certain behavioral traits of the *Anopheles* vector - e.g., entering houses at night to feed and rest. Much of the malaria transmission in Central America occurs outdoors by *Anopheles* species that are as likely to feed on non-humans as humans (=zoophagic). To address this issue, additional tactics need to target these behaviors. Livestock management may hold the key. Our project examines the potential of endectocides to help reduce zoophagic *Anopheles* populations and eliminate residual transmission of malaria in Central America. Endectocides are chemicals widely used in the livestock industry to control intestinal nematodes and ticks. The most widely-used endectocide, ivermectin, has been shown to reduce the survival and fecundity of several *Anopheles* species when ingested in a bloodmeal. Ivermectin is increasingly important for vector control in Africa. We compared the dose-responses to ivermectin between

the Central American vector, *A. (Nyssorynchus) albimanus*, and the Asian vector, *A. (Cellia) stephensi*. Toxicity of ingested ivermectin in *A. albimanus* (oral IC-50=1,349 mg/ml) was significantly less than in *A. stephensi* (oral IC-50=11 mg/ml) and far exceeded the normal range of ivermectin plasma concentration typically found in treated cattle (20-50 ng/ml). Ivermectin was 10x more toxic when injected into the thorax of *A. albimanus* (parenteral IC-50=100 ng/ml), but was still less toxic than when injected into *A. stephensi* (parenteral IC-50=16 ng/ml). This suggests that ivermectin is not readily absorbed across the gut in *A. albimanus*, and that the molecular targets of ivermectin (i.e., glutamate-gated chloride channels) in *A. albimanus* are more resistant to the effects of ivermectin than are those in *A. stephensi*. Another related endectocide, abamectin, proved much more toxic to *A. albimanus* (oral IC-50=107 ng/ml) than ivermectin. Thus, abamectin may be a better choice of endectocide to reduce zoophagic vector populations in Central America.

## 1471

### INSECTICIDE RESISTANCE IN JAMAICAN *Aedes aegypti*

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For the past thirty years, Jamaica has been using the insecticide malathion in its mosquito control programme in an effort to minimize the spread of dengue. Recently, the introduction of the Zika and chikungunya viruses prompted the switch from malathion to a permethrin-based insecticide. Resistance to insecticide has been identified as an impediment to successful vector control programs world-wide, as such we undertook to investigate the status of resistance in the most populous parish of Jamaica. We report for the first time, metabolic and target site to permethrin- and malathion-resistance in *Aedes aegypti* from St. Andrew, Jamaica. The Center for Disease Control bioassay revealed that Jamaican *Ae. aegypti* collected from five sites in the parish of St. Andrew were resistant to permethrin (15 µg/bottle). 100 - 92% of the mosquitoes survived up to 120 minutes of contact. Surprisingly, contact with malathion (50 µg/bottle) revealed 57 - 87% survival after 15 minutes, which decreased to 5 - 16% after 30 minutes. The standard susceptible New Orleans (NO) strain exhibited 0% survival within 15 minutes contact with either permethrin or malathion. The activities of enzymes, the mixed function oxidases and *p*-nitro phenyl-acetate esterases, commonly over-expressed in insecticide resistant mosquitoes, were significantly greater in most Jamaican populations in comparison to the NO strain, however, the activities of glutathione-S-transferase, acetylcholinesterase,  $\alpha$ -esterase and  $\beta$ -esterase were relatively equal to- or lower than that of the control strain. Mutations in the voltage dependent sodium channel gene, which are usually observed in permethrin resistant *Aedes*, revealed that phenylalanine to cysteine substitution (Cys1534) was fixed in all Jamaican *Ae. aegypti* populations, while the valine to isoleucine mutation (Ile1016) was 56% heterozygotes and 33% homozygotes. The results show that *Aedes aegypti* from St. Andrew Jamaica are resistant to permethrin with variations in the mode of mechanism, and possibly developing resistance to malathion. Such findings will be useful for developing an effective vector control program in Jamaica.

## 1472

### OUTCOMES OF A SURVEY OF AGRICULTURAL INSECTICIDE USE PRACTICES IN A MALARIA ENDEMIC SETTING IN RURAL COTE D'IVOIRE

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At a time when insecticide resistance threatens the gains made in the fight against malaria and facing a limited array of available vector control tools; the introduction of new insecticide modes of action is crucially important. Certain repurposed agricultural compounds are already under evaluation within the WHOPES-PQ evaluation scheme for use in IRS and LLINs and thus there is some promise of respite on the selection pressure to existing chemistry. However there is also much debate about the role agricultural insecticide usage plays in the selection of resistance in malaria vectors and as these new repurposed agricultural compounds are being developed it is important to understand the environment into which they will be introduced - most notably the degree to which those families of compounds are already used in agriculture in malaria endemic areas and what propensity that might convey to any reduced susceptibility in a given mosquito population. Neonicotinoids are one such family of repurposed agricultural insecticides which are being developed for IRS application; neonicotinoids are also one of the most widely used insecticide classes in agriculture. There is little sharing of knowledge between the agricultural sector and the public health sector in terms of insecticide usage in many (most) malaria endemic countries. Previous studies have collated circumstantial data around insecticide usage based on either the brands stocked within distribution outlets or on registrations of insecticides in countries. This does not take into account actual practice. This paper reports on results of surveys carried out with farmers involved in production of the main crops of cocoa, cotton, vegetables and rice in 11 districts in Cote D'Ivoire. It addresses some of the challenges of previous studies by engaging direct surveys with farmers - reflecting actual usage patterns. It reveals that neonicotinoids are used quite commonly in certain cropping systems. The implications of these results, in terms of next steps and considerations for introduction of repurposed agricultural compounds, is discussed.

## 1473

### ANOPHELES GAMBIAE S.L. INSECTICIDE RESISTANCE OF IN KINSHASA, IDJWI ISLAND (SUD KIVU), LUBUMBASHI (HAUT KATANGA) AND KWILU-NGONGO SUGARCANE PLANTATIONS (KONGO CENTRAL) IN DEMOCRATIC REPUBLIC OF THE CONGO

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Malaria is a major public health problem with serious consequences for the economy of a post-conflict country. Long lasting impregnated nets (LLINs) is the principal vector control intervention in Democratic Republic of Congo (DRC). For almost 5 years, million nets were distributed. However, knowledge of insecticide resistance level in *Anopheles gambiae s.l.*, the main malaria vector, is limited. Insecticide resistance is recorded throughout the world in many malaria vectors populations such as *Anopheles gambiae*. As current control methods against malaria currently depend on LLINs and vector resistance The study objective was to characterize insecticide resistance in five sites. Wild *Anopheles gambiae s.l.* mosquitoes were collected, reared to adults and tested with different insecticides for their susceptibility using the WHO standard protocol under optimum conditions Insecticides tested included pyrethroids (0.05% deltamethrin and 0.75% permethrin), organochlorines (4%

DDT) and carbamates (0.1% bendiocarb). In addition the susceptibility of populations of *An.gambiae* from deltamethrin and permethrin resistance sites were tested with after pre exposure with PBO during 1 hour to assess the involvement of P450 metabolic mechanisms. Resistance or suspected resistance was observed in all visited sites specifically on pyrethroids (permethrin and deltamethrin). This resistance was very high in Kwilu-Ngongo (31% - 51%) and Kimpese (33,7% - 53,%) a sugarcane plantation region. Carbamate remains susceptible in all sites tested. Resistance to DDT was observed in all sites (11,5-33%) Synergists (PBO) were able to restore efficacy of deltamethrin or permethrin in different sites that suggest metabolic mechanisms (P450s) are partially or fully involved in the observed resistance. In conclusion, further analysis should be done on *Anopheles* of different sites to obtain more information. Insecticide resistance is an emerging problem in DRC. The susceptibility of malaria vectors to insecticide must be considered in the choice of vector control tools such as LLIN or IRS.

## 1474

### METHANOL KILLS *ANOPHELES COLUZZII* MOSQUITOES DURING IN VITRO FEEDING EXPERIMENTS

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Our previous experiments suggested that sulfadoxine fed to laboratory reared *Anopheles stephensi* mosquitoes during standard membrane feeding assays had a significant lethal effect of these malaria vectors. In the present study we attempted to replicate these experiments with field *Anopheles coluzzii* mosquitoes in Mali. Sulfadoxine was dissolved in methanol. The final tested concentrations of sulfadoxine were equivalent to *in vivo* plasma concentration reached on days 1, 3, 7 and 14 after a single oral dose of sulfadoxine-pyrimethamine. Five hundred anopheles, were fed through an artificial membrane with a solution containing the respective concentrations of sulfadoxine dissolved in methanol. As Control No1 five hundred mosquitoes were fed on blood without neither sulfadoxine nor methanol. As control No2, five hundred mosquitoes were fed on blood without sulfadoxine but containing methanol. Cumulative mosquitoes' mortality was assessed by 72h after the feeding experiments. Seventy-two hours after feeding with day 1 (65.9 ug/ml), day 3 (54.9 ug/ml), day 7 (30.4ug/ml) and day14 (12.8ug/ml) concentrations of sulfadoxine the observed mosquitoes' mortality compared to the control No1 (blood without methanol) were 70.5% vs. 2.1% ( $p < 10^{-3}$ ); 14.7% vs. 3.4% ( $p < 10^{-3}$ ); 5.3% vs. 5.7% ( $p > 0.05$ ), and 3.6 vs. 2.5% ( $p > 0.05$ ). However, when compared with the control No2 (blood with methanol), observed mosquitoes' mortality with day 1 and day 3 concentrations of sulfadoxine were 34.4% versus 39.4% ( $p > 0.05$ ) and 12.7% vs. 13.8%, ( $p > 0.05$ ), respectively. This study indicates that mosquitoes' mortality was due to a toxic effect of the methanol used to dissolve sulfadoxine rather than to an effect by the antimalarial drug itself. We show that methanol kills *Anopheles coluzzii* mosquitoes.

## 1475

### INSECTICIDE RESISTANCE AND MECHANISMS IN *AEDES* ARBOVIRAL VECTORS: A WORLDWIDE SYNTHESIS

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*Aedes aegypti* and *Ae. albopictus* are major vectors of multiple important arboviruses and control using insecticides is a critical component of disease management and prevention. As part of a WHO-TDR commission to the Worldwide Insecticide resistance Network, we review all accessible datasets to investigate the geographical distribution of *Aedes* insecticide resistance and underlying mechanisms. Resistance to all four main classes

of neurotoxic insecticide has been detected in the Americas, Africa and Asia. Interacting target site mutations are especially important in *Ae. aegypti* and show evidence of geographical patterning, whilst major resistance-linked detoxification gene overexpression appears more widespread in both species. Resistance-linked metabolic genes are highlighted from the meta-analysis to prioritise candidates for functional assessment and diagnostic development, key steps in understanding their relative impact on phenotypes relative to target site mutations. Estimating insecticide resistance in unsampled locations is currently hampered by a lack of standardisation and diagnostic doses, but could be greatly assisted by calibration and predictive application of existing DNA diagnostics for resistance. Widespread resistance calls for the careful use of existing formulations and implementation of insecticides with alternate modes of action, as part of well-planned resistance management programmes.

## 1476

### EXON-ENRICHED LIBRARIES OF DELTAMETHRIN RESISTANT *AEDES AEGYPTI* REVEAL STRONG POSITIVE SELECTION AT THE VOLTAGE GATED SODIUM CHANNEL

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Resurgence of pyrethroid resistance in *Aedes aegypti* mosquito populations threatens our ability to control several arbovirus diseases, including dengue, Zika and chikungunya. Mechanisms of pyrethroid resistance involve knockdown resistance (kdr) and increased metabolism by multifunction oxidases. Two kdr-associated mutations at the pyrethroid target site, the voltage gated sodium channel gene (*vgsc*) rapidly increased across mosquito populations in Mexico as a result of continuous permethrin applications from 1999 to 2010. Currently, alternative pyrethroids are used in mosquito control campaigns, including deltamethrin (pyrethroid type 2). In this study, we identified replacement coding polymorphisms associated with deltamethrin resistance in *Ae. aegypti*, using an exome-targeting sequencing approach. Mosquito females from Yucatan México were exposed to 3 µg deltamethrin in a bottle bioassay during one hour. DNA from alive and dead mosquitoes was pooled to build duplicate high throughput sequencing (HTS) libraries. A total of 7,392 replacement substitution SNPs were significantly different between the resistant and susceptible mosquitoes. We identified a cluster of SNPs with high allelic differentiation in chromosome 3. The Phe1,534Cys, Val1,016Ile and two novel replacements at *vgsc* were strongly associated with deltamethrin resistance. A cluster of replacement SNPs near *vgsc*, corresponding to genes coding for a phosphatase, RNA binding, trypsins and metalloproteinases were identified in this region. An analysis of heterozygosity in the resistant relative to the susceptible mosquitoes suggests that nearby gene-replacements have swept by positive selection of *vgsc* resistant haplotypes.

## 1477

### NOOTKATONE: A NATURALLY OCCURRING, NEXT-GENERATION PEST MANAGEMENT STRATEGY

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Thanks in large part to the global outbreak of Zika virus, gaps in pest management are once again drawing attention from consumers and Public Health experts alike. Many of these concerns are once again focused upon growing insecticide resistance, community compliance, gaps in public engagement and education, and environmental impacts. For years, the most ubiquitous and cost-effective pest management

strategies have been built around legacy repel-and-kill products for biting pests. One of the key challenges to this construct is that the all-natural pest control agents that consumers and the media view most favourably, produce limited results, while the petrochemical-based synthetic agents that these same constituencies often mistrust, produce tangible results, albeit at the expense of target-vector resistance. The naturally-occurring sesquiterpene nootkatone carves a path between these two legacy constructs. This familiar, pleasant-smelling citrus fragrance can be extracted in minute quantities from the skin of grapefruit or from the bark of the Alaska Yellow Cedar tree (*also known as the Nootka cypress*). The challenge is that an estimated 400,000 grapefruits are needed to produce a single kilogram of nootkatone; and extraction from tree bark is equally unsustainable and economically problematic. The Swiss-American company Evolva has demonstrated that nootkatone can be produced on an industrial scale from biotechnology and yeast fermentation, using essentially the same production platform used to brew beer or make wine. Working closely with the CDC and others, Evolva has been compiling data for EPA registration (and other geographies) that also demonstrates the broad spectrum activity of nootkatone against a range of biting pests (including resistant vectors) such as the mosquitoes that transmit diseases like Zika, chikungunya, and Dengue fever, and the black-legged tick that transmits Lyme disease.

## 1478

### IMPACT OF HOLE SIZE AND LOCATION AND INTERACTION WITH INSECTICIDE CONCENTRATION ON THE PERSONAL AND COMMUNITY PROTECTION PROVIDED BY BEDNETS AGAINST PYRETHROID SUSCEPTIBLE, AND RESISTANT *ANOPHELES GAMBIAE* AND *ARABIENSIS*

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Data on mosquito net durability in the field in terms of physical integrity (holes) and insecticide content is only useful if we can understand how this affects the ability of these nets to protect against transmission of vector borne diseases. In an experiment in Tanzania, nets were treated with a series of concentrations of the insecticide deltamethrin (0, 5, 15, 25 and 55mg/m<sup>2</sup>) and deliberately torn, either in the top or side panels, over a range of total hole surface area (0-5800cm<sup>2</sup>) to reflect the range of net integrity and insecticidal content observed in a nationwide longitudinal study in Tanzania. Whole nets in each category were tested in an ambient chamber to measure the protective efficacy to people resting underneath them overnight with released *Anopheles gambiae* s.s. susceptible to pyrethroids, low resistant (80% mortality with deltamethrin in standard WHO susceptibility tests) and high resistant (20% mortality) *An. arabiensis*. The relative importance of hole size, hole location, and insecticidal content of nets for mosquito entry in resistant and susceptible mosquitoes is presented, as well as the impact on personal and community protection through the use of a mathematical malaria transmission model. Early data demonstrate a linear relationship between pyrethroid resistance and feeding success as well as mortality, and also a strong preference for entry through holes in the roof of the nets. We present a modified method for measuring the quality of nets in the field by teams performing routine surveys.

## 1479

### DIFFERENTIAL TRANSCRIPTOMIC RESPONSES ASSOCIATED WITH DENV EIP IN *Aedes aegypti*

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The extrinsic incubation period (EIP) is an important determinant of vectorial capacity. In *Aedes aegypti* mosquitoes, the EIP of dengue virus has been shown to be heritable. EIP is also genetically correlated with decreased lifespan, suggesting negative fitness consequences are associated with rapid body infection. This suggests that evolutionary pressures may limit the persistence of mosquitoes with short EIP in a population. To investigate whether EIP trait can be altered by selective breeding, we created 38 families with a half-sib breeding design, phenotyped them for EIP and selected the extremes for interbreeding. We found that despite selection for short EIP lengths, the trait shifted toward longer EIP lengths within just one generation. Selection for long EIP led to no change in phenotype. To understand the mechanisms driving the evolutionary pressures around EIP, we then performed transcriptome sequencing on mosquitoes characterised for their EIP phenotypes (6, 8, 10, or 12 days post-infection) and studied their transcriptomic responses to dengue infection. Functional clustering analysis performed on mosquitoes with the extreme EIPs of 6 and 12 DPI revealed very distinct biological processes. Mosquitoes with short EIP exhibited higher rates of transcription for a range of genes encoding structural components of the ribosome. Given viral dependence on host ribosomal machinery, these changes may enhance the rate of viral replication and spread. The evidence of reduced fitness for short EIP mosquitoes may potentially be explained by the extra costs associated with their enhanced protein synthesis activities. The profile of long EIP mosquitoes featured an increase in protein degradation, a process shown to be associated with flaviviral replication in mammalian cells. Our results suggest multiple physiological responses of mosquitoes to virus that may determine the balance between viral success and host fitness. The specific genes identified may underpin mosquito transmission of virus and hence warrant further functional study.

## 1480

### TARGETED DELIVERY OF CRISPR/CAS9 INTO THE ADULT MOSQUITO GERMLINE

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Cas9-mediated gene editing is a powerful technique for addressing research questions in arthropods of medical importance. There is a critical need to develop methods for Cas9 delivery that are simple, accessible for many researchers, and generally compatible for a large variety of arthropod species. Here, we propose to deliver Cas9 into the mosquito germline via hemolymph injections into adult females. We generated a peptide ligand (P2C) with highly specific tropism for the female mosquito germline. When fused to Cas9, the fusion protein is internalized into oocytes after injection into the hemolymph of *Ae. aegypti* and *An. stephensi* mosquitoes during oocyte development. The optimization of Cas9 delivery via maternal injections will benefit researchers who wish to perform genome editing in mosquitoes and other disease vectors.

## GENETIC ANALYSIS OF MOSQUITO ITCH

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The mosquito, *Aedes aegypti*, can spread devastating viruses when it injects saliva into human skin while blood-feeding. Mosquito saliva is a pharmacopeia of biologically active molecules that not only facilitate the spread of disease, but also affect skin physiology and induce swelling and itching. Yet few individual salivary molecules have a known function. Here, we take advantage of natural variation between two subspecies (or forms) of *Aedes aegypti* to understand the role of mosquito saliva in generating itch in humans. The forest form feeds mainly on non-human animals, while the recently-evolved domestic subspecies is anthropophilic and specializes in biting humans. Interestingly, we recently discovered that domestic mosquito bites are judged by humans to be less itchy and painful than forest bites in side-by-side comparisons. Additionally, humans are slower to detect a biting domestic mosquito. We hypothesize that milder bites are an adaptation that helps domestic mosquitoes avoid detection when they blood-feed on human hosts. These natural differences in bite severity are striking and present a unique opportunity to capitalize on evolutionary divergence to uncover the molecular basis of mosquito itch. Because reactions to mosquito bites are known to have an immunologic component, the causal agents in mosquito saliva may act on the human immune system, thus having implications for the spread of disease. We are taking a transcriptomic and proteomic approach to search for the molecular basis of differences in saliva between these two mosquitoes. Additionally, we are using QTL mapping to identify mosquito *loci* that mediate human itch. Our results promise to shed light on how mosquitoes have evolved to overcome host defenses and may provide new information helpful in fighting mosquito-borne illness.

## STRUCTURAL VARIANT DETECTION BY READ-CLOUD SEQUENCING IN THE ZIKA VECTOR *Aedes aegypti*

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The recent re-emergence of Zika virus has focused attention on the arbovirus vector *Aedes aegypti*, a mosquito which is also the transmits dengue virus, yellow fever and chikungunya. Yet compared to the better-studied *Anopheles* mosquitoes that transmit malaria, population genetics studies in *Aedes aegypti* have been hampered by a lack of genome-scale resources. A large and repetitive genome, high diversity, and potentially extensive structural variation combine to hamper genomic analyses, and until this year had prevented even the full assembly of the genome. Structural variants themselves have proven to be key features for studying dipteran population structure. Across the full range of the order, large chromosomal inversions exist both as clearly identifiable markers distinguishing sub-populations, as well as potential sites of divergence that could underlie speciation or local adaptation. Although the intractable genome of *Aedes* has prevented any such survey being carried out in the past, characterising structural variation will be an important asset for mapping insecticide resistance markers, examining population structure, or identifying suitable targets for gene-drive systems, among other approaches. Building on the work performed by the *Aedes* Genome Working Group, we have performed the first large-scale investigation of structural variation in *Aedes aegypti*. Using linked-read (10X) technology and a variety of algorithmic approaches we have identified segregating inversions in a world-wide sample set along with a large number of duplications and deletions. By studying SVs within mosquito colonies

sampled from across the globe, this work will provide the foundation to subsequent studies of *Aedes* population structure and the potential for disease transmission by this vector.

## WHOLE GENOME SEQUENCING OF THE ANOPHELES FUNESTUS SUBGROUP REVEALS ANCIENT INTROGRESSION

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*Anopheles funestus* is one of the four most important and widespread vectors of human malaria in tropical Africa, but unlike *An. gambiae*, it remains understudied. Whole genome sequencing of *An. gambiae* sibling species yielded important insights into genomic changes that contribute to making it a major malaria vector. A similar approach applied to the *Funestus* Subgroup, including sequencing both vector and non-vector species, would likely provide further insights into anopheline biology and malaria epidemiology. The current state of genomic data on *An. funestus* consists of a single reference genome. There is no genomic information on other *Funestus* Subgroup members, and no whole genome data from wild-caught species necessary to characterize genetic diversity. The *Funestus* Subgroup consists of six recognized species: *An. funestus*, *An. funestus-like*, *An. parensis*, *An. vaneedeni*, *An. aruni*, *An. longipalpus* C. and *An. confusus*. The potential contribution of these species to malaria transmission is uncertain as only *An. funestus* has been implicated as a major malaria vector. The first step to understanding species diversity is to reconstruct the evolutionary relationship between species using phylogenetics. A phylogenomic approach leverages information from the entire genome, but requires genomic data on all subgroup members. To this end, we assembled 20 *de novo* genomes by implementing a novel pipeline for assembling highly heterozygous genomes. Our preliminary results demonstrate a high discordance among the mitochondrial and nuclear genomes. We hypothesize that historical introgression among the *Funestus* Subgroup has led to reciprocal mitochondrial capture events. Future analysis is continuing to uncover the extent of the introgression between Subgroup species and reconstruct a likely species topology. The continued study of anopheline species groups that contain both vector and non-vector members will allow us to better characterize malaria vectors at a genomic level and therefore more effectively identify and eliminate potential vectors from malaria endemic areas.

## SPATIO-TEMPORAL GENETIC STRUCTURE OF ANOPHELES GAMBIAE IN THE NORTHWESTERN LAKE VICTORIA BASIN, UGANDA: IMPLICATIONS FOR GENETIC CONTROL TRIALS IN MALARIA ENDEMIC REGIONS

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It is anticipated that genetic control approaches will complement existing malaria vector management approaches in the near future. However, before outright deployment of such tools, prior field tests in isolated sites in endemic areas are recommended. We assessed the level of spatial and temporal genetic differentiation between island and mainland *Anopheles gambiae* populations in the Northwestern Lake Victoria basin (LVB) in Uganda, using whole genome sequences (WGS) of samples collected in 2015 and the ND5 region of the mitochondrial DNA (mtDNA) for these and samples from 2002 and 2012. Most pairwise *F*<sub>ST</sub> values between mainland and island *An. gambiae* populations were low but significant (Average *F*<sub>ST</sub>=0.07 and 0.02 for mtDNA and WGS respectively) while

most mainland-mainland pairwise FST values were much lower and not significant (FST=0.007 and 0 for mtDNA and WGS respectively). Temporal variation in genetic structure was not significant over the three sampling periods. The differentiation observed on this and other island *An. gambiae* contrasts with shallow structure seen across the continent in the species, reflective of the species' recent history of population expansion, extremely large population sizes, and remarkably high genetic diversity. These conditions depress FST values even between mainland samples separated by 7,000 km, but our study and previous work in the LVB suggests that under-appreciated structure may exist on lacustrine islands. A combination of a water barrier presented by L. Victoria and/or other local adaptive factors (see also Bergey et al., abstract) could account for the observed genetic substructure in the LVB making these islands suitable sites for future genetic control trials against malaria.

NB: \* Both ML and CMB contributed equally

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### WHY INDELS MATTER: INSERTION-DELETION VARIANTS IN THE ANOPHELES GAMBIAE COMPLEX

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Analysis of single nucleotide polymorphisms (SNPs) within vector genomes is now common, but longer insertion-deletion variants (indels) are often ignored. While indels may present more analytical challenges than SNPs, they also have the potential to cause greater disruption to the genome. Research in other species, including humans and *Plasmodium*, has shown that indels are common and important sources of variation. To extend this research to malaria vectors, we used data from phase 1 of the *Anopheles gambiae* 1000 Genomes Project to identify a set of indels segregating within the parents and progeny of a cross between *An. coluzzii* and *An. gambiae*. Exploiting the nature of the cross, we filtered these indels by removing any variants that displayed violations of expected Mendelian segregation. Further quality filtering produced a set of high-confidence indels found in coding regions; as expected, these indels were strongly enriched for length changes in multiples of three. Using field-caught specimens of *An. gambiae*, we confirmed that the vast majority of indels in this high-confidence set also segregate in the wild. The high-confidence coding-region indels overlapped 2,285 genes. Functional enrichment analysis of these genes showed enrichment of genes involved in transcriptional regulation, as well as of genes whose protein product includes a coiled coil motif. Interestingly, a number of the genes overlapped by high-confidence indels are known or believed to have a role in reproduction, including AGAP009002, which interacts with the receptor of 20-hydroxyecdysone; AGAP009368, a component of the mating plug; and AGAP005194, which interacts with the mating plug.

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### STRUCTURE OF SELECTED VARIATION IN ANOPHELES GAMBIAE ON LAKE VICTORIA ISLANDS AND IMPLICATIONS FOR GENETIC CONTROL FIELD TRIALS

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Geographically isolated islands have been proposed as sites for initial transgenic mosquito release to test the dynamics of the spread of beneficial introduced transgenes while limiting their movement beyond the study population. However, antecedent studies of population structure and connectivity of potential release sites are necessary to evaluate the success of such field trials, as well as to quantify the risk of escapee

transgenic insects carrying constructs designed to propagate across mosquito populations and country borders. A limited number of such field assessments has been conducted on lacustrine and oceanic islands. In the present study, we sequence the full genomes of 116 *Anopheles gambiae* mosquitoes living near and on islands in Lake Victoria to analyze signatures of selection and explore how beneficial alleles have moved through geographically semi-isolated populations and variable genetic backgrounds. Despite the geographic isolation (see also Lukindu *et al.* abstract), we find that alleles having undergone recent selective sweeps have colonized the islands to different extents, with the migration of the carriers likely aided by human transport. We hypothesize that the islands' differing environments, in both natural and anthropogenic dimensions, may have influenced the timing and intensity of such sweeps. These regions with signatures of selection include *loci* with known roles in insecticide resistance as well as a locus involved in sensory processing, retinal degeneration A. The dynamics of these beneficial alleles can serve as a model for those of a transgenic construct, allowing us to assess the islands' suitability as a potential site for field trials of genetically modified mosquitoes.

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### INVESTIGATING THE EFFECTS OF LATITUDE AND TEMPERATURE ON THE LIFE HISTORY TRAITS OF THE MAJOR LATIN AMERICAN MALARIA VECTOR, ANOPHELES DARLINGI

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Brazil has the second largest burden of malaria in Latin America, with most cases occurring in the Amazonia biome. The primary vector in Brazil is the widespread species *Anopheles darlingi*. Both a conservative and an extreme ecological niche model have predicted an expansion of *An. darlingi* and *Plasmodium falciparum* distribution into southern Brazil by the year 2070. It is unclear whether this invasion will arise from local adaptation of southern populations or from an invasion of northern populations. Laboratory experiments at three fixed temperatures were conducted on *An. darlingi* populations distributed along a latitudinal gradient to test for the presence of a plastic response using a suite of life history traits. Variation in life history traits can have a substantial impact on vectorial capacity. Adult female mosquitoes were collected from replicated localities spanning three latitudes and three biomes in Brazil (Amazonia, Cerrado, Mata Atlântica). Eggs from individual females were reared at 20, 24 and 28°C. Six life history traits were analyzed: larval growth rate, preadult developmental time (hatch to emergence), starved adult life span, adult size, daily survival, and sex ratio. Across all sites in response to temperature, the fastest larval growth rate, fastest preadult development, and the shortest adult life span of both sexes occurred at 28°C. At 24°C all populations had the same survival probability. Mosquitoes from the Mata Atlântica (most southern) biome developed slowest of the three biomes tested at 20 and 28°C. Sex ratio was not affected by biome, latitude or temperature. Despite having the most rapid development, the smallest body size and shortest life span, *An. darlingi* from Amazonia (lowest latitude) had the highest survival to adulthood across all temperatures, likely contributing to its role in malaria transmission. Models of climate change and vector-borne disease distribution must utilize data from multiple populations to increase their accuracy.

### DISTRIBUTION, INFECTION STATUS AND BLOOD-FEEDING BEHAVIOR OF ANOPHELES SPP. MOSQUITOES IN SOUTHERN MALAWI

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Despite multiple nationwide campaigns that distributed long lasting insecticide treated bed nets, malaria infection and disease remain a large problem in Malawi. Although malaria transmission occurs year-round, Malawi experiences elevated transmission during the rainy season, typically from November to April. Transmission is highest in rural and humid lowland areas. We collected and analyzed entomological data in relation to human infection at the end of five rainy seasons (2012-2016) and three dry seasons (2013-2015) from cross-sectional studies in southern Malawi. Adult mosquitoes were captured indoors by aspiration during all surveys and by CDC light trap during the last three surveys. The number, species and sex of mosquitoes was determined by visual identification and species was confirmed by PCR. The blood-feeding status was also determined visually, and the blood-meal source was determined for blood-fed mosquitoes by PCR. Finally, presence or absence of sporozoites in the mosquito salivary glands was determined by ELISA. 4,047 mosquitoes were collected, nearly all of which were identified as *Anopheles funestus* s.s. or *An. arabiensis*. More mosquitoes were captured during the rainy than the dry season, and *An. arabiensis* was more common during the dry season while *An. funestus* constituted a greater proportion of collected mosquitoes during the rainy season. Both species fed extensively on humans, with an overall human biting index of 0.96 and the remaining meals coming from cattle or goats. However, overall sporozoite infection levels were low, at 1.4% as measured by CSP ELISA. These patterns are similar to those seen in neighboring countries such as Zambia. Despite the low level of sporozoite infections, the large preference for human biting and the presence of *An. arabiensis* during the dry season allows malaria transmission to continue at these sites.

### ABILITY OF COMMERCIALY AVAILABLE HUMAN RAPID DIAGNOSTIC TESTS (RDTs) TO DETECT DENGUE AND MALARIA IN ARTHROPOD VECTORS

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Malaria and dengue are the two most significant worldwide infectious disease threats; both have global endemicity, cause morbidity and mortality, and are transmitted by mosquito vectors. The primary infection countermeasure against these vector-borne diseases is bite protection including bednets, insect repellent, and permethrin-treated uniforms although user compliance can compromise efficacy. Such bite protection efforts can be strengthened and prioritized if disease agents are detected in the local circulating vectors, verifying an immediate risk. Multiple rapid diagnostic tests (RDTs) are commercially available for detection of malaria parasites (*Plasmodium falciparum* and *Plasmodium vivax*) or dengue virus (DENV) in human blood samples; some of these detect antigens that are also expressed by mosquito-stage parasites and viruses. We hypothesized that clinical RDTs exploiting those antigens would be capable of detecting infection in mosquitoes. To test this, we seeded single infected mosquitoes

in pools of 24 uninfected mosquitoes, mechanically homogenized them in buffer, and applied the homogenate to the RDTs in lieu of a blood sample and three independent analysts read each test. Three DENV RDTs were capable of detecting in-vector virus, with one achieving 100% detection. We downselected this RDT for further assessment of cross-reactivity with other arboviruses and limit of detection. Of three malaria RDTs tested thus far, none detect in-vector *Plasmodium*. In sum, this indicates at surveillance of at least one vector-borne disease can be achieved both in vector collections and in the clinic using the same RDT.

### SOCIOECONOMIC AND LIFESTYLE DRIVERS OF Aedes Aegypti Abundance Across Diverse Urban Landscapes in Los Angeles, California: A Cross-Sectional Study

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Since the start of the current widespread Zika virus outbreak originating in South America and as of April 2017, 4,886 travel-associated infections and 222 locally-acquired infections have been reported in the continental United States, including > 500 cases of travel-associated infections in California, which is the 3rd highest number among U.S. states. Due to the recent increase in the number of Zika virus-infected travelers returning to areas of the U.S. with the primary Zika virus vector, *Aedes aegypti*, there is an urgent need to understand the genesis of local chains of transmission. Low dengue incidence in the U.S. has been attributed in previous studies to lifestyle factors such as improved housing quality and air-conditioning usage that may limit humans' exposure to *Aedes aegypti* biting. Biting is a function of *Ae. aegypti* abundance and availability of bloodmeal hosts, and in this study, we consider whether socio-economic status and lifestyle factors explain differences in *Ae. aegypti* distribution and abundance in two contrasting neighborhoods in Los Angeles, California. We conducted surveys in randomly selected households and compared relative abundance of *Ae. aegypti* indoors vs. outdoors to determine whether total abundance and endophily can be explained by socio-economic variables that could be mapped at broader scales to guide surveillance and control efforts.

### ROAD-SIDE CATCH BASINS AS SENTINELS FOR WEST NILE VIRUS INFECTED Culex spp. Mosquitoes

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CDC-gravid traps are a standard vector surveillance tool for West Nile virus (WNV) detection in the United States because they can capture a substantial number of gravid (i.e. previously blood fed) mosquitoes. However, the testing of a large number of specimens can be a financial and personnel burden for vector control districts, and trap placement may be limited by the availability of public spaces and permissions from land owners, which can influence the design of virus surveillance programs. In an effort to devise a more targeted and accessible approach to the surveillance of urban arboviruses transmitted by *Culex spp.*, we compared morphological identification success and WNV detection of paired gravid traps and targeted Prokopack aspiration collections inside road-side catch basins in 4 neighborhoods in the City of Atlanta (Georgia, USA) from 2014 to 2016. For each method, all collected mosquitoes were morphologically identified, and specimens were pooled (up to 25 individuals) by species, date, location, and trap type. Pools were tested for WNV through virus isolation, Vec-Tests, and RT-PCR. A total of 8,262 mosquitoes were

collected from weekly aspiration in 47 catch-basins and 55,709 were collected in 775 trap-nights. On average, 69.7% of *Culex spp.* collected from catch basins were successfully identified to species compared to 48.8% from gravid traps. Weekly WNV minimum infection rates (MIR, number infected per 1,000 individuals) ranged from 6.1 to 143.6 for catch basin collections and from 1.3 and 28.9 for gravid trap collections. Though the percentage of positive pools was higher for gravid traps (2.2%) than catch basins (1.2%), weekly MIRs were significantly higher for catch basins than for gravid collections (Wilcoxon Test,  $W=417$ ,  $p<0.01$ ). Total costs of testing mosquitoes in the parks was reduced by 63% when aspirating in catch basins (\$44,000 to \$15,000). Thus, aspirating catch basins may be a cost-effective approach for vector and virus surveillance as the lower number of collected mosquitoes translates into fewer pools that need to be tested without lowering the rate of WNV detection.

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### DESIGN OF STRATEGIES FOR SURVEILLANCE AND EFFICIENT MANAGEMENT OF *Aedes aegypti*

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The goal of entomological surveillance is to generate necessary information for the organization of vector control actions in order to prevent, predict and ameliorate the consequences of an epidemiological outbreak, as well as to evaluate the implemented control measures. Due to epidemiological dynamism, characteristic of diseases like dengue, chikungunya and zika, all of them transmitted by *Aedes aegypti* mosquitoes, monitoring of populations of this vector must be performed in a continuous manner, in order to obtain timely information about its distribution, abundance, and local characteristics that modulate its life cycle. Since 2002, Mundo Sano Foundation, implements surveillance and monitoring strategies of *Ae. aegypti* populations in localities affected by seasonal outbreaks of dengue. These strategies are planned and implemented in a continuous manner, generating information on the temporal population dynamic of the vector and its breeding sites, weekly gonadotrophic activity and recognition of persistent infestation areas, as well as the main urban and sociocultural determinants that influence the abundance of the mosquito. All this information permits the detection of associations with environmental variables obtained with remote sensors that can then be modelled due to the availability of temporally consistent data. The management of data is performed through the use of an online platform in a way that the information obtained from larval sampling is digitalized *in situ* and analyzed remotely within 24 hours, constituting an important operational tool that allows the manager to count with precise and timely information for quick decision making and guidance of control actions. In this poster, the main results of the activities developed in an uninterrupted manner during seven years of implementation of the "Program of Surveillance and Control for *Aedes aegypti*" in Tartagal City are presented as well as the impact these actions had on the vector and the incidence of the diseases it transmits.

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### BIONOMICS OF *Aedes aegypti* IMMEDIATELY PRECEDING THE 2016 DENGUE OUTBREAK IN OUAGADOUGOU, BURKINA FASO

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The dengue outbreak in Ouagadougou in late 2016 resulted in 2,600 cases and 21 deaths. As elsewhere, an evidence-based vector control response would have enabled a rapid and more effective impact. We report on a three-month surveillance study of *Aedes spp.* and other mosquito populations in three localities (urban, periurban and rural) in and around Ouagadougou, prior to and overlapping the outbreak. Together with characterisation of insecticide resistance profiles, the data provide an essential contemporary evidence base, fundamental to development of a locally-specific vector control strategy. *Aedes aegypti* and other mosquitoes were collected indoors and outdoors using vacuum aspirators and all potential breeding sites were sampled for immature stages. All *Ae. aegypti* were assessed morphologically to investigate their subspecies status (*i.e.* whether *Ae. a. formosus* or *Ae. a. aegypti*). To assess susceptibility to commonly-used insecticides, standard WHO bioassay tests were performed on larvae and adults. Blood meal origins were identified by PCR and the mechanisms underlying resistance to insecticides investigated using qPCR. *Ae. aegypti* exhibited more outdoor than indoor biting and, although mixed human-animal blood meals were detected, showed a strong preference for human hosts. An increasing gradient of *Ae. aegypti* density was recorded from urban to rural localities, which was also seen in the variation in abundance and diversity of productive breeding sites. Larval stages displayed susceptibility to organophosphates but adults showed high levels of resistance to pyrethroid insecticides, a difference that was at least partly explained by metabolic resistance mechanisms. The results provide much-needed data on *Ae. aegypti* bionomics in Ouagadougou to enable targeting of efforts to prevent or to mount a vector control response to outbreaks of dengue, or other arboviruses transmitted by the same vector, in Burkina Faso.

## 1495

### IMPACTS OF VECTORS ABUNDANCE AND WEATHER ON RISK OF DENGUE AND CHIKUNGUNYA INCIDENCE ACROSS KENYA

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Dengue (DENV) and chikungunya (CHIKV) viruses are arboviruses of increasing global concern that are transmitted by *Aedes spp.* mosquitoes. Attributing human disease incidence to *Aedes* abundance is rarely possible. We investigated how climate and vector abundance affect risk of DENV and CHIKV seroconversion among healthy and febrile child cohorts in Kenya. Children aged 1-17 were enrolled in an ongoing study beginning in January 2014 at four Kenyan study sites (rural west, rural coast, urban west, urban coast). Surveillance was both active, with children tested every six months to document asymptomatic CHIKV and/or DENV infections via IgG ELISA testing, and passive at local hospitals to capture symptomatic disease via PCR and IgG ELISA testing. Questionnaire data were collected to describe demography, socioeconomic status, and household environment. *Aedes spp.* vectors at all life stages were collected monthly in each site, and immatures were reared to adulthood for identification. Weather variables were collected locally at each site using HOBO loggers. DENV incidence (104/2131) increased with abundance of immature *Aedes spp.* outdoors ( $p<0.001$ ), primarily due to ovitrap sampling, and mature *Aedes spp.* indoors ( $p<0.001$ ), primarily due to prokopack sampling. Incidence also increased during rainfall anomalies ( $p<0.001$ ), but decreased with average temperature, relative humidity, and dew point.

CHIKV seroconversion (28/4983) risk increased with age ( $p < 0.05$ ) and was higher in females ( $p < 0.05$ ) but not linked to climate or vector variables, likely due to low case numbers. We hypothesize that incidence is also related to the built environment such as house construction, population density, urbanity, and land use. This study provides an important link between vector abundance and disease incidence due to robust sampling among humans and vectors over a prolonged period at the local level. Comprehensive data collection systems that include human, vector, and climate inputs can provide important guidance on predictors of human risk.

## 1496

### PILOT PROJECT OF AN ENTOMOLOGICAL AND MOLECULAR SENTINEL SURVEILLANCE SYSTEM BASED IN THE ENTOMOLOGIST CITIZEN IN PUERTO RICO

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40,000 cases (rate 1,172 x 100,000) of arboviral disease were reported in Puerto Rico in 2016. The vectors *Aedes aegypti* and *Aedes albopictus* are endemic and reported in Puerto Rico respectively. Among other actions, the Technical Advisory Group on Public Health Entomology of the Panamerican Health Organization/WHO recommends "strengthen and enhance" the entomological surveillance and to adapt the "integrated vector management of Dengue model to both Chikungunya and Zika disease prevention and control" including the community. At present Puerto Rico has not an entomological-molecular surveillance system aimed at the prevention and control of the arboviral diseases. This study was undertaken to assess the feasibility of an entomological-molecular surveillance system based on the Entomologist Citizen in Puerto Rico. Entomologist Citizen (EC) is a "person of the community, who has no experience or advanced knowledge in entomology and is trained in the basic skills and knowledge of entomology aimed to be active part of a vector sentinel surveillance system". Fourteen EC in sixteen zones of Puerto Rico collected information about: mosquitos, bites, larvae and pupae and other variables. We calculated: mean, SD, absolute and relative frequency, ANOVA, female/male ratio, classification of mosquitoes and RT PCR analysis are being performed to assess the presence in the mosquito of arbovirus (dengue, chikungunya, zika). In 87.5% of the zones under study (ZUS) were observed mosquitoes (*Aedes spp.* (72%)) and in 53% of the ZUS were reported bites. Regarding the quantity of mosquitos observed a  $p < 0.05$  was found between ZUS, the same result was found to the quantity of bites reported. The male:female ratio was 0.31. The classification of collected mosquitoes confirmed the observations made by EC. A potential (real world evidence) mosquito insecticide resistance was reported by an EC in one ZUS. We still processing the RT PCR analysis. In conclusion, 1) the project is feasible; 2) sentinel surveillance based in EC bring information about arboviral diseases risk; 3) The community increased its vector control and prevention empowerment.

## 1497

### INTEGRATING METEOROLOGICAL DATA IN A NEW MODEL OF ENTOMOLOGICAL-MOLECULAR SENTINEL SURVEILLANCE SYSTEM IN PUERTO RICO

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The increase in temperature, precipitation and humidity are factors that positively influence the incidence of vector-borne diseases. In Puerto Rico the arboviral disease is endemic (dengue, chikungunya (cases reported from 2014) and zika (cases reported since 2015)) and the vector is the *Aedes aegypti* (in 2006 was reported the presence of *Aedes albopictus*). The climate change, trade and developmental model can increase the risk of transmission of vector borne diseases. This study of meteorological variables correlated to the distribution of mosquitoes as vectors will allow to identify vulnerable areas that requires interventions to prevent future outbreaks of emerging vector borne diseases impacting the health disparities that suppose these diseases. This study was undertaken: 1) to evaluate the distributions of mosquitos by cluster and identifying whether meteorological variables such as mean temperature, precipitation and evaporation are associated with these distributions. This study use the same methodology of the *Arbovirus Project* (Entomologist-Citizen Sentinel Surveillance System ECSSS). To this study seven areas across Puerto Rico were studied. The meteorological data is from NOAA. Latitude and longitude for mosquito was identified in ArcGIS and meteorological variables were correlate to each cluster. The mosquitoes caught were classified, a spatial analysis was done and the statistical analysis was: mean, SD, ANOVA, absolute and relative frequency. Arecibo and Yauco notified the major quantity of mosquitoes observed with a  $p < 0.05$  when comparing with the others areas. The mean of temperature in Arecibo was 64.70F (zone of development zero is 570F), and this factor can be the reason of notify just one bite/day while Yauco (74.90F) notified 4 bites/day. Analysis of the other areas still in process and currently a RT-PCR analysis to evaluate arbovirus in mosquito is in process. In conclusion, 1) the climate characteristics and the data from the ECSSS can be a proxy of transmission risk in real time; 2) establish the *hot spot* of risk can improve both prevention and control actions.

## 1498

### OUTDOOR BARRIER COLLECTION IN NCHELANGE DISTRICT, ZAMBIA

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Nchelenge District of northern Zambia experiences high levels of malaria transmission despite decades of control, including artemisinin combination therapies, insecticide treated nets, and indoor residual spraying. This district is under study by the Southern African International Centers for Excellence in Malaria Research (ICEMR) as part of an effort to understand the mechanisms underlying such persistently high transmission. Among the major challenges faced in the global drive to eradicate malaria is the existence of vectors exhibiting behaviors that allow avoidance of indoor-directed vector control. In Nchelenge the only vector control measures deployed are ITNs and IRS, and so one possible contributor to transmission in this district may be non-canonical outdoor foraging or resting behaviors exhibited by the known vectors, *An. funestus s.s.* and *An. gambiae s.s.*, as well as previously unrecognized and potential vectors in the region. In order to study outdoor foraging vectors, we trialed barrier screens to

sample mosquitoes mid-flight. We characterized mosquitoes from four hourly barrier collections conducted on each of two trap nights from 8 households. Time series showed that vectors are potentially present outdoors from 8PM until 6AM, with an overall peak of vector numbers seen around 1AM. This is a range of vector presence that includes times when household residents are outdoors and unprotected by either bed nets or residual spray programs. The overall proportion of *An. gambiae* to *An. funestus* was 1:1, in contrast to the roughly 1:2 composition seen in indoor collections conducted during the same time period. Species composition was skewed more heavily toward *An. gambiae* at collections taken close to nearby Lake Mweru than for those taken at inland locations. The overall vector infection rate was ~2%, lower than had been previously observed from indoor collections.

## 1499

### CLIMATE CHANGE MAY DECLINE PREVALENCES OF DISEASE VECTORS IN ECUADOR

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Vector-borne diseases are of global concern with >50% of the world's population at risk, and health consequences that include mortality and disabilities. In Ecuador, vector-borne diseases are a growing public health drawback owing to high prevalences of diverse vectors species across the country carrying new emerging pathogens such as chikungunya and zika viruses, and re-emergent epidemic threats such as yellow fever. Other diseases such as dengue and leishmaniasis pose important ongoing burdens to the Ecuadorian population; moreover although malaria and Chagas disease have been reduced in past years, a raise in cases have been identified more recently. To incorporate environmental features of Ecuador on the potential distributions of vectors for arboviruses, malaria, leishmaniasis, and Chagas disease, we used worldwide reports of 14 vector species coupled with climate information to calibrate ecological niche models (ENMs). Models were used to forecast present-day and future-climate distributions of such disease vectors. ENMs were developed using a logistic-regression like algorithm with species-specific configurations selected via an information theory metrics. We found a strong signal of shrink in the distribution of several vectors (e.g., *Aedes aegypti*) under future climates, and identified new areas of distribution for some species, suggesting a plausible shift to Andean regions (e.g., *Anopheles darlingi*). From shrink and shift of vector ranges, we identified reductions of people potentially exposed to vectors, which will resemble an uncommon scenario for climate change offering a likely benefit in terms of people expose to vector-borne diseases. Our findings may be provocative, but also may encourage a reconsideration of climate change as main concern for these diseases and, instead, may help prioritize prevention strategies at landscape level in the short term, including improvements in health disparity and mitigations of the rapid land cover change as drivers of epidemics.

## 1500

### MIDGUT BACTERIA EXERT IMMUNE PRIMING WITH A CERTAIN LEVEL OF SPECIFICITY IN *ANOPHELES GAMBIAE*

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Mosquito immune repertoire has been built upon encounter with various mosquito associated microbes throughout evolution. The interactions with midgut symbiotic microbial residents contribute to the evolution of mosquito immune system. In this study, we tested that immune priming effects of gut bacterial residents *Cedecea* sp. Ag1 and *Serratia fonticola* S1. Oral ingested bacteria can prime mosquito immunity by providing protection against a homocoel infection by bacterial injection. Intriguingly, the protection shows a pattern of specificity. *Cedecea* primed mosquitoes were resistant to *Cedecea* infection with a survival of 92.2% vs 62.5% in unprimed control mosquitoes (P<0.01). However, *Cedecea* primed mosquitoes were less resistant to *Serratia* infection with a survival of 56.8% (P<0.01). Similarly, the *Serratia* primed mosquitoes showed a survival of 76.6% upon *Serratia* infection higher than the survival of 45.4% upon *Enterobacter* infection (P<0.05). Both bacteria are Gram negative and have smooth type of lipopolysaccharide (LPS) with O antigen. The O antigens of the two bacteria show different banding patterns on a PAGE gel. *waaL* gene encodes an O antigen ligase that ligates O antigen to the LPS core. The *waaL* mutant of *Cedecea*, which lacks O antigen, showed a reduced priming effect against *Cedecea* challenge. Furthermore, the *waaL* mutant was less effective than wildtype in priming mosquitoes against rodent malaria *Plasmodium berghei*. The data indicate that midgut bacteria exert immune priming with a certain level of specificity, and O antigen may be involved in immunogenicity.

## 1501

### CLIMATE SERVICES FOR HEALTH: SUPPLEMENTING LOCAL AND REGIONAL DENGUE EARLY WARNING SYSTEMS IN THE SOUTH EAST ASIA WITH OCEAN NINO INDEX IMPROVES OUTBREAK PREDICTIONS

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Dengue, Chikungunya, Zika, and Yellow Fever are of serious public health concern in many regions of the world, and may threaten populations in Southeast Asia. Climate services can inform actionable, data-driven decisions to protect public health in a myriad of manners, which may include augmenting the early warning of vector-borne disease outbreaks. Some climate signals may be more predictable and have longer lead-time than meteorological and surveillance only methods, which may detect outbreaks only after they are already underway. Posing an exacerbated threat to human health, corresponding to greater variability and intensity of infectious disease outbreaks, climate change may further complicate the existing public health problems associated with *Aedes* vectors in communities of Southeast Asia. Our study aimed to examine the utility of spatial and time-series analysis including specific Oceanic Nino Indices (ONI) in five countries at the local level, to determine stable associations needed for reliable disease early warnings systems based on combinations of climate signals, meteorological observations and disease surveillance. In this analysis, for best regional comparison, we used notifications of dengue in the public health surveillance system, however, some methods and conclusions may be conserved for other warnings of *Aedes*-borne arboviruses. Analysis from Malaysia, Singapore, Indonesia, Sri Lanka, and Vietnam during 2009 through 2015 yielded largely converging findings especially during larger dengue outbreak years corresponding to trends in ONI. Year to year epidemiological analysis revealed seasonality, trend,

and cycle in many study areas were persistent throughout the datasets, indicating strong potential for climate and meteorological services to be mainstreamed used in dengue early warnings systems utilized by public health planners working both in vector control and clinical management of arboviruses. Having early reliable disease predictions, public health practitioners may better plan, budget and coordinate efforts to prevent large scale dengue epidemics.

## 1502

### HEALTH SEEKING BEHAVIOR AMONG UNDER-2 CHILDREN IN VELLORE HEALTH UNIT DISTRICT

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Health Seeking Behaviour (HSB) of children in India is influenced by multiple factors. Governments constantly try to innovate and incentivize services to make them accessible and acceptable to target populations. A survey among parents/ care-givers of 3123 (2403- urban, 720- rural) under-2 children was done in Vellore health unit district to understand utilization of antenatal and immunization services; place of birth, illness in preceding 4-weeks, health center visited and health centres preferred for hypothetical future mild/ severe illness. A majority (2552, 81.7%) had used government antenatal services while 2623 (84%) had utilized government immunization services. Of the 2192 (70%) who recalled illness in the preceding 4-week recall 2120 (96.7%) sought care. The commonest health center visited was private (1733, 81.7%) while 378 (18%) visited government centers, both urban and rural population had preferred private health care during prior illness (87%, 69% respectively). For hypothetical future mild illnesses, 2342 (75%) preferred private health care while 624 (20%) preferred government health services. For hypothetical severe illnesses, 2498 (80%) preferred private health care with the rest choosing government services. Private health care was the most preferred health care irrespective of urban rural divide. While most preferred government centers for antenatal and immunization services, they preferred private health care for curative services. Among private health care seekers, majority preferred to consult a specific physician for most illnesses. Among those preferring government health services most 353 (56.7%) visited the medical officer at the closest primary health center with very few (<5%) preferring the nurse run health sub-center while the rest preferred the government hospitals. Centers preferred by more than 10% of respondents identified 17 for mild and 8 for serious illness. Incentivized schemes have shown an increase in institutional antenatal and immunization services, more innovation in required to increase acceptability of curative services offered by public health institutions.

## 1503

### QUALITY AND INTEGRATED SERVICE DELIVERY: A CROSS-SECTIONAL STUDY OF THE EFFECTS OF MALARIA AND ANTENATAL SERVICE QUALITY ON MALARIA INTERVENTION USE IN SUB-SAHARAN AFRICA

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Service integration remains an underexplored area of study for systems strengthening and improved health outcomes. In the field of malaria service delivery, a pertinent gap in knowledge exists with respect to the role of quality of integrated malaria and antenatal services. This knowledge deficit warrants attention, given persistent gaps in national coverage and use targets for malaria interventions in pregnancy and childhood in sub-Saharan Africa. Using regionally-linked, cross-sectional facility and

household data from the Demographic and Health Surveys, we aimed to determine whether quality of integrated malaria with antenatal services in Kenya, Namibia, Senegal, and Tanzania predicted malaria intervention use during pregnancy and in children under-five. We assessed quality of these services using a novel combination of quality frameworks to select suitable indicators, and modeled the relationships of interest using pooled and country-stratified multilevel mixed effects modified Poisson models for three outcomes: insecticide-treated bed net use in currently pregnant women or children under-five, and receipt of two or more doses of intermittent preventive treatment in last pregnancy. We found modest, positive associations between malaria in pregnancy quality and study outcomes across pooled models and for most countries, with evidence of heterogeneity in strength of associations and relevant confounding factors. On average and using our indicator set, there was significant room for improvement in the quality of malaria in pregnancy services. Study findings indicate quality improvement of maternal health services delivering well-accepted interventions like malaria prophylaxis and bed nets is necessary to address gaps in national coverage and use targets for pregnancy and childhood. Consensus on a set of malaria in pregnancy service quality indicators and best practices for measuring integration of these services with antenatal care are needed.

## 1504

### TWITTER REACTIONS TO GLOBAL HEALTH NEWS RELATED TO FIVE DIFFERENT COUNTRIES: A CASE STUDY OF #POLIO

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Social media has become a vital tool for global health communication, given the penetration of the internet and mobile phones across the world. In addition to disseminating health information, public health professionals also monitor traditional media and social media to assess the communication environment. Prior research showed that outbreak-related social media contents were largely driven by traditional media reports. However, we hypothesized that different types of news contents could trigger different levels of reaction on social media. In this study, we retrospectively examined a 40% random sample of Twitter data containing the hashtag #polio from January 2014 to April 2015 (N=79333), from which we extracted five sub-corpora each with a co-occurring hashtag #India, #Iraq, #Nigeria, #Pakistan, and #Syria respectively. We also retrieved 104 polio-related traditional news stories from 2 newspapers, 2 television news stations, and 2 radio news stations within the same time frame. We assessed the relationship between polio-related news from traditional news sources and the Twitter content. We hypothesized that polio-specific Twitter conversations differed by the location of interest and they were reactions to traditional media news articles. Descriptive analyses and unsupervised machine learning were conducted on the 5 Twitter sub-corpora to elucidate their underlying topics. Traditional media articles were grouped according to the country of interest and were categorized into the following topics: celebrations or achievements; violence or crises; political actions; vaccinations or other programs/aid; new cases or spreading of polio; and miscellaneous. Strong Twitter reactions were observed following a few news stories published by traditional media but not the others. Our evidences suggest a nuanced relationship between outbreak-related traditional media stories and Twitter contents. Evidence from our study helps inform media monitoring and communication surveillance during global public health crises, such as infectious disease outbreaks, as well as reactions to health promotion campaigns.

## 1505

### A QUALITATIVE STUDY OF THE ACCEPTABILITY OF WEEKLY IRON SUPPLEMENTATION PRIOR TO THE FIRST PREGNANCY IN BURKINA FASO

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Reducing anaemia in women of reproductive age is a global priority as iron deficiency anaemia (IDA) is a leading cause of years lived with disability. To reduce IDA iron supplementation for menstruating women is recommended. This includes adolescent girls as adolescence may be the optimal time to build iron stores in readiness for pregnancy. The success of such interventions largely depends on their feasibility and acceptability to adolescents and their communities. This study explored the acceptability of weekly iron supplementation given preconceptionally for up to 18 months to non-pregnant adolescents (mean age 16 years) enrolled in a randomised controlled trial. Before and during the trial systematic information on the role of iron in health was provided. In-depth interviews were conducted with young women, selected according to their level of adherence in either trial arm (iron and folic acid versus folic acid alone) by field workers who had provided directly observed capsule intake. Tape recorded transcripts were translated from Mooré into French and analyzed thematically. 38 interviews were conducted with young women, some of whom had become pregnant during follow-up. At the outset, the research focus on adolescents suggested to communities that supplements were contraceptives and this led to drop out by some, but not other, interviewees. Adolescents expected curative and health benefits from the supplements which, when not experienced, engendered negative views. Commitment to the intervention was affected by mobility. Some were students who returned to homes outside the study area during holidays. Unplanned pregnancy and domestic assistance to relatives living elsewhere affected the mobility patterns of other interviewees. Despite health education, iron supplementation was not seen as a high priority by communities nor did the benefits persuade adolescents themselves of its importance.

## 1506

### STRENGTHENING NURSING AND MIDWIFERY TRAINING THROUGH IMPLEMENTATION OF CONTINUOUS QUALITY IMPROVEMENT PROCESS: THE TANZANIA EXPERIENCE

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Tanzania is one of the countries with critical shortage of human resource for health. The coverage of skilled birth attendants is about 50%, which connote suboptimal quality of care contributing to poor neonatal and maternal outcomes. Training and deploying adequate numbers of competent health workers is one of the objectives of the Tanzania National Health Policy. The government of Tanzania and partners like Jhpiego led Maternal and Child Health Survival Program, are working to improve the training environment hence competent graduates. In support of quality trainings; Jhpiego in collaboration with the Ministry of Health are implementing the Continuous Quality Improvement (CQI) process which encourages health training institutions to improve quality teaching and learning by focusing on Classroom and Practical Instructions,

Clinical instruction and practice, Institutional Infrastructure, Learning and Teaching Materials and Institutional Governance and Administration. A baseline assessment was done using the CQI guide in 10 training institutions to assess the quality of training and educational process, output and outcomes for the provision of quality nursing and midwifery care. Results indicated substandard outcomes with scores less than 50% at most schools. All 10 schools were oriented on CQI process including its implementation. Quarterly assessment by a team comprised of institutions' quality improvement teams, Ministry of Health and Jhpiego were done and gaps identified addressed through supportive supervision and mentorship. Training institutions are progressively improving their training capabilities. The teaching learning environment has improved substantially with an average score 95% during external verification from 45% baseline score. After eighteen months of program implementation, three schools have been recognized for their outstanding performance and were presented with trophies and certificates as a motivation. This translates into increased number of skilled health care workers from rural nursing schools with required competency to avert maternal and neonatal deaths.

## 1507

### COMMUNITY HEALTH VOLUNTEERS PROVIDE ESSENTIAL PRIMARY HEALTH CARE SERVICES IN MADAGASCAR

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Community Health Volunteers (CHVs) are the pillar of Madagascar's health system, as they meet the health needs of underserved and vulnerable populations who live more than 5km from a formal health facility. CHVs provide information, education, and services for family planning, maternal and newborn health care, and child health services, including the prevention, diagnosis, and treatment of malaria. The USAID Mikolo project assists and supports CHVs to provide services and promote health in their communities in 8 regions. Overall, CHVs expand access to crucial health services beyond the reach of the formal health system and thus help improve maternal and child health outcomes. Routine service data reported by CHVs were compared to routine service statistics reported by basic health centers between 2014 and 2016. The data were recorded and reported through the health information system and obtained by USAID Mikolo. Data were entered into an electronic database and analyzed in MS Excel. Between 2014 and 2016, the number of CHVs supported by USAID Mikolo rose from 4,489 in 375 communes to 6,694 in 506 communes. The number and proportion of children under the age of five years who were treated for malaria by CHVs and health centers, respectively, increased from 2014 (93,257) to 2016 (130,841). The same is true for children treated for diarrhea—increasing from 66,043 cases in 2014 to 101,969 cases in 2016. The number of referrals made for long-acting and permanent methods (LAPM) of FP rose from 4,009 in 2014 to 5,993 in 2016. The number of pregnant women referred to the nearest health facility by CHVs for and receiving ANC increased from 10,071 in 2014 to 30,728 in 2016. Finally, the number of newborns referred by CHVs for and obtaining emergency care increased from 2,032 in 2014 to 3,138 in 2016. The data also shows an important increase in the proportions of clients reached by CHVs relative to health centers over time. Ultimately, the evidence demonstrates that CHVs play a critical role in the provision of FP; reproductive, maternal, newborn, and child health; and referral services, stimulating both access to and use of services in Madagascar.

### PSYCHOLOGICAL DISTRESS AND ZIKA, DENGUE, AND CHIKUNGUNYA INFECTIONS FOLLOWING 2016 EARTHQUAKE IN COASTAL ECUADOR

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Stress from catastrophic events can decrease people's capacity to mount an effective immune response to infectious pathogens. In April 2016, a 7.8 magnitude earthquake struck coastal Ecuador, which resulted in significant mortality and morbidity, damages to housing structures and piped water infrastructure, and psychological trauma. This event occurred in an area with a historically high burden of dengue fever and coincided with the first outbreak of Zika fever. This cross-sectional analysis aims to describe the co-occurrence of psychological distress from the 2016 earthquake and viral transmission of Zika, Dengue and Chikungunya, and to determine whether greater distress was associated with a higher average number of self-reported disease. In July 2016, heads of household from four communities were interviewed in face-to-face surveys over a seven-day period in Bahía de Caráquez, Ecuador (n = 573 people) as part of a post-disaster evaluation by the Ministry of Health. Survey questions included demographics, psychological symptoms, physical damages caused by the earthquake, healthcare-seeking behavior, and self-reported symptoms of Zika fever, Dengue fever and Chikungunya. Results showed the prevalence of reported viral symptoms was 23% across the entire study population, and over 50% survey participants reported symptoms of psychological distress. Bivariate analyses showed the average number of psychological distress symptoms was higher among people with arbovirus symptomatology than among people without those symptoms (1.48 vs. 0.95; p=0.003). This is observed in the communities of Junco (1.96 vs. 1.12; p=0.003) and Bella Vista (1.25 vs. 0.70; p=0.003), among women (1.68 vs. 1.00; p=0.002) and in participants 19-39 years old (1.34 vs. 0.69; p = 0.03). In conclusion, we documented a high burden of psychological stress, and positive associations with self-reported arboviral infections. The results of this study highlight the need for integrated health responses following natural disasters that include mental health professionals and infectious disease physicians.

### AN EXAMINATION OF THE BARRIERS AND ENABLERS TO HEALTH AND WELLBEING IN THE COMMUNITIES ALONG THE INTER-OCEANIC HIGHWAY IN MADRE DE DIOS, PERU

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The interoceanic highway in Madre de Dios, Peru, paved in 2006, has driven dramatic change in the Peruvian Amazon basin with increases in movement of people and goods and decreases in biodiversity, flora, and fauna. The impacts of the interoceanic highway on the health and wellbeing of people living nearby, however, has been less characterized. Our study examines threats, barriers, and enablers to health and wellbeing in these communities, using a modified socio-ecological model, which stratifies the threats, barriers and enablers at the individual, community, and societal levels. The mixed methods include a survey (N= 522), key

informant interviews (N=34) and 12 focus group discussions (N= 83 individuals). At the individual level, we find that enablers to improved health and well-being are improved access to health services, food, and economic opportunities. Sixty-six percent of survey participants list poor personal health as the greatest threat to their well-being, and the most common barrier is decreased access to natural resources. At the community level, the key enabler is improved cultural exchange and the key barrier is poor access to water and sewage facilities. Enablers at the societal level are access to health insurance (68% of survey respondents have basic insurance) and the presence of health campaigns, including a local campaign to eliminate mosquito breeding areas following a positive dengue case. The key barrier to health and well-being is the limited quality and availability of health services. These findings reveal dynamic and rapidly changing communities, which would benefit from improved sewage and potable water services. Furthermore, focusing on improving health care access, service, infrastructure, is necessary to respond to changes in this region. Health education messages need to address the decrease in natural resources (bush meat, fishing, and forest products) in order to mitigate the impact of environmental changes on health and well-being.

### MANAGEMENT OF THE QUALITY OF TRICHIASIS SURGERY SERVICES IN A COMMUNITY SETTING IN CAMEROON: IMPLEMENTING A QUALITY ASSURANCE APPROACH

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Several quality approaches exist for patient care, such as total quality management, the Plan-Do-Check-Act (PDCA) or Deming wheel approach, and the business process reengineering. To ensure the delivery of high quality trachomatous trichiasis (TT) surgery services at the community level, in collaboration with the Ministry of Health Helen Keller International (HKI) has implemented the PDCA approach in Cameroon, through its USAID-funded MMDP Project. Plan: The Project evaluated the capacity of the health care system from the outset to provide an idea of the resources (physical, human, infrastructural) available for TT surgery. Do: A training of master trainers of surgeons followed by a training / certification of surgeons was undertaken through the use of the Trachomatous Trichiasis surgery manual (WHO 2015, 2nd edition) and the HEADSTART surgical simulator. Attention was paid on the selection process of the master trainers and surgeons based on their profile and experience. For field practice, the surgeons each operated on 15 eyes under the observation of the master trainer. The Project designed a trichiasis surgery calculator to estimate the quantities of consumables required and helped train a dedicated health team responsible for waste management and equipment sterilization. Check: At the end of each campaign, an evaluation meeting was conducted with the surgeons, district and region level managers, program managers and partners. A post-operative follow-up evaluation activity was conducted 3 to 6 months after each surgical campaign. Results were analyzed and discussed with the surgeons and program managers to identify areas for improvement. The average per surgeon surgical failure rate was estimated to be between 20-60%. Act: Based on these findings, a recommendation was made to provide supportive supervision and a training of surgeon supervisors was developed and conducted including the use of HEADSTART. A refresher training of surgeons was also recommended before the next surgery campaign. The PDCA approach has proven to be very useful and adaptable to improve the quality of community TT surgery services as part of the MMDP Project.

## 1511

### PRE-TRANSMISSION ASSESSMENT SURVEY OF LYMPHATIC FILARIASIS IN THREE HEALTH DISTRICTS IN INSECURITY ZONE IN NIGER

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Lymphatic filariasis (LF) is endemic in 31 health districts (HDs) across all regions of Niger. Annual mass drug administration (MDA) with ivermectin and albendazole for LF elimination began in 2007 and progressively reached full national geographical MDA coverage in 2014. Diffa region, where a state of emergency was put in place due to the insecurity caused by Boko Haram, started MDA in 2010 in all three HDs (Diffa, N'Guigmin and Mainé Soroa) regardless of the insecurity, and completed 5 rounds of MDA by 2015. The national program conducted the pre-transmission assessment survey (pre-TAS) in these HDs in January 2016. One sentinel site (SS) and one spot check site (SC) from each HD was surveyed by nocturnal microfilaria examination: Asaga and Gueskerou for Diffa HD, Kablewa and Bilabrine for N'Guigmi HD, and Boudoum and Djanguiri for Mainé Soroa HD. Night blood samples were collected between 11pm to 4am from each site and thick blood smear was prepared and examined for the presence of microfilariae. The survey teams moved house to house to perform blood sampling. The number of persons examined was 303 in Asaga, 304 in Gueskerou, 305 in Kablewa, 317 in Bilabrine, 304 in Boudoum, and 301 in Djanguiri. The prevalence of microfilariaemia was 0% in all 4 sites in Diffa and N'Guigmin HDs, while it was 0.33% in Boudoum and 0.66% in Djanguiri in Mainé Soroa HD. The results showed that these HDs in Diffa region qualified for conducting transmission assessment survey to assess whether MDA can be stopped. The survey was conducted in the context of a nocturnal curfew due to insecurity. The movement of people was prohibited from 8pm to 6am. Special authorization and assistance was obtained from the military services for securing the zone of the survey. These villages are less than 5 kilometers from the Boko Haram carpet lines in the green meadows of the rice paddies. 20% of the households awakened refused to open their doors for lack of confidence. 5% of people climbed their walls to escape when the teams passed. However, the national program successfully conducted the pre-TAS, a step forward in achieving the LF elimination objective in challenging situation in Niger.

## 1512

### THE MOST IMPORTANT ELEMENTS OF INFORMED CONSENT PROCESS AS RATED BY NEW AND EXPERIENCED RESEARCHERS: AN ONLINE SURVEY

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Informed consent process is a requirement when conducting human research. The researchers have to respect basic ethical principles of autonomy while balancing risks and benefits of study procedures. The objective of this study was to explore the opinions of researchers about the importance of ethical elements of informed consent process. An anonymous online questionnaire was sent to biomedical and health researchers in various academic and research institutes in Thailand on March 2017. Based on the importance level identified with a 5-point Likert rating scale, the ratings per item were analyzed. The ratings differed among 61 researchers, classified by years of experiences as: 22 with 1-5 years, 18 with 6-10 years and 21 with >11 years. For ethical considerations in informed consent process, the ratings for "most important" among the 3 groups of researchers with different experiences included: comprehension of information (82%,44%,72%), communication of risks and risk minimization (77%,67%,76%); decisional authority for consent

to research (82%,61%,62%); communication and power inequities (67%,24%,57%); timing for making decision (64%,17%,43%); request for maintaining specimen for future use (59%,44%,43%); and process in approaching research participants (64%,44%,65%). The consent form was rated differently as "most important" by the three groups for language (73%,50%,75%); form length (64%,33%,62%); completeness of the information given in relation to the protocol (73%,67%,71%); provision of alternatives and different choices (77%,61%,71%); having contact person regarding deviation/violation (82%,35%,87%), and handling of documentation (82%,28%,76%). The study results showed different weights of the elements given by the researchers. Interestingly, more new researchers with experiences 1-5 years tended to give most importance ratings for all elements compared to those working 6-10 years. This suggests gaps in their perceptions of informed consent process. The communication of these findings would help plan supports for researchers who may need to understand the importance of informed consent requirement.

## 1513

### DEVELOPMENT OF MULTIPLEX TAQMAN ARRAY CARDS FOR THE CHILD HEALTH AND MORTALITY PREVENTION SURVEILLANCE (CHAMPS) PROGRAM

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Every year, approximately 5.9 million children under the age of five die; two-thirds of these deaths are due to infectious causes. The Child Health and Mortality Prevention Surveillance (CHAMPS) program, supported by a Bill & Melinda Gates Foundation award to Emory Global Health Institute, aims to increase understanding of why children die, including identifying the specific pathogens contributing to child mortality. CHAMPS is focused on strengthening public health capacity in developing countries in Sub-Saharan Africa and South Asia. Laboratory data includes pathology analysis of tissue specimens obtained from minimally invasive tissue sampling (MITS) and molecular testing of tissue and non-tissue specimens collected post-mortem. Five unique multiplex TaqMan Array Card (TAC) configurations were developed for the molecular detection of pathogens from various specimen types: Respiratory (nasopharyngeal/oropharyngeal swabs and lung tissue), Enteric (stool and rectal swabs), Blood/CSF Tier 1 and 2 (whole blood and cerebrospinal fluid (CSF)), and Neonatal (whole blood and CSF specimens from neonates and stillbirths). Over 100 assays were developed, modified, and/or optimized for detection of bacterial, viral, fungal, and parasitic agents. Each assay underwent comprehensive evaluation, including determination of analytical sensitivity and specificity. Application of multiplexing to TAC increased the number of potential individual test results from 48 to as many as 144 per specimen while also allowing for multiple replicates to maximize detection in clinical specimens that may contain very low amounts of targeted nucleic acid. When possible, previously characterized clinical specimens were used to evaluate assay performance. Optimization of oligonucleotide sequences and modifications and multiplex assay combinations based on preliminary findings from CHAMPS sites is ongoing. Adaptation of innovative pathogen detection methods is planned to evolve over the duration of CHAMPS surveillance to increase sensitivity and specificity in assigning infectious causes of child death.

## 1514

### STRENGTHENING IMPLEMENTATION OF ROUTINE IMMUNIZATION PROGRAMS: PERSPECTIVES OF PRIMARY HEALTHCARE FACILITY MANAGERS IN WESTERN KENYA

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Vaccine-preventable diseases, including diarrhea and pneumonia, cause a high burden globally. Despite development of several strategies to increase vaccine coverage, current coverage is not optimal in many developing countries. Routine immunization programs rely on primary healthcare systems and primary healthcare facility managers (PHFM) constitute a critical link in the impact pathway of immunization programs in developing countries. Yet, few studies elucidate their perspectives on intervention targets for improving vaccine delivery in primary healthcare facilities. We explored perspectives of PHFMs on how human resource factors might influence delivery of immunization programs. We conducted in-depth interviews with 14 purposively selected key informants in Kakamega County, Kenya. Interviews were transcribed, coded and explored for salient themes. Emergent themes from the interviews were used to guide questionnaire development for a cross sectional survey with 94 PHFMs. Descriptive analyses of survey data were carried out. Most PHFMs were female (72%) and registered nurses (77%). Most frequently reported consequences of high workload were reduced accuracy of vaccination records (47%) and poor client counseling (47%). Hiring more clinical staff was identified as an effective remedy to high workload (69%). Few respondents (20%) felt highly motivated to ensure full immunization coverage in their communities. Increasing frequency of immunization-relevant supervisory visits and acting on the feedback received during those visits were most frequently perceived as important measures to improve effectiveness of immunization programs. PHFMs can provide insight into contextually relevant intervention targets to improve immunization programs. Besides increasing clinical staff, acting on feedback garnered from more frequent immunization-relevant supervision visits was perceived as a measure to increase effectiveness of immunization programs. Future studies should compare impact of developing coverage interventions based on PHFM perspectives to current intervention development approaches.

## 1515

### CLIENT SATISFACTION WITH COMMUNITY CASE MANAGEMENT OF UNCOMPLICATED MALARIA IN BUNGOMA COUNTY, KENYA

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Owing to the burden of malaria in Kenya, community case management of Malaria (CCMM) has been adopted to overcome barriers to prompt access to Malaria treatment as recommended by World Health Organisation. This initiative is part of the contributions to achieving malaria eradication. Community members' feedback is essential in evaluating the process as implemented by Amref Health Africa. This study therefore sought to evaluate the extent to which clients were satisfied with Community Case Management of Malaria. A cross-sectional study was conducted whereby a client satisfaction tool was administered to 381 clients offered CCMM services at household level. All suspected malaria tested by Community Health Volunteers (CHVs) were asked to consent to participate in the assessment. The inclusion criteria included individual or child must have been sick or presented with a new health problem or does not require urgent referral. Parameters used to measure satisfaction were availability of CHVs, convenience of getting CCMM service and promptness to respond to a call by CHVs. Data was analysed using descriptive statistics. Average age of the respondents was 40

years, 81% were female and majority of respondents practised farming (61%). Majority (93%, 94% and 91%) of the clients were satisfied with availability (obtainability/readiness), convenience (suitability/ease) and promptness (timeliness/punctuality) to respond to a call by CHVs. They further felt that the time taken to conduct the test, explanations given on treatment and friendliness during CCMM was good (94%, 90%, and 95% respectively). Most (98%) of the clients considered CHVs a regular source of basic healthcare on Malaria. Health education received was highly perceived to be helpful (93%). The community was satisfied with CCMM due to accessibility to diagnosis and treatment of uncomplicated malaria in relation to convenience, promptness and additional health education services received.

## 1516

### EFFECTS OF DEWORMING ON MATERNAL AND CHILD HEALTH: A LITERATURE REVIEW AND META-ANALYSIS FOR THE LIVES SAVED TOOL

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Soil-transmitted helminth infections affect an estimated 1.3 billion people worldwide. The World Health Organization recommends mass drug administration as the primary method of control for soil-transmitted helminth due to the expense of helminth diagnosis and low cost of deworming drugs. In 2014, over 400 million preschool-aged and school-aged children were targeted for deworming globally. The Lives Saved Tool is a software package that uses a linear deterministic mathematical model to estimate the effect of scaling up interventions on maternal and child health outcomes. This review and meta-analysis investigates the scope of available evidence for benefits of deworming treatments to assess the advisability of including deworming in the Lives Saved Tool. We searched for studies in PubMed, the Cochrane Library, Google Scholar, and published reviews. We included studies that reported pre/post data in children younger than five years, or pregnant women for outcomes related to mortality and growth. Studies that did not include a non-antihelminthic comparison group, or did not report post-intervention outcomes were excluded. We categorized articles by treated population, experimental versus observational studies, mass drug administration versus treatment, multiple versus single dose, length of follow up, and reported outcomes. Disagreements exist about the appropriate effect estimates to include in deworming meta-analyses. To address this, we conducted sensitivity analyses regarding the impact of different combinations of effect estimates. We identified 27 trials that investigated children younger than five years and 11 that investigated pregnant women. We conducted meta-analyses of relevant outcomes in children younger than five years. Our analyses suggest that deworming does not show consistent benefits at the population level for indicators of mortality, anemia, or growth in children younger than five or women of reproductive age. Although benefits of deworming may exist, we do not recommend including the effect of deworming in the Lives Saved Tool.

## 1517

### AN INNOVATION IN PRIMARY HEALTH CARE: A STEP TOWARDS UNIVERSAL HEALTH COVERAGE

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A UN resolution passed in 2012, endorsed universal health coverage as a pillar for sustainable development and global security. The World Health Organization's Alma Ata declaration in 1978, identified primary health care as key in delivering equitable health for all. Decades of research have also shown that investments to strengthen primary health care (PHC) are vital in achieving universal health coverage, lower inequality in access to services and improve health of populations. Philips through collaboration with local government has set up the Community Life Centre (CLC)

intervention as a PHC solution, intended for low-resource settings, which aims to address some of the challenges confronting health and health systems in developing markets. The CLC is an integrated primary health care solution aimed at providing sustainable improvement in health and well-being for entire communities across the health care continuum. The CLC Project started in 2014 as a community hub offering primary health care with the main aim of improving access to services, improving quality and efficiency of primary health services in low resource settings and enhancing connected care to the community and referrals to higher facilities. Through a co-creation approach between the county, local community and Philips, the first CLC was developed and deployed in Githurai, Kiambu County, Kenya. The intervention was deployed through a modular approach that saw improvements in infrastructure, staff capacity building, innovations in energy solutions, monitoring and evaluation and community outreach. Since deployment, utilization of services in outpatient department has increased by 30%; child welfare clinic by 250% and antenatal services by over 600%. In addition, increased involvement of community health workers has seen improvement of community-referrals and early identification of diseases, including non-communicable diseases. Based on the learnings from Githurai, other CLCs are in the process of development and implementation in Kenya, DRC and Zimbabwe.

## 1518

### IMPROVING QUALITY OF CARE AND PERCEIVED CLIENT SATISFACTION WITH PERFORMANCE-BASED FINANCING IN LESOTHO

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The Lesotho Ministry of Health initiated a World Bank funded Maternal and Newborn Health Performance-Based Financing (PBF) project in 2014, later re-structured as Health Sector Performance Enhancement Project. The project is operational in 6 of the 10 districts of Lesotho and aims to improve both the quantity of services in Health Centers (HC) and the quality of care in HC and District Hospitals (DH). The project incentivizes health workers by providing individual monetary incentives and promotes operational autonomy of their Health Facilities (HF) by making direct performance-based payments. The project's impact on quantity of services (measured through 14 indicators) has shown mixed results, although certain indicators have clearly improved; for instance, the percentage of pregnant women delivering in PBF participating HF rose from 53% to 74% between December 2009 and December 2016. Moreover, one of the highlights of the project has been an increase in the quality of care provided by the HF. This is measured by quarterly assessments using specifically designed quality checklists, an innovative approach to trace patients and conducting quarterly satisfaction surveys in the communities. The methodology also includes analysis of reports from exchange workshops and field visits to assess the motivation of health workers. Overall, HF are able to improve quality of care by implementing PBF concepts such as: contracting-in with relevant government authorities, improving record keeping and reporting, developing quarterly business plans to strategies on which indicators to improve, involving the community in decision making while rewarding client satisfaction. In addition, the staff motivation methods resulted in improved team work and workers motivation. In the coming two years of project implementation, we aim to develop a clearer understanding as to what extent the increase individual incentives and increased autonomy of the facilities has positively impacted on the quality of care and the perceived client satisfaction.

## 1519

### AVAILABILITY OF TREATMENTS IN MANAGING DIARRHEA, PNEUMONIA IN CHILDREN IN KENYA

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Kenya has made significant progress in reducing child mortality over the last decade. Child mortality declined from 74 to 52 deaths per 1000 live births between 2009 and 2014. Among children under 5 (CU5), major causes of death are diarrhea (21%), pneumonia (16%) and malaria (11%). The Kenya policy on management of diarrhea in CU5 recommends the use of zinc and oral rehydration salts (ORS) for the treatment of diarrhea. Studies show that when ORS is given to a sick child with diarrhea it can reduce mortality by up to 93% and Zinc is effective in the reduction of mortality by diarrhea of up to 23%. The 2014 KDHS survey shows that only 8% of children with diarrhea were treated with both ORS and zinc with 66% of CU5 having acute respiratory infections. A National outlet survey was carried out in June-August, 2016 among 84 clusters targeting public and private sector. Representative samples of locations was selected in each research domain using one-stage cluster sampling. 84 census locations were selected where a sample of 2,272 outlets completed interview amongst eligible outlets. In the findings, availability of any ORS in public health facilities dropped from 80.1% (2014) to 76 % (2016) and in private health facilities from 7% to 4%. A similar downward trend on availability of ORS and Zinc packaged differently across the sectors was observed. ORS and zinc stock was found in 70% of public health facilities in 2016 and stocked by 80% of registered pharmacies. Availability of QA ACT, ORS, zinc, and Amoxicillin DT was quite low or non-existent across all public- and private-sector outlets in 2016. However the availability of any antibiotic in Public sector was 92.8% and 6.8% private sector. Proven interventions exist to mitigate deaths caused by diarrhea and pneumonia including antibiotics for pneumonia and ORS/Zn for diarrhea. In conclusion, there is need for commodity and allocation of adequate resources, political will and focused action for ending preventable childhood deaths. For new interventions like Amoxyl DT, public private partnerships need to be embraced towards growing a market for adequate product market traction.

## 1520

### SPATIAL ASSOCIATIONS OF LEPROSY AND SCHISTOSOMIASIS AND POTENTIAL EFFECTS OF THE CO-ENDEMIC HELMINTH ON THE TRANSMISSION OF LEPROSY IN THE MICROREGION OF GOVERNADOR VALADARES, BRAZIL

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Brazil has the second highest prevalence of leprosy (Hansen's Disease, HD), but factors contributing to transmission remain unclear. Pilot data suggest a potential spatial association between HD and schistosomiasis in Brazil. Studies have also shown a predisposition to the more infectious multibacillary leprosy (MB) in those co-infected with helminths. An ecological study using public health surveillance and census data was conducted to investigate whether the occurrence of HD-and specifically MB disease-is related to the presence of schistosomiasis in the community in 41 municipalities of the state of Minas Gerais, Brazil, 2011 to 2015. Multivariate logistic regression analysis and spatial cluster analyses using geographic information systems (GIS) were performed. The average annual incidence was high for HD at 35.3 per 100,000. *Schistosoma mansoni* average annual incidence was 26 per 100,000. Bivariate local indicator of spatial autocorrelation (LISA) analysis identified 15 high-high clusters

of HD and schistosomiasis and 11 of MB and schistosomiasis. However, there was no overlap between significant most likely clusters of HD or MB disease with schistosomiasis in Kulldorff's spatial scan. In census level multivariate analysis, the risk of MB presence was over 1.5 time greater in tracts with reported schistosomiasis than in tracts without it reported, adjusted for population density, household density, and household income (aOR=1.66, 95% CI 1.01, 2.71). This study provides a novel means to study HD transmission using GIS to analyze co-occurrence of schistosomiasis which may affect HD transmission in an area with clusters of hyperendemic HD. LISA clusters provide a depiction of areas of high risk for co-occurrence. Furthermore, census tract multivariate analysis show association of schistosomiasis with MB disease warrant more detailed analysis through co-infection studies. These findings not only suggest that helminth infections can influence HD transmission, they can guide integrated control programs in co-endemic areas to decrease the burden of these infections.

## 1521

### EVALUATING THE EFFECTIVENESS OF A VILLAGE GOVERNANCE MODEL FOR IMPROVING NEGLECTED TROPICAL DISEASE (NTD) AND WATER, SANITATION AND HYGIENE (WASH) RELATED OUTCOMES IN PWANI REGION, TANZANIA

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While the importance of improved Water, Sanitation, and Hygiene (WASH) for Neglected Tropical Disease (NTD) control is well-established, the WASH and NTD sectors currently operate independently. Additionally, the governance of NTD control programs has been largely top-down in nature, with relatively little focus at the village-level. We created and implemented a governance structure, called the Enhanced Development Governance (EDG) model, that facilitates multi-sectoral coordination at the village level in an attempt to improve performance, reach, and outcomes of both the NTD and WASH sectors. In this study, we quantitatively evaluate whether the EDG model was associated with improved health and WASH-related outcomes. We conducted a case-control study in Tanzania whereby the six-month intervention was implemented in four villages of Rufiji district while four villages in Mkuranga district served as the control. Questionnaire surveys were conducted among schoolchildren and parents before and after the intervention to assess disease and WASH-related outcomes. To analyze the effect of the EDG model on the change in outcomes between the intervention and control districts, we utilized logistic generalized estimating equations regression models. Our results show that the intervention was associated with a significantly larger reduction in schistosomiasis prevalence compared to the control district (ROR = 0.67, 95% CI= 0.48 – 0.93). Incidence of diarrheal diseases was reduced in both districts, resulting in an insignificant intervention effect. Several key WASH indicators experienced significantly greater improvements in the intervention district, including reported handwashing and soap utilization. We conclude that the intervention was associated with several favorable health and WASH-related outcomes; however, additional research is needed to understand the exact mechanism by which these changes occurred and the reasons for outcome changes in control villages. These results suggest that the EDG model has the potential to improve both NTD and WASH-related outcomes, warranting further study.

## 1522

### EVALUATION OF SURGICAL INSTRUMENT DISINFECTION SYSTEMS AT THE EMERGENCY DEPARTMENT OF HIGHER LEVEL HOSPITALS IN SANTO DOMINGO, DOMINICAN REPUBLIC

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The evaluation of the surgical instrument disinfection processes is a key element for the elimination of any microbial form and to avoid nosocomial infections. The aims of this study was to evaluate the application of home-based methods of sterilization across five hospitals that offer attention at the emergency department for wounds and other injuries. An observational, descriptive, cross-sectional study was performed before and after disinfection of reusable devices at the emergency department of higher level hospitals in Santo Domingo, the capital city of Dominican Republic. A total of sixty samples were collected. Sterile transport sampling swabs were used to be cultured, and isolation of any microorganisms. The process was also described per each hospital. Three types of disinfection and sterilization were identified: a) hot air oven, b) 3% glutaraldehyde submersion, in hospital A and B respectively, and c) autoclave in C, D, and E, all after mechanical disinfection. Direct observational evaluation showed that 6 out of 36 (16.7%) sterilized instruments showed residues, and in 17 (47.2%) it was impossible to determine the presence of residues because of rust, while 7 out of 60 (11.7%) instruments showed bacterial growth, all of them before disinfection procedures. In 3 (42.9%) of them moderate colonies (3+) *Staphylococcus* spp were isolated; 2 (28.6%) *Staphylococcus* spp with low growth (1+); in one swab collected was isolated (14.3%) *Streptococcus* spp with abundant growth (4+) and 1 (14.3%) *E. coli* in abundant growth (4+). Although there is no standard guidelines of disinfection in use in the Dominican Republic; during the period of the study, pathogens were isolated. There is a lack of evidence-based interventions to reduce nosocomial transmission of bacteria, and this will impacted the flora of co-infections, leading to multiresistant strains, and other complications. It is recommended the creation of a standardized system of sterilization across the country, and enforcement in the application of International regulations for these departments.

## 1523

### OPTIMIZATION OF EXTRACTION PROCEDURES FOR DIVERSE CLINICAL SPECIMEN TYPES AND GLOBAL IMPLEMENTATION OF MULTIPLEX TAQMAN ARRAY CARDS FOR THE CHILD HEALTH AND MORTALITY PREVENTION SURVEILLANCE (CHAMPS) PROGRAM

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The Child Health and Mortality Prevention Surveillance (CHAMPS) Program, funded by the Bill and Melinda Gates Foundation, provides the opportunity to build local laboratory capacity and promote global health partnerships in Sub-Saharan Africa and South Asia in order to track preventable causes of mortality among children under five years of age. Global laboratory capacity building is impacted by numerous technical and logistical challenges, particularly in low-income settings. We have expanded laboratory capacity at five CHAMPS sites to identify common infectious causes of childhood deaths by implementing methods to detect diverse pathogens in multiple specimen types. We optimized and standardized specimen processing and testing protocols and transferred

the TaqMan Array Card (TAC), a multiple-pathogen detection system, to each site. We conducted field trainings at four global sites, instructing over 17 laboratorians. Specifically, we developed a more efficient method for simultaneous inactivation and lysis of parasitic, bacterial, viral, and fungal organisms in respiratory swab specimens, whole blood, cerebrospinal fluid, stool, and lung tissue for the capture of total nucleic acid. Various mechanical disruption methods were evaluated to replace expensive lysis enzymes for extracting relatively small amounts of pathogen nucleic acid from specimen types with abundant human nucleic acid (e.g. whole blood), from difficult to lyse microorganisms (e.g. gram-positive bacteria, fungi, and parasites), and from specimen types requiring extensive homogenization (e.g. lung tissue and stool). We adjusted the extraction protocol for use with various automated platforms in order to standardize procedures across field sites with different equipment and reagent resources. Together, optimizing technical aspects and conducting hands-on field site trainings for CHAMPS specimen processing has strengthened laboratory capacity. This capacity-building, along with newly forged scientific collaborations, will promote sharing of high-quality data and the ability to improve and adapt molecular detection methods in the future.

## 1524

### PROJECT TYCHO 2.0: A NEW OPEN ACCESS, GLOBAL DATA INFRASTRUCTURE FOR INFECTIOUS DISEASES TO IMPROVE RESEARCH CAPACITY AND INNOVATION THROUGH NORTH-SOUTH PARTNERSHIPS

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Project Tycho 2.0 is a new iteration of the open access Project Tycho database that was released in 2013 and now includes case counts of dengue fever for 100 countries between 1955 and 2010 at subnational and subannual resolution, obtained from the World Health Organization and country health agencies. This expansion has made Project Tycho 2.0 a new, global-scale, infrastructure for infectious disease data. In addition to new dengue data, the database now includes case counts for 45 additional notifiable conditions in the US, resulting from an extensive standardization process: we classified all diseases according to the Systematized Nomenclature of Medicine -- Clinical Terms (SNOMED-CT) and the International Classification of Diseases (ICD) version 10; we linked each disease with its causative pathogens and their NCBI Taxonomy ID numbers; we also standardized location names according to existing ISO and Global Administrative Boundary (GADM) definitions. This standardization makes Project Tycho data interoperable with other datasets and improves the reusability of these data. The impact of open access to infectious disease data is illustrated by our 3,000+ registered users, 16 peer-reviewed papers, and 235 media articles that used or mentioned Project Tycho data. We added the collection of global dengue data through partnerships between research institutes in the US and low- and middle-income countries in Asia and Latin America that involved joint research and infrastructure development. Project Tycho 2.0 data can be accessed through an online user interface, an application programming interface, an R-package, and a Github repository, to maximize the discoverability and reuse of this new data resource for global health research and innovation.

## 1525

### INVESTIGATING THE SATISFACTION OF REPUBLIC OF BENIN MINISTRY OF HEALTH FIELD STAFF PAID THROUGH THE MOBILE MONEY PLATFORM

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Benin's MoH started receiving support from the USAID funded project, ENVISION, for NTD programs in 2013. Back then, at completion of

activities, all involved MoH field staff received per diem payments through a local Credit Union, FECECAM. Due to the delay in receiving supporting documentation for payments, in 2014 the country started using another platform to pay per diems: Mobile Money (MM). MM sends money using a cellular phone subscribed to the MTN network. The objective of this study was to determine if MM is a viable and satisfactory method of payment for NTD programs. A questionnaire was designed and administered to 852 MoH field staff in different districts. Data was collected between February and March 2017. About 78% of participants reported being satisfied with the MM platform. Most of participants found the money recovery process through MM easy (90.3%), fast (82.3%), and safe (88.8%). Satisfaction varied according to education level and title. Participants who completed vocational training or university reported being more satisfied with the MM platform (respectively 94% and 86%) than those who completed high school or primary school only (73% and 80% respectively,  $p < 0.0001$ ). Issues frequently experienced with MM were: delays in reception of per diem (31%) and difficulty of withdrawing money due to technical problems such as poor MTN network coverage, slow or unstable internet connection- (13%). Besides the technical problems, the most frequently reported causes of complication during money retrieval (59%) were cash unavailability or slow service in some MM service delivery points. About 12% also reported non reception of per diem as a cause of complication for money retrieval. Despite the problems reported, almost all the participants (91%) judged that MM was the best way to pay because of its ease of use, the security it offers and other advantages in terms of services. MM proved to be a very good platform to transfer per diem payment. The availability of complete attendance lists and improved network coverage and internet quality would likely lead to an increase in payments received on time and therefore in user satisfaction.

## 1526

### SUPPORTIVE SUPERVISION FOR MALARIA CASE MANAGEMENT IN ZAMBIA - THE EFFECTS OF FOCUSED CAPACITY BUILDING ON INDICATORS OF DIAGNOSTIC AND CLINICAL PERFORMANCE

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Zambia was the first country in Africa to introduce the use of Artemether Lumefantrine as a first-line ACT and subsequently called for parasite-based diagnosis in order to curb over-prescription of ACTs due to clinical misdiagnosis of malaria. From 2008-2012 the Zambia NMCC, with support from the international community, implemented an external quality assurance (EQA) scheme to support malaria case management across a spectrum of health facilities performing laboratory-based malaria diagnostics. Supportive supervisory assessments, coupled with focused on-the-job training, were conducted on a quarterly basis to measure changes in health facility staff performance over time and to collect diagnostic and clinical data. A total of 88 health facilities (36 health centers and 52 provincial/district hospitals) with 4 repeated supervisory assessments were included in this analysis. Bivariate analysis (McNemar's test) shows significant differences from assessment 1 to assessment 4 for stock-outs of microscopy reagents/consumables (35 vs 7,  $p < 0.0000$ ) and instituting internal quality assurance measures such as use of microscopy positive controls (3 vs 17,  $p < 0.0026$ ), conducting parasite counting (6 vs 19,  $p < 0.0146$ ), and converting from the plus system to an alternative counting methodology (e.g. parasites/ $\mu$ l) (2 vs 20,  $p < 0.0001$ ). Improvements to malaria diagnostic and clinical performance (i.e. RDT use (mean(diff)=15.89%,  $p < 0.0000$ ), blood slide preparation (mean(diff)=12.90%,  $p < 0.0001$ ), blood slide staining and reading (mean(diff)=13.48%,  $p < 0.0000$ ), febrile case management (mean(diff)=8.38%,  $p < 0.0006$ ) and prescriber adherence to diagnostic test results (mean(diff)=7.18%,  $p < 0.028$ ) were also found to be significantly different from assessment 1 to assessment 4 via paired t-tests.

The EQA scheme provided periodic, cross-sectional representations of clinical and laboratory staff performance. Supportive supervision with focused capacity building can lead to improved malaria diagnostic skills and strengthened febrile case management practices leading to more appropriate treatment with ACTs.

## 1527

### TREATING MALNUTRITION IN THE COMMUNITY: A FEASIBILITY STUDY OF LOW-LITERACY COMMUNITY HEALTH WORKERS TREATING SEVERE ACUTE MALNUTRITION USING SIMPLIFIED TOOLS AND PROTOCOL IN SOUTH SUDAN

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UNICEF estimates that 270,000 children in South Sudan are currently suffering from severe acute malnutrition (SAM). Coverage surveys in Northern Bahr el Ghazal State found that roughly 60% of severely malnourished children were not enrolled in treatment programs. Long distance, rough terrain, and high opportunity costs deter caregivers from accessing essential treatment. The International Rescue Committee (IRC) assessed through a two-part process the feasibility of community-based treatment for uncomplicated SAM cases, provided by community health workers (CHW). First, innovative tools for low-literacy CHWs were developed through an iterative, human-centered design process. More than 50 CHWs and 30 nutrition staff participated in five field tests in Mali, Chad, and South Sudan. Participants practiced using prototypes of the tools, gave feedback, and revisions were further tested until a toolkit was finalized. In the second stage, the IRC evaluated in Aweil South County the CHWs' performance in treating SAM children using the finalized toolkit and the outcomes of children treated. 57 CHWs, all with no formal education, were trained and tested at an outpatient clinic and their performance scored against a checklist of critical actions. After six days of training, CHW performance score was an average 93.4% and ranged from 67.5% to 100%. 52 of the 57 CHWs (91.2%) passed at an a priori determined standard of 80%, and 28 of 57 CHWs (49.1%) had perfect scores. As of April 2017, 44 of the highest scoring CHWs were recruited to treat uncomplicated cases of SAM in their communities. Their on-the-job performance scores and the treatment outcomes of the children enrolled in the study (expected sample size of 320 children) will be available in September 2017. Current outpatient treatment programs for SAM can be taxing on families, based on distance to the clinic and weekly frequency of visits. A successful community-based treatment program can alleviate the burden of SAM worldwide.

## 1528

### DRUG COVERAGE AND FACTORS ASSOCIATED WITH MDA IN PAPUA NEW GUINEA

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Papua New Guinea (PNG) bears one of the highest burdens of Lymphatic Filariasis (LF) in the South Pacific region and experiences extensive logistical barriers to implementing repeated, high-coverage Mass Drug Administration (MDA) against LF. High drug coverage during MDA is considered a key predictor of successful LF elimination. This study investigates factors that influence population coverage in East and West Sepik provinces currently implementing MDA to improve LF elimination programs. Seven villages currently implementing MDA were randomly selected with a WHO-based cluster-survey protocol to assess MDA coverage in households. A key respondent per household answered questions relating to knowledge of lymphatic filariasis, reasons for participation and perception of the MDA program. Households were

divided into 3 groups for analysis: (i) Households who received MDA only (ii) Households who did not receive MDA (iii) Households with incomplete MDA participation. There were 506 households within the 7 villages with a total population 2271 people. Six of the 7 villages had population coverage > 70- 95%. Only one village coverage fell below the programmatic minimum of 65% (57% reported coverage). The factors that were significantly associated with having all eligible members per household receiving MDA across all villages were; Being informed by health workers (OR= 3.2, p=0.0009); Concern about acquiring LF (OR= 2.5, p=0.002); and distribution site close to homes (OR=2.1, p=0.010). The factor that decreased the probability of all eligible members to receive MDA per household was; (i) High number of people per household (OR= 0.8, p<0.0001). Some individual villages had unique factors associated with all eligible members receiving MDA per household including knowledge of LF prevention (OR= 13.6, p=0.003). The results indicate that concerns about acquiring LF and close MDA distribution site were positive factors in all 7 villages. There variation in significant factors unique to individual villages highlights the importance of delivering MDA information tailored to specific communities.

## 1529

### EVALUATION OF THE IMPACT OF THE 2014-2015 EBOLA OUTBREAK ON ACUTE FLACCID PARALYSIS SURVEILLANCE IN LIBERIA

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The Global Polio Eradication Initiative (GPEI) was launched in 1988 with a goal to eradicate poliomyelitis worldwide. Since its inception, the number of WPV cases dropped from 350,000 in 1988 to 37 in 2016. Acute flaccid paralysis (AFP) surveillance is the GPEI tool used for detecting poliovirus circulation. In 2014, the Ebola outbreak infected over 28,000 people in Guinea, Liberia and Sierra Leone. The outbreak disproportionately affected health workers who were 21-32 times more likely to be infected than the general population. This resulted in an overall reduction in the health workforce of 8% in Liberia, 7% in Sierra Leone, and 1% in Guinea. In order to quantify the effects that the outbreak had on AFP surveillance activities in Liberia, we assessed the performance of AFP surveillance before (2012-2013) and during the Ebola outbreak (2014-2015). AFP surveillance data obtained from the Expanded Program on Immunization (EPI) were analyzed to calculate five World Health Organization (WHO) AFP surveillance performance indicators: the non-polio AFP rate, notification, investigation, stool collection, and stool adequacy. Logistic and Poisson regressions were run to detect associations between changes in surveillance indicators and the Ebola time periods (before and during the outbreak). ArcGIS (Version 10.3.1) was used to visualize the overlap between number of Ebola cases reported in each county and corresponding NP AFP rates. During the Ebola outbreak, the overall national NP AFP rate dropped from 2.7 to 1.1 (RR: 0.41; 95% CI: 0.24, 0.69; P=0.001). The NPAFP rate dropped below the WHO recommended target in 10 out of 15 counties during the Ebola outbreak period. Among these 10 counties, 8 of them had high number of Ebola cases detected. Lofa County did not report any AFP cases in 2014 and 2015, while Bong, Grand Cape Mount, Margibi and Rivercess counties did not report any AFP cases in 2015. The other 4 surveillance indicators, except NPAFP rate, met the WHO target with some exceptions in counties that had high numbers of Ebola cases. These findings highlight the association in time between a decline in sensitivity of AFP surveillance and the Ebola outbreak.

## 1530

### USE OF AN UNMODIFIED OFFICE SCANNER FOR DIGITALIZATION OF CHEST X-RAY FILMS FROM TUBERCULOSIS PATIENTS

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When managing tuberculosis (TB) patients in sites where clinical records are maintained physically, the digitalization of the archived chest x-rays films provides major benefits in both efficiency and accessibility. There are several ways to digitalize chest x-rays films: using commercial medical x-ray film scanners, photo-camera or smartphone cameras coupled with light sources and special software. In all instances transmitted light is used to create the image. The reliability of radiologist's interpretations of the x-ray depends on the quality of the scanned image. In this study, we propose a method that uses an unmodified office scanner with no special software and no adhoc-light-source to obtain useful digital images from chest x-rays films from TB patients. In this method the x-ray film is placed on the scanning area of a scanner (Epson XP-100 multifunction printer + scanner), then a paper sheet (A4, white paper bond 70gr) is placed on top of the film, and the x-ray film is scanned maintaining the scanner cover open during the process. It was found that this process provides enough illuminance of 360lx on the scanning surface (measured using a digital lux meter CEM DT-1309) to obtain useful quality scans. We obtained that illuminance, simply locating the scanner on a desk under the office spotlight or close to a window during the day. We tested this procedure scanning three x-ray films with different qualities based on human perception (good, medium and medium-low contrast). The scanned digital images had different average Contrast to Noise Ratio (CNR=55.5, 33.6, 30.3). A radiologist evaluated and filled a report of 45 items (number of cavities, consolidations, etc.) for each film, and digital image (JPEG format). Cavitation items were matched perfectly for the middle and high CNR, and failed detecting one cavity in the low quality image. With this image was possible to match 90.2% of the items and with the images with highest CNC=55.5 and CNC= 33.6 was possible to match 95.6% and 97.8% respectively.

## 1531

### STIGMA AMONG BATEY RESIDENTS IN THE DOMINICAN REPUBLIC: IMPLICATIONS FOR MALARIA ELIMINATION

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Malaria elimination efforts often require engagement with groups that may already experience stigma, such as the rural poor or migrants. Consequently, elimination programs can inadvertently worsen stigma if they feed into narratives of blame. To avoid adverse social impacts and strengthen community engagement, a first step is to assess how stigma is already felt and interpreted. The island of Hispaniola, shared by Haiti

and the Dominican Republic (DR), is the last malaria-endemic island in the Caribbean. In the DR, malaria outbreaks occur along the Haitian border and in *bateyes*, agricultural areas historically reliant on migrant labor from Haiti. A legacy of stigma against migrants and their descendants exists in the DR. The goals of this study were to: 1) measure stigma among *batey* residents; 2) determine which stigmatizing experiences were most relevant across social groups; and 3) compare explanations for stigma. In 2016, a stratified cluster survey was conducted in *bateyes* across the DR. The sample population (n=780) contained three distinct social groups: Haitian-born persons (33.1%), persons of Haitian descent (29.4%), and those without Haitian descent (37.5%). Mean total stigma score was 17.3 out of maximum 55 (range 0-55). Highest scores were noted among Haitian-born persons, followed by those of Haitian-descent, and lastly by those without Haitian-descent. Factor analysis within each group revealed two main components: 1) feeling treated with less courtesy and respect, and 2) feeling humiliated. While these experiences were common to all participants, their explanations differed. Skin color or origin were more readily endorsed as reasons for stigma among persons born in Haiti or of Haitian-descent, whereas poverty or lack of education were cited by those without Haitian descent. For all groups, disease-related stigma was less important. In this setting, stigma is common but interpreted differently. To build trust and maximize engagement, elimination programs should recognize these underlying stigma processes and avoid reinforcing them.

## 1532

### FEASIBILITY OF IMPLEMENTING POINT-OF-CARE ULTRASOUND IN A MSF HOSPITAL-TEACHING LUNG ULTRASOUND TO CLINICAL OFFICERS IN SOUTH SUDAN

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Medecins Sans Frontieres has been operating a pediatric inpatient service in Aweil State Hospital, Northern Bahr al-Ghazal state in South Sudan for more than 8 years. This includes 150 inpatient beds, which expand to over 300 beds during peaks of malnutrition and malaria. This is a low-resource area, with the only x-ray services 15 minutes by car. There are over 900 admissions annually for Lower Respiratory Tract Infections (LRTIs). But the current MSF/WHO protocol is quite broad and includes disease processes that mimic LRTIs, frequently necessitating further diagnostic testing. Bedside Lung US is a novel tool available to clinicians to aid diagnosis. As Clinical Officers (COs) are responsible for the primary evaluation of most patients, we evaluated the ability to teach COs a lung algorithm at an acceptable level. We trained 6 COs to perform a protocolized Lung Ultrasound. Subsequently, each CO submitted 3 sets of 20 Lung US exams. Expert reviewers evaluated both the image acquisition and image analysis. A CO would be deemed successful if he reached 90% positive scores in any of the three sets of 20 exams. We used the Philips Lumify US machine, a tablet based portable portable ultrasound. The training was a protocol-based with an algorithm of various internationally standard Lung US findings, including A-lines, B-lines, sub-pleural consolidations, consolidations and effusions to conclude an overall impression according to the following list: Normal Lungs; Bronchiolitis or Viral Pneumonia; Consolidation/Pneumonia (Possibly Bacterial); Pulmonary Interstitial Disease; Pleural Effusion; Pneumothorax. We did not alter patient management based on the CO ultrasounds. The study is ongoing. Each CO has finished his 60 exams and the Experts are reviewing the images – expected completion is mid-May 2017. POCUS is becoming an essential diagnostic tool in resource-poor settings. This study is intended to show that with proper tools, training and protocols, Clinical Officers can

safely and effectively perform a key POCUS application. If successful, this study will set a model for future trainings of clinicians in resource-limited settings.

## 1533

### EVD OUTBREAK PREPAREDNESS OF LABORATORY AND HEALTHCARE WORKERS IN SIERRA LEONE

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The 2014 West African Ebola outbreak was unparalleled with over 14,000 cases of Ebola Virus Disease (EVD) in Sierra Leone resulting in approximately 4000 deaths before waning in 2016. According to WHO estimates, healthcare workers (HCWs), including laboratory staff, were more than 20 times more likely to become infected with EVD than the general adult population in Sierra Leone. Tabletop Exercise (TTX) events have been employed as a means of discussing and evaluating emergency scenarios, and in the past proved valuable and effective in gathering HCW information, particularly useful identifying preparation needed for future EVD events. In accordance with the National Laboratory Strategic Plan, key stakeholders including the Sierra Leone National Public Health Rapid Response Team (NPHRRT) and healthcare workers associated with the EVD response were invited. The informal TTX event was held on September 17, 2016 to facilitate candid discussions on the EVD diagnostic laboratories in Sierra Leone between 40 stakeholders as part the national EVD response (Laboratory staff, Ministry of Health and Sanitation (MOHS) Ebola Virus Disease (EVD) staff, National Rapid Response Team (NRRT), and the World Health Organization (WHO)). HCWs described their understanding of current national outbreak response capabilities in relation to themselves, MOHS, and other EVD diagnostic laboratories. At the TTX event, 75% (n=30) completed a preparedness survey which compared and contrasted laboratory capabilities with solutions addressing functional gaps in training and EVD diagnostics workflow within the EVD response system. TTX participants recognized gaps and challenges to better support EVD diagnostics efforts in the future. In addition to an acknowledgement of the local concerns and challenges to EVD diagnostics in Sierra Leone, the exercise also provided a better understanding of what the local experts consider important shortfalls in their system, thereby allowing for formal preparedness recommendations of appropriate solutions to long-term and sustainable EVD laboratory functionality.

## 1534

### ASSESSMENT OF HYDROCELECTOMY SURGICAL CAPACITY IN SUB-SAHARAN AFRICA USING THE WHO SITUATIONAL ANALYSIS TOOL

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Diagnosis of hydroceles is closely linked to lymphatic filariasis in endemic areas. Managing morbidity and disability from hydroceles is halted by poor access to essential surgical care in sub-Saharan Africa, resulting in significant psychosocial sequelae. Our aims were to evaluate hydrocelectomy capacity in sub-Saharan Africa using the WHO SAT by comparing facilities that provide hydrocelectomy to those that do not; and determine factors associated with increased hydrocelectomy delivery. Using a retrospective analysis of the WHO SAT administered between 2010 and 2013, facilities were organized by whether or not they perform

hydrocelectomy, and each group was compared to determine differences in workforce, infrastructure, and reasons for referrals if the procedure was not provided. Significance was determined using student t tests. Data was collected from 365 facilities from 25 countries in sub Saharan Africa. 231 facilities performed the procedure, the majority were district hospitals (37%) and mission hospitals (20%). 134 facilities did not perform the procedure, 75% of them were health centers and 25% district and general hospitals. Reasons for referrals were lack of equipment (16.42%), lack of skills (25.37%) lack of both (35.82%), undisclosed (22.39%). Comparing facility types, those performing hydrocelectomy had more qualified surgeons (means 4.63 vs 0.45, p<0.01) and non physician providers (means 6.62 vs 0.74, p<0.01); more qualified anesthetists (means 0.92 vs 0.08, p<0.01) and non physician providers (means 5.06 vs 0.41, p< 0.01), and more functioning operating rooms (means 2.9 vs 0.9, p< 0.01). Facilities performing hydrocelectomy had significantly more providers and functioning operating rooms. Lack of skills and equipment were the most common causes of referrals to other facilities. Efforts to improve surgical care and hydrocelectomy provision should include building a surgical workforce, including mid-level providers and improving infrastructure, including equipment and supplies.

## 1535

### THE ROLE OF PARTICIPANT TRACKERS IN THE PREVAIL EBOLA VACCINES TRIAL IN LIBERIA

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A quality clinical research has the potential to mitigate mortality and accelerate the end of outbreaks as well as contribute to the prevention of future outbreaks. The West African Ebola outbreak began in December 2013 in Guinea and went on to cause over 28,000 cases and claim over 11,000 lives. Conducting quality research in the setting of an infectious disease outbreak requires systematic process in the setting of dynamic outbreak challenges. The Liberia-US Joint Clinical Research Program (PREVAIL) was established in October 2014 to accelerate the development of Ebola vaccines and therapeutics. With the Liberian scientists identified Social Mobilization and Community Engagement (SMC) as one of those key processes necessary for a more successful conduct of a clinical research program, the PREVAIL team worked with outbreak response structures in both community and the Incident Management System (IMS) to establish a unique robust community support for a clinical research. Working with the District Community Ebola Task Force and community leaders, provided a smooth way forward in establishing a social and cultural valued SMC for the research. Having Communicators, Mobilizers and Trackers for a comprehensive message dissemination and follow-up on participants regularly, led to the study achieving a 98% follow-up retention rate.

## 1536

### EVIDENCE-BASED GUIDELINES FOR SUPPORTIVE CARE OF PATIENTS WITH EBOLA VIRUS DISEASE

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The 2013-2016 Ebola virus disease (EVD) outbreak in West Africa was associated with unprecedented challenges in the provision of care to EVD patients, including lack of pre-existing isolation and treatment facilities, patients' reluctance to present for medical care due to fear of a high risk of mortality in treatment units, lack of effective Ebola virus-specific therapy and limitations in provision of supportive medical care. Case fatality rates (CFR) in West Africa were initially greater than 70% but over time decreased with increasing clinical and health system experience that included improvements in supportive care. To inform optimal care in a future EVD outbreak, we employed the Grading of Recommendations Assessment, Development and Evaluation (GRADE) methodology to develop evidence-informed guidelines for the delivery of supportive care to patients admitted to Ebola treatment units.

1537

### GEOHEALTH: A GEOSPATIAL SURVEILLANCE AND RESPONSE SYSTEM RESOURCE FOR VECTOR-BORNE DISEASE IN THE AMERICAS

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Implementation of a geospatial surveillance and response system data resource for vector borne disease in the Americas (GeoHealth) is proposed using geographic information systems and ecological niche modeling, available global databases on climate-hydrology and NASA earth observing satellite data systems to characterize the environmental suitability and potential for spread of endemic and epizootic vector borne diseases. The initial focus is on developing prototype geospatial models for visceral leishmaniasis, an expanding endemic disease in Latin America, and geospatial models for Dengue and other *Aedes aegypti* borne arboviruses (Zika, Chikungunya), emerging arboviruses with potential for epizootic spread from Latin America and the Caribbean and establishment in North America. Use of geospatial surveillance and response system open-source data bases and models will be made available through the International Society for Geospatial Health-GnosisGIS, with training courses, to other investigators interested in mapping and modeling other vector borne diseases in the western hemisphere. GeoHealth is designed to be part of the NASA public health societal benefit area and the Global Earth Observation System of Systems (GEOSS) architecture and voluntary open source data sharing concept of the Group on Earth Observations (GEO).

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1538

### A NEW APPROACH FOR PROVIDER PERFORMANCE IMPROVEMENT WITH CLINICAL AND QUALITY ASSURANCE COMPONENTS IN MORAMANGA, MADAGASCAR

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Malaria is endemic in 90% of Madagascar. However, the entire population is considered to be at risk for the disease, with pregnant women particularly vulnerable. Madagascar adopted the IPT in pregnancy policy in 2004. The Malaria Indicator Survey in 2016 in Madagascar showed only 10% of Malagasy pregnant women receiving three doses of SP (IPT3). MCSP previously conducted health provider training in antenatal care in workshop style, with usually one provider per facility attending each training. To increase the number of providers offering standardized service according to the new WHO recommendations for IPTp, in 2016 MCSP Madagascar began implementing a new low dose/high frequency training approach, with routine supportive supervision. On-site training and supportive supervision provide the opportunity for the trainer and providers to discuss the barriers to delivery of IPTp-SP. Additionally, with the addition of a new IPTp indicator in the facility dashboard, the trainer/supervisor can monitor the indicator, and discuss plan of action with providers as needed. To date, in Moramanga District, 16 providers in six facilities have benefited from this performance improvement approach. The qualitative and quantitative study of provider performance and results from January 2017 to May 2017 shows Adequate action plans were implemented to prevent SP stock out and to commit community health workers. The increase of antenatal care utilization rate in these facilities. The IPT uptake increased. This intervention is expected to show that IPT uptake and other maternal and newborn outcomes are improving in Madagascar.

1539

### A PVDBP MONOCLONAL ANTIBODY RECOGNIZES A CONSERVED EPITOPE IN *PLASMODIUM FALCIPARUM* AND *P. CHABAUDI* ANTIGENS

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The *Plasmodium falciparum* parasite has evolved numerous adaptations to survive in its human host. A key adaptation is the cytoadherence of infected erythrocytes (IE) to host tissues, allowing the parasite to escape detection by the immune system and resulting in the significant pathologies associated with malaria. In pregnancy, *P. falciparum* expresses the protein VAR2CSA on the surface of IEs, which allows the parasite to adhere to chondroitin sulfate A (CSA) expressed on the surface of the placenta. In Africa, where *P. falciparum* is holoendemic, antibodies to VAR2CSA are acquired through successive exposure to *P. falciparum* in pregnancy and are able to protect the mother and child by blocking parasite adhesion to CSA. Recently, we discovered a novel mechanism for protection against malaria in pregnancy through exposure to a surface protein from *P. vivax* that is structurally related to VAR2CSA. We found that a monoclonal antibody (mAb) against the *P. vivax* protein, PvDBP, recognized VAR2CSA through shared DBL domains. Here, using live and fixed CS2 IEs, we show that there is partial overlap between the recognition sites of a polyclonal antibody against VAR2CSA and the PvDBP mAb. In addition, prior incubation of IE with the VAR2CSA antibody did not block recognition by the PvDBP mAb, suggesting there are epitopes, possibly cryptic, that are exclusively recognized by the mAb. We further show that cross-recognition extends beyond VAR2CSA. As

demonstrated by flow cytometry, the PvDBP mAb recognized a significant proportion of unselected 3D7 IEs. Lastly, using ELISA and western blot methods, we found that the mAb recognized a recombinant protein from the murine malaria parasite *P. chabaudi*, which we identified based on sequence homology with PvDBP. These results suggest that there is a highly conserved epitope in proteins expressed by several species in the *Plasmodium* genus, which could offer important insight into malaria immunity and for vaccine development.

## 1540

### PITTING-RELATED PERSISTENCE OF *PLASMODIUM FALCIPARUM* HRP2 IN PERIPHERAL BLOOD PREDICTS POST-ARTESUNATE DELAYED HEMOLYSIS IN SEVERE MALARIA

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Post-artesunate delayed hemolysis (PADH) occurs in 7-25% of patients treated with artemisinin derivatives and 60% of them requiring blood transfusion in severe imported malaria. This adverse event do not compromise the use of artemisinins because it remains the most effective drug against malaria. However, its evaluation is becoming an important public health problem. In severe malaria patients treated with intravenous artesunate, we reported that PADH is related to the lifesaving effect of artemisinins: The dead parasites are expelled from the host red cells by the spleen. These pitted red cells that previously contained a parasite persist in circulation and retain the parasite protein HRP2 as an imprint. Dipstick-based titration of HRP2 predicts frequent, potentially severe hemolysis induced by artemisinins. This recent finding was initially confirmed retrospectively in more than 100 patients with severe malaria in France where the highest number of imported malaria is annually recorded. Through an observational and prospective analysis in the severe malaria program surveillance, we propose a field use of this HRP2-based RDT method for the prediction of PADH. The standardization of this method with the widely available HRP2-based RDTs would help to predict this potentially severe adverse event, several days before it occurs and would significantly improve healthcare in patients treated with artemisinin derivatives.

## 1541

### *PLASMODIUM FALCIPARUM* ERYTHROCYTE MEMBRANE PROTEIN 1 (PFEMP1) VARIANT EXPRESSION PROFILES IN KENYAN CHILDREN WITH SEVERE MALARIAL ANEMIA FROM WESTERN KENYA

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Annually millions of individuals are infected with *Plasmodium falciparum*, the majority of whom reside in sub-Saharan Africa. *P. falciparum* infection in children under 5 years of age in holoendemic regions, such as western Kenya, can cause severe malarial anemia (SMA, hemoglobin [Hb] ≤ 5.0 g/dL). Although the complex host-parasite interactions that culminate in SMA are largely undefined, previous investigations suggest that variant expression of *P. falciparum* erythrocyte membrane protein 1 (PFEMP1) may influence malarial disease outcomes. PFEMP1 is expressed on the surface of

infected erythrocytes, and is important for parasite immune evasion, and erythrocyte adhesion to the microvasculature. We determined if expression levels of five PFEMP1 domains previously associated with disease outcomes, were associated with the development of SMA in Kenyan children. *P. falciparum* RNA was extracted from erythrocytes collected on their first hospital visit prior to antimalarial treatment. Domain-specific primer and probe sets were used to quantify DBLα and CIDRα subdomains (and serylRNA as an endogenous control) with the qRT-PCR ΔΔC<sub>t</sub> method. Samples were selected from a cohort (n=1,218, aged 3-36 months) and stratified into discrete 'polarized' groups of non-SMA (Hb≥5.0 g/dL, Avg: Hb, 9.98 g/dL; n=18) and SMA (Hb<5.0 g/dL, Avg: Hb, 3.78 g/dL; n=18, cases), excluding children with co-morbidities. These studies revealed that expression levels for the CIDRα1.1 domain was ~3-fold lower in the SMA group (P=0.050). Although differences in expression levels between the groups were not significant for the DBLα0, DBLα1, DBLα2/ α1.1/2/4/7, and DBLβ1.2 domains (P=0.59, P=0.73, and P=0.81, respectively), interesting patterns emerged. As expected based on previous results, DBLα0 was used as an internal control and did not differ between the groups (P=0.956). Studies are currently ongoing in a larger sample size to validate these initial findings and determine the impact of variation in the PFEMP1 on the cross-sectional and longitudinal development of SMA.

## 1542

### SEVERE DEFICIENCIES OF AMINO ACID PRECURSORS FOR L-ARGININE DE NOVO BIOSYNTHESIS IN PEDIATRIC *FALCIPARUM* MALARIA: IMPLICATIONS FOR NITRIC OXIDE INSUFFICIENCY

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Work by us and others has previously demonstrated nitric oxide (NO) insufficiency and low plasma levels of the NO synthase substrate L-arginine in severe falciparum malaria. Arginine is derived from several sources—diet, protein catabolism, and *de novo* synthesis primarily in the small intestine and kidney through the glutamine → glutamate → ornithine → citrulline → arginine pathway. Here we assessed this biosynthetic pathway in Tanzanian healthy children (HC, n=109), and in Tanzanian children with falciparum malaria [mild (uncomplicated) malaria (UM, n=61) and cerebral malaria (CM, n=45)]. All children were 6 months to 6 years of age. HC subjects had no malaria parasites and were without fever or signs of illness. Detailed criteria for categorizing HC, UM, and CM subjects were as we have noted before (J Exp Med 184:557-567, 1996). Plasma amino acids were quantified by ion-exchange chromatography with detection using spectrophotometry after reaction with ninhydrin. Wilcoxon rank-sum was used to determine statistical significance. Compared to HC children, measurements of each amino acid in the pathway was significantly lower in the UM and CM patients. Glutamine measurements (μM) were as follows (median [interquartile range]): 457 [400-508] in HC, 300 [256-365] in UM, and 257 [195-320] in SM (p<0.02 for all pairwise comparisons). Glutamate measurements showed a similar pattern: 73 [58-105] in HC, 42 [29-60] in UM, and 28 [17-65] in SM (p<0.04 for all pairwise comparisons). Ornithine, citrulline and arginine levels were decreased in children with malaria (all pairwise comparisons of HC vs. UM and HC vs. CM p<0.001; UM vs. CM p>0.05). Relative to pediatric normal reference range for glutamine with a lower limit of 410 μM, glutamine was markedly decreased in children with malaria. These findings suggest that low precursor amino acids, most notably glutamine, contribute to low arginine and NO insufficiency in severe malaria. The underlying mechanism(s) of glutamine deficiency in malaria warrants further study. Adjunctive therapy to replenish glutamine levels may improve NO bioavailability in severe malaria, and improve outcomes.

## 1543

**DIFFERING RECEPTOR EXPRESSION IN BRAIN MICROVESSELS DERIVED FROM WHITE AND GRAY MATTER: IMPLICATIONS FOR CEREBRAL MALARIA**Midrelle Nandjou<sup>1</sup>, Bianca Galasso<sup>2</sup>, Monique Stins<sup>1</sup><sup>1</sup>Johns Hopkins University/School of Public Health, Baltimore, MD, United States, <sup>2</sup>Johns Hopkins University/Krieger School of Arts and Sciences, Baltimore, MD, United States

Cerebral malaria (CM) is a severe complication of malaria with mortality rates of up to 30%. It affects mostly children in sub-Saharan Africa where it causes neurological impairment. CM occurs when *Plasmodium falciparum*-infected red blood cells (Pf-IRBC) sequester into the brain microvasculature via binding of *Plasmodium falciparum*-encoded erythrocyte membrane protein-1 to intercellular adhesion molecule-1 and endothelial protein C receptor (EPCR) on the surface of brain endothelial cells. The pathogenesis of CM is still not well understood and its comprehension will be helpful in the development of appropriate neurological interventions. Sequestration of Pf-IRBC in CM is observed in both the white matter (WM) and gray matter (GM) of the brain although both regions have distinct cellular environments. Importantly, the pathology of CM in the WM is predominantly hemorrhagic, whereas this is not observed in the GM. In this study, we hypothesized that the difference in CM pathology observed in the GM versus WM of the brain is due to differences in the protein receptors expression profile of their respective vasculature. We isolated microvessels from different brain areas, including corpus callosum, deep white matter, frontal cortex white matter, and frontal cortex gray matter. These microvessels were then stained for CD31 (endothelial marker), GLUT-1 (glucose transporter type 1), EPCR and AP (alkaline phosphatase). The expression of GLUT-1, CD31, EPCR, and AP in these areas was also quantified by western blot. Overall, microvessels from different areas of the brain differed in their expression of EPCR, GLUT-1 and AP. This difference might indicate that specific vessels in various brain areas will be more susceptible to sequestration of Pf-IRBC in CM and may explain the observed differential pathologies of the GM vs WM in CM patients. Knowing that the brain microvasculature differs in the expression of some receptors/transporters will lead to a better understanding of CM pathology and may have implications for other neurological diseases involving vessel pathologies, such as multiple sclerosis and stroke.

## 1544

**BIOENERGETIC ANALYSIS REVEALS OXPHOS ACTIVITY IN THE LATE STAGE GAMETOCYTES**

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*Plasmodium falciparum* has a complicated life cycle consisting of multiple developmental stages in both the human host as well as the mosquito vector. During these developmental and host changes, the parasites are exposed to dramatic environmental shifts. Previous reports have indicated that blood stage parasites in the human host rely on glycolysis as their primary energy source, while mosquito stages fully depend on mitochondrial respiration, or oxidative phosphorylation (OXPHOS). These observations suggest that malaria parasites achieve a huge metabolic shift from human to mosquito life-cycle stages, but raise the question of when this shift occurs. Previously, we developed a robust bioenergetic assay for asexual blood stage parasites utilizing an Extracellular Flux Analyzer. It enables simultaneous investigation of mitochondrial respiration and glycolysis in a physiologically relevant microenvironment with readouts of an oxygen consumption rate (OCR) and extracellular acidification rate (ECAR). With this assay system, we have discovered strong glycolytic activity, inefficient OXPHOS, and a glucose-regulated metabolic shift in asexual blood stage parasites. In this study, we applied our assay system to tightly synchronized gametocytes to evaluate OXPHOS and glycolysis activities in each stage of gametocytogenesis. We found a strong increase of basal OCR over the course of gametocyte maturation. In addition, we

observed that oligomycin A decreased OCR in day 5 gametocytes but not in earlier stage gametocytes and asexual stage parasites. The values of oligomycin A-sensitive OCR increased and became maximal around day 12 (stage IV), suggesting that late stage gametocytes actively conduct OXPHOS. Altogether our observation reveals the bioenergetically active nature of late stage gametocytes and the significant increase of OXPHOS activity that occurs during gametocyte maturation. This additional OXPHOS activity is presumably required for successful transmission to mosquitoes where the parasite is dependent upon OXPHOS, suggesting a promising target for transmission blocking therapeutics.

## 1545

**THE PLASMODIUM FALCIPARUM 130 KDA MAURER'S CLEFT PROTEIN IS A RESIDENT MAURER'S LEFT PROTEIN PERIPHERALLY ASSOCIATED WITH THE MEMBRANES OF THE CLEFTS**Raghavendra Yadavalli<sup>1</sup>, John W. Peterson<sup>2</sup>, Judith A. Drazba<sup>2</sup>, Tobili Yvonne Sam-Yellowe<sup>1</sup><sup>1</sup>Cleveland State University, Cleveland, OH, United States, <sup>2</sup>The Cleveland Clinic, Cleveland, OH, United States

Maurer's clefts (MCs) are membranous structures formed immediately after *Plasmodium falciparum* merozoite invasion into the erythrocyte host. The virulence marker PfEMP-1 is trafficked through the MCs to the surface of the IE in the asexual blood stage. In this study, we investigated a soluble 130 kDa MC protein. Previously, Immunoelectron microscopy localized the 130 kDa protein underneath knobs and associated with the MC in the asexual blood stage. The protein colocalized with known resident MC proteins suggesting it is a resident protein of the clefts. The stage specific expression and pattern of association of the 130 kDa protein with the MC membrane is not known. In addition, the gene encoding the 130 kDa protein has not been identified. In the present study, we performed biochemical experiments to analyze the topology, solubility and trafficking of the protein from the parasite to the erythrocyte cytoplasm using brefeldin A (BFA). Protease digested bands of the 130kDa protein were also subjected to LC-MS/MS analysis followed by data base searches using Mascot and Sequest to identify the gene encoding the 130 kDa protein. Peptides corresponding predominantly to the high molecular weight rhoptry protein 2 (RhopH2; PF3D7\_0929400) were obtained. Additional peptide matches were obtained with Falcilysin (PF3D7\_1360800). The solubilization and permeabilization studies show that the 130 kDa protein is synthesized and trafficked into the erythrocyte cytoplasm as a peripheral membrane protein insensitive to BFA, and that it associates peripherally to the MCs. Immunofluorescence (IFA) microscopy shows the 130 kDa protein is a resident MC protein due to its colocalization with the MC protein REX-1. Our results also show that the 130 kDa protein is associated with the infected erythrocyte membrane. IFA colocalization of monoclonal antibodies specific to the 130 kDa MC protein with Rhop-3 specific antibodies showed independent distribution of both proteins within the infected erythrocyte suggesting that the 130 kDa protein is not localized to the rhoptries and is not a member of the high molecular weight rhoptry protein complex.

## 1546

**ROLE OF PKA SIGNALING IN ARTEMISININ INDUCED DORMANCY IN RING STAGE PLASMODIUM FALCIPARUM**

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The cell cycle control mechanisms remain largely unexplored in *Plasmodium falciparum*, but the progression through the developmental stages of erythrocytic life cycle of the parasite seems to involve an intricate interplay between the parasite's changing environment and its cellular regulation. Artemisinin induced dormancy, is a stress response that allows parasites to sustain the cytotoxic effects of the drug. Artemisinin induces dormancy in the ring stage parasite, during which the parasite is under

temporal growth arrest and resumes growth between 9 to 12 days after drug treatment. This quiescent state seems analogous to a G1 cell cycle arrest and provides a plausible explanation to recrudescence reported in field, following artemisinin monotherapy. There is an increasing evidence of involvement of cAMP signaling in the regulation of stress response pathways like gametogenesis in parasites. In this study, we investigated the implication of cAMP signaling in dormancy induction. Dormancy was induced in transgenic parasites overexpressing the regulatory subunit of PKA and their recovery rates were compared to the control strain. We observed that the percentage of surviving parasites on day 8 (after the drug treatment) for PfPKA-R overexpressing strain was 1.7 times of the control strain, resulting in faster recovery from dormancy. This phenotype was reversed by treatment of cultures with PDE inhibitor IBMX that prevents the degradation of cAMP. We conclude that, downregulation of PKA pathway might be aiding parasites' entry into dormant phase, analogous to yeast, this would allow higher percentage of parasites to evade the drug pressure. On the contrary, the constitutively active PKA pathway in IBMX treated cultures might be interfering parasites' ability to enter dormancy, thereby making them more susceptible to the drug pressure. To further investigate the role of PKA pathway in dormancy, our future focus is to test the PKA kinase activity, cAMP modulation and transcription levels of key players of PKA pathway in dormancy by RT-qPCR.

## 1547

### RESISTANCE TO A NONCOVALENT SELECTIVE PROTEASOME INHIBITOR IN *PLASMODIUM FALCIPARUM*

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The proteasome is a multi-subunit complex (26S) with a regulatory cap (19S) and a catalytic core (20S). Proteasome inhibition leads to the accumulation of unfolded or oxidatively modified proteins in the intracellular environment causing endoplasmic reticulum overload and stress and eventually cell death. Proteasome inhibitors, currently used as anti-neoplastics, have also been identified as novel and potentially potent anti-protozoal agents. We have been working with a compound that selectively targets the *Plasmodium falciparum* proteasome over the human constitutive and immune proteasomes, PKS21004. To further assess this compound and the role of the proteasome in the parasite lifecycle we set out to generate parasites that are resistant to our malaria specific inhibitor. Parasites were grown *in vitro* under continued and escalating drug pressure. Regrowth was only found in one of our test flasks. Two clonal lines were isolated and used in further studies. Repeat drug assays revealed a stable 130 fold change in EC50 to PKS21004 compared to the parental line. There was no change in EC50s to other antimalarials surveyed. Phenotypic studies revealed a potential fitness cost associated with resistance with slower growth of the resistant parasite line compared to controls. Resistant parasites were subjected to whole genome sequencing revealing a mutation in PF3D7\_0518300, the Beta 6 subunit of the proteasome core (20S). This mutation is associated with an A117D amino acid change. The identification of a mutation associated with PKS21004 resistance away from the Beta 5 active site is consistent with the noncompetitive mode of action previously identified for this compound. When compared with other eukaryotic Beta 6 sequences this alanine was found to be highly conserved suggesting a conserved function within this primarily structural, non-catalytic 20S subunit. Further studies should reveal more about the impact of this mutation on proteasome function and parasite growth.

## 1548

### 3D BRAIN MICROVESSEL MODEL FOR THE STUDY OF CEREBRAL MALARIA PATHOGENESIS

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Cerebral malaria is the most life-threatening disease complication, and still carries 15-20% mortality rates. *Plasmodium falciparum* sequestration in the brain microvasculature is considered the main pathogenic event leading to brain damage, but the lack of a suitable animal model has hampered research on cerebral malaria. Taking advantage of the recent advances in the generation of 3D microvascular platforms, we have developed an engineered 3D human brain microvessel model to study cerebral malaria pathogenesis. Primary human brain endothelial cells grow in the three dimensional collagen matrix and establish complex tight junctions, as revealed by transmission electron microscopy and ZO-1 staining. The geometry of the 3D microvessels generates a shear stress gradient within the device, and reproduces the complex microfluidics of the brain vasculature. Clonally variant *P. falciparum* lines present a distinctive binding behavior to endothelial cells that is both shear-stress and endothelial activation status dependent. While ICAM-1 binders present a strong binding in regions of medium shear stress, EPCR binders adhere maximally in regions of low shear stress. In addition, activation of the endothelial cells causes a significant increase in the cytoadhesion of ICAM-1-binding strains, while other parasite variants present similar or weaker binding to activated endothelium. Our findings with 3D human brain microvessels reveal considerable heterogeneity in parasite interaction with resting and activated endothelium and establish a novel *in vitro* model for cerebral malaria.

## 1549

### THE TOXIC TABLET: ACCIDENTAL ANTI-MALARIAL POISONINGS IN FAMILIES OF FRENCH AND U.S. VIETNAM VETERANS

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The control and prevention of malaria among French and American troops were key military objectives in colonial and post-colonial Indochina. Among the most common anti-malarials included atabrine, chloroquine, and primaquine. "Malaria discipline" provided an effective way to minimize malaria among the ranks. Its rationale was simple: If malaria cases remained in the rear, then they were of little combat-use. In American units, medics weekly distributed anti-malarials to their members, watching closely to ensure that troops had swallowed the medication. Among U.S. troops who soon would rotate state-side after completion of their 365-day tour, a simple urine test was used to ensure compliance with the weekly chloroquine regimen. Medical personnel employed the Edeson-Wilson Test to detect the presence or absence of chloroquine (the most commonly used drug among American troops) metabolites. A *negative* chloroquine test suggested that an individual had not complied with the weekly chloroquine regimen, thus disobeying a standing order that dated from 1965. A *negative* chloroquine assay could delay their return stateside indefinitely. Most troops passed the chloroquine test. As they departed for home, positive results in hand, they received a six-week supply of chloroquine to continue as directed once stateside. (Their signatures also affirmed that they were under orders to continue the medication until finished.) Soon, however, reports began to appear in the lay and professional press about accidental chloroquine poisonings—many fatal and among children—in the homes of returned veterans. (A similar phenomenon had occurred in 1950s families of French troops who had served in colonial Indochina.) The scenario (in a pre-childproof medicine-

bottle era) had become all too common: A returned vet would carelessly leave an unsecured bottle of chloroquine tablets on a kitchen table or counter. A family member--not least a small child--then might accidentally ingest them. This presentation will examine the rash of chloroquine poisonings among the children or newly-returned French and American vets veterans from the late 1950s to the mid-1970s.

## 1550

### HUMANIZED MOUSE MODELS TO BOOST ANTIMALARIAL DRUG DISCOVERY

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Malaria is caused by the erythrocytic stages of protozoa of the genus *Plasmodium* and is transmitted by infected female *Anopheles* mosquitoes. To counter this disease, animal models of malaria are essential tools particularly for drug discovery. Understanding and determining efficacy parameters using preclinical models is critical to estimate effective human doses and allow an effective progression of candidate antimalarials. The generation of SCID mice has allowed successful engraftment with human red blood cells (hRBCs). SCID mice have enabled the generation of *in vivo* *P. falciparum* blood-stage infections that can be used to improve rational drug discovery efforts. Some of the potential uses of this model are: i) Build PK/PD relationships of preclinical candidates: assessment of *in vivo* parasite clearance with simultaneously PK sampling allows determining *in vivo* critical drug concentrations producing efficacy. *In vivo* clearance and time to recrudescence can be used to determine MPC and MIC. These parameters can be used to inform clinical trials and select the most appropriated human doses. ii) Monitor *in vivo* parasite rate reduction (PRR) profiles: *ex vivo* culturing of parasites after drug treatment and determination of viable counts (*ex vivo* PRR) allows to determine killing kinetics in an *in vivo* setting and can be used to assist modeling of curative doses. iii) Selection on *in vivo* resistant mutants: to gain pre-clinical understanding of resistance issues for new chemical entities (NCE's). Propensity to select resistance in an *in vivo* environment and effects of resistance on efficacy parameters can be used to anticipate and try to prevent clinical failure. iv) Translational model for transmission blocking potential: *Anopheles stephensi* mosquitoes can directly fed on SCID mice engrafted with *P. falciparum* gametocytes and develop oocysts. This direct feeding assay can be exploited to develop an *in vivo* transmission blocking assay with translational meaning. All these uses place humanized mouse models at the forefront of antimalarial drug research as a state-of-the-art tool to boost the discovery of novel antimalarial drugs.

## 1551

### A NATIONAL MOLECULAR SURVEILLANCE PROGRAM FOR THE DETECTION OF *PLASMODIUM FALCIPARUM* MARKERS OF RESISTANCE TO ANTIMALARIAL MEDICATIONS IN HAITI

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Chloroquine (CQ) and sulfadoxine-pyrimethamine (SP) remain the 1st and 2nd line treatments for uncomplicated malaria in Haiti. Few studies have reported the presence of molecular markers for resistance in *Pf* parasites, and *in vivo* therapeutic efficacy studies (TES) have been limited. Recognizing the history of antimalarial (AM) resistance worldwide, and the challenges of implementing TES in low-endemic areas, the Ministry of Health established a surveillance program to detect molecular markers of AM resistance in Haiti. Sentinel sites were selected in each of Haiti's 10 administrative departments, and an 11th site in Grand'Anse, the

department with the highest number of reported cases. Factors for site selection included the number of malaria cases identified, observed skills of laboratory technicians conducting rapid diagnostic tests (RDTs), stock/storage conditions of RDTs, accuracy of data reporting to the national surveillance system, and motivation to participate. Sentinel site staff administered the RDTs, obtained consent, collected filter paper blood samples, and completed the surveillance forms. Associated costs included the time and supplies for the project manager, supplies for collecting blood spots, transportation, and molecular sequencing. Filter paper samples were stored on site at room temperature and retrieved quarterly for storage at the national public health laboratory. Samples from 919 RDT positive patients were collected from Mar-Dec 2016. Of these, 381 (41.5%) patients reported having taken medication since the onset of their illness prior to diagnosis; 43 (4.7%) reported taking an AM. Six patients (0.01%) reported sleeping away from home for at least 1 night in the month before diagnosis; 1 was outside of their home department. Among the 668 samples successfully sequenced for *Pfcr* to date, no markers of CQ resistance (C72S, M74I, N75E, K76T) were detected; SP analysis pending. Establishing a molecular surveillance program proved practical and feasible in a resource-limited setting, and will provide the evidence needed to make informed treatment policy decisions at the national level moving forward.

## 1552

### RANDOMIZED CLINICAL TRIAL: EFFICACY OF ARTEMISININ COMBINATION THERAPIES FOR UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN SELINGUE, MALI, 2016

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In 2016, a therapeutic efficacy study using artemether-lumefantrine (AL) and artesunate-amodiaquine (AS-AQ) for treatment of uncomplicated *Plasmodium falciparum* malaria was conducted as a randomized clinical trial in Sélingué, Mali. Four hundred and eighty patients were enrolled in this study, with 240 patients enrolled in each treatment arm (AL and ASAQ). Of the 387 completing the study at 28 days (93 of those enrolled were lost to follow-up), 3 (all of whom had received AL) were recrudescence late treatment failures (days 14, 14, and 25) using microsatellite markers. For the 309 day 0 samples successfully sequenced for the *k13* gene, only one possessed a non-synonymous mutation. This mutation was A578S, which is not associated with artemisinin resistance. For the 309 day 0 samples successfully sequenced for the *Pfmdr1* gene, 68 (27.1%) possessed a non-synonymous mutation: 6 with N86Y, 61 with Y184F, and 1 with D1246Y. For the recrudescence patients, all 3 had the NFD haplotype in the day 0 samples. In the recrudescence samples, 2 of these had the NFD and one had the NYD haplotype. In summary, we observed no *k13* mutations associated with artemisinin resistance and high efficacy of AL and ASAQ treatment in Sélingué, Mali. Continued monitoring

for molecular markers of resistance to artemisinin-based combination therapies is critical in supporting therapeutic efficacy studies and providing timely evidence-based malaria treatment policies.

## 1553

### COMPARATIVE EFFICACY OF ARTEMETHER-LUMEFANTRINE AND DIHYDROARTEMISININ-PIPERAQUINE FOR TREATMENT OF UNCOMPLICATED MALARIA IN CHILDREN IN UGANDA

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*Plasmodium falciparum* resistance to artemisinin derivatives and artemisinin partner drugs is emerging in Southeast Asia. In Uganda, artemether-lumefantrine (AL) is the first-line therapy and dihydroartemisinin-piperaquine (DP) the second line therapy for the treatment of uncomplicated malaria. Recent changes in treatment practices and evidence of shifting drug sensitivities prompted a reassessment of the relative efficacies of these regimens. This study was a randomized, single blinded, longitudinal phase IV clinical trial. A total of 600 children aged 6-59 months was randomized to receive treatment for uncomplicated malaria with AL or DP (100 per treatment arm in Arua, Mbale, and Gulu, Uganda), and followed for 42 days. The primary outcome was the risk of treatment failure unadjusted and adjusted by genotyping to distinguish recrudescence and new infection at day 42. Parasite clearance was evaluated by blood smears every 6 hours. Recruitment and follow-up have been completed at all sites and genotyping of recurrent infections to distinguish recrudescence from new infection and to characterize *Plasmodium falciparum* resistance-mediating polymorphisms is ongoing. Of 600 children enrolled, 587 (98%) completed follow-up. Preliminary results showed no early treatment failures. Time to fever clearance and parasite clearance were similar in the two treatment groups. The uncorrected 42-day risk of treatment failure was significantly higher for children treated with AL than for those treated with DP at Arua (42.0% vs 15.0 %;  $p < 0.005$ ), Mbale (38.0% vs 23.0 %;  $p < 0.005$ ) and Gulu (72.0% vs 37.0 %;  $p < 0.005$ ). Overall, fever clearance and parasite clearance were similar in the two treatment groups, but DP resulted in fewer recurrent infections. Studies to assess comparative adjusted treatment efficacy, safety, and prevalence of drug resistance-associated *P. falciparum* genetic polymorphisms are ongoing.

## 1554

### ASSESSMENT OF EFFICACY OF ARTESUNATE AMODIAQUINE IN DISTRICT OF IFANADIANA MADAGASCAR

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Since 2012, a study to monitor the therapeutic efficacy of Artemisinin Combined Therapy (ACT) recommended as first line treatment for the uncomplicated *falciparum* malaria has been conducted by National Malaria Control Program (NMCP) Madagascar. The aim is to improve the orientation of treatment policy. This was a cohort study that was conducted from March to June 2016 in Tsaratanana village, district of Ifanadiana, an endemic area of Malaria. The criteria of inclusion (WHO 2009) was all outpatients between six months to fifteen years old who consulted at the health facilities for fever  $\geq 37.5^\circ\text{C}$  or history of fever  $< 24$  hours, diagnosed for uncomplicated malaria to *Plasmodium falciparum* and parasitemia between 1 000 - 200 000 p/μl. The criteria of assessment were the rates of parasitological and clinical failure in day 3, day 7, 14, 21 and 28 of follow up by microscopy then after adjusted by PCR. The

risks of these failures were analyzed by the Kaplan Meier. The clearance of fever and parasites, as well as the incidence of side effects was analyzed during follow up. Also hemoglobin rate was calculated in day 0 and day 28. A total of 450 patients were tested for malaria and RDT positivity rate was 44.2%. For 67 patients recruited, 65 (97%) patients were followed up until day 28. Two patients was voluntary withdrawn from the study. 58 (89.2%) of patients presented fever at day 0 and none from day 3. The median parasitemia was 32 796 p/μl [Min 1 591; Max 185 103]. The clearance of parasites by microscopy was 0% from Day 3, but by PCR was 55.4 % in day 3, then 98.5% in day 7 and 0% from day 14. The improvement of anemia in day 0 compared to day 28 was observed (76.9% vs 38.5%). No side effects were noted. The results of the follow-up demonstrated the efficacy and safety of the use of as ACT (ASAQ) in Madagascar. Regular monitoring of safety and therapeutic efficacy is useful to observe the failure rate of recommended national policy treatment for *Plasmodium falciparum*.

## 1555

### SINGLE DOSE SUPERIOR PHARMACODYNAMICS OF PYRONARIDINE COMPARED TO ARTESUNATE, CHLOROQUINE AND AMODIAQUINE IN A HIGH DENSITY MURINE MALARIA-LUCIFERASE MODEL

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The majority of previous preclinical malaria drug studies have relied on drug inhibition of low parasites numbers numerically compared to untreated controls both *in vitro* and *in vivo*. In contrast, human malaria drug studies measure the decline or killing of high parasite densities near 100 million per mL. Here we compared the *in vivo* single dose pharmacodynamic properties of artesunate and 4-aminoquinolines-pyronaridine, chloroquine, and amodiaquine, in a *Plasmodium berghei*ANKA-GFP-Luciferase-based murine malaria blood stage model. In this study, pyronaridine exhibited dose-independent killing, achieving parasite reduction near 5-6 log drop at 48 hours post dosing with complete cure at 10 mg/kg compared to artesunate, which exhibited a 48 hour dose-dependent killing with a 2 log drop at the noncurative high dose of 250 mg/kg. Chloroquine (noncurative) and amodiaquine (partially curative) have nearly the same initial dose-independent killing with a lag phase of no parasite reduction between 6 and 24 hours post dosing, but a 2.5 log reduction at 48 hours. In drug treated, washed infected blood transfer experiments to a naïve mouse, chloroquine and amodiaquine showed less viable parasites at the 24 hour compared to 8 hour transfer measured by a prolonged return to measured parasitemia, despite a similar log reduction, in contrast to the correlation of parasite log reduction to viable parasites with artesunate and pyronaridine. Artesunate in combination with pyronaridine exhibited a slightly weak antagonistic effect, while the combination with chloroquine or amodiaquine, showed an additive effect. Single oral dose pyronaridine was much more potent *in vivo* than artesunate, chloroquine and amodiaquine.

## 1556

### OPPORTUNISTIC PHARMACOKINETIC DETERMINATIONS OF LUMEFANTRINE FROM DRIED BLOOD SPOTS BY LC-MS/MS FOR PHARMACOKINETIC-PHARMACODYNAMIC MODELING OF MALARIA

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Study of the clinical effects of combination therapy for malaria is aided by measuring infected persons' exposure to individual partner drugs. Existing methods for measuring lumefantrine (LF) from dried blood spots (DBS) rely on pretreatment of paper with tartaric acid to facilitate drug elution. Untreated DBS may offer some utility when pretreatment was not done but pharmacokinetic-pharmacodynamic (PKPD) analyses are retrospectively considered. We assessed various methods for LF quantitation from untreated DBS using different combinations of solvents and mixers. LC-MS/MS methods were adapted from an existing protocol for plasma. Clinical application was assessed by exploratory PKPD analyses using post-treatment day 7 PK samples, parasite clearance times estimated from serial blood smears, and recurrence of malaria following treatment in a drug efficacy trial of artemether-lumefantrine for children with uncomplicated falciparum malaria. Optimal recovery was achieved by extraction from 3 mm punches of DBS (0.5-1 cm dia) with 5% formic acid in acetone, vortex mixing, sonication, and centrifugation. The lower limit of quantitation was 100 ng/ml. The median day 7 concentration among study participants (n=71) was 145 ng/ml (IQR: 120-233 ng/ml). There were no associations between drug concentration and location of the punch (center or periphery), weight of the punch, or storage duration (range: 16-24 mo). Controlling for participant age, gender, and parasite burden, each log increase in LF day 7 concentration corresponded to a decrease of 3.3 hrs in mean parasite clearance time (95% CI: 0.3-6.3 hrs, P=0.03). A nested case-control study of time-matched participants (n=18) with and without recurrent malaria showed mean post-treatment day 7 concentrations of 174 ng/ml and 223 ng/ml, respectively, but the difference was not significant (P=0.48). A method for LF determinations from post-treatment day 7 collections of DBS on untreated filter paper stored up to 24 months yielded results comparable to those previously reported. Clinical application was tentatively demonstrated in an exploratory PKPD analysis of parasite clearance.

## 1557

### INVESTIGATING THE PRESENCE OF MUTATIONS IN THE *PFKELCH13* GENE IN CHILDREN FROM UGANDA AFRICA WITH SEVERE MALARIA AND ASYMPTOMATIC PARASITEMIA

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Artemisinin resistance was first observed in Southeast Asia (SEA) and could pose a threat to malaria treatment all over the world. Recently mutations in the propeller region of *Pfkelch13* gene have been used as a genetic marker for resistance observed in SEA. We investigated the presence of mutations in the *Pfkelch* gene in children in Kampala, Uganda with severe malaria (SM) treated with intravenous quinine, or with asymptomatic *P.falciparum* infection (AP) treated with artemether-lumefantrine. We sequenced the *Pfkelch* gene (2178bp) in 176 children with SM and 49 children with AP infection. In children with SM and AP we identified 106 (60.8%) and 27 (55.1%) parasites with mutations upstream of the *Pfkelch13* propeller region. The two most prevalent mutations were 142NN (26.1% in SM, 32.6% in AP) and K189T (16.5% in SM, 12.2% in AP). In SM, only a single infection had a mutation in the propeller region (A578S), while in AP, mutations in the propeller region included A578S (n=1) and S522C (n=1). In children with SM, parasites with 142NN insertion compared to 3D7 *Pfkelch13* parasites had lower parasite density (median [IQR], 20780 parasites/ $\mu$ l [2000-63570] vs 57100 parasites/ $\mu$ l [12300-224600], p=0.006) and lower parasite biomass (*Pf*HRP2, ng/ml, median [IQR]; 295 [113-3142] vs. 3251 [281-6612] p=0.02). Children with SM who either had 142NN or K189T mutation cleared parasites after quinine treatment faster than those with the 3D7 *Pfkelch13* genotype

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(median, interquartile range, in hours; 142NN: 24, [24-48]; K189T: 24, [24-48]; 3D7: 48, [24-72]), P<0.001 for both mutations compared to 3D7). In this cohort mutations upstream of the *Pfkelch13* propeller region were common. The *Pfkelch13* 142NN insertion in children with severe malaria was associated with decreased parasite density, decreased parasite biomass and more rapid parasite clearance with quinine. Future studies will assess the presence of *Pfcr*t and *Pfmdr* mutations in this cohort, and how these relate to the *Pfkelch13* mutations and to parasite clearance.

## 1558

### CONTINUED ABSENCE OF *PF CRT* GENE MUTATIONS WITHIN THE HAITIAN *PLASMODIUM FALCIPARUM* POPULATION

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Previous reports have shown little indication that the island nation of Haiti harbors *Plasmodium falciparum* parasites bearing genetic resistance markers to chloroquine-based therapies. This has provided cautious optimism for the national control program and partner organizations seeking to accomplish malaria elimination in the near future, as having this chemotherapy tool still available remains an asset. However, as resistance mutations to *Pfcr*t are nearly fixed in South America and other regions of the world, surveillance efforts are needed to monitor this parasite population continually for potential emergence or importation of chloroquine resistant alleles. Recently, a national drug surveillance strategy was developed for *P. falciparum* in Haiti, including 11 health facility sentinel sites strategically selected to monitor for changes in drug resistant alleles. Throughout the course of 2016, 706 persons who tested positive for *P. falciparum* malaria by histidine-rich protein 2 (HRP2) rapid diagnostic test (RDT) were enrolled from seven health facilities throughout Haiti to attempt *Pfcr*t gene sequencing and identification of any functional mutations. Of these 706 RDT positive persons, 693 (98.2%) were found to contain *P. falciparum* DNA, and the *Pfcr*t gene was successfully sequenced in 668 (94.9%). All 668 isolates were found to be the wild-type CVMNK genotype with no putative chloroquine resistance markers (C72S, M74I, N75E, or K76T) detected. These data represent the largest recent screening of the Haitian *P. falciparum* population for functional *Pfcr*t mutations, and provide further evidence of the unique genetic signature of the Haitian parasite population in the Western hemisphere. Continued absence of chloroquine resistant *Pfcr*t alleles in this population is consistent with the current policy to use chloroquine as the primary drug for treatment of uncomplicated malaria in Haiti.

## 1559

### PHENOTYPIC AND GENOTYPIC ANALYSIS OF DIHYDROARTEMISININ-RESISTANT MALARIA PARASITES *PLASMODIUM FALCIPARUM*

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Artemisinin-based combination therapies (ACTs) are the first-line drugs for treatment of malaria parasites, *Plasmodium falciparum*. Unfortunately, clinical resistance to artemisinin and its derivatives has emerged in Southeast Asia. The clinical manifestation of artemisinin resistance as delayed parasite clearance is mediated by mutations in the kelch-domain protein PfK13. To identify whether additional resistance mechanisms are involved, we selected dihydroartemisinin (DHA) resistance in the P.

falciparum Dd2 strain using a step-wise procedure with continuous DHA exposure. The resistant clones grew stably in culture medium with 50 nM of DHA. In addition, they had significant increases in IC50 to most of commonly used antimalarial drugs in an in vitro assay. Further, ring-stage survival assay and mature-stage survival assay revealed DHA resistance occurred at both of ring and trophozoites stage. The resistant parasites showed prolonged ring stage. Using whole-genome sequencing, we showed that the parasites showed increased copy number in several drug resistance genes. RNA-seq analysis was conducted to determine transcriptomic changes associated with the acquisition of DHA resistance. This study identified phenotypic and genotypic changes in vitro selected DHA-resistant *P. falciparum* and provides important information for monitoring artemisinin resistance under field settings.

## 1560

### EVALUATION OF THE USE OF PARENTERAL ARTESUNATE BY CARE PROVIDERS FOR THE MANAGEMENT OF SEVERE CASES OF MALARIA IN SENEGAL

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Since 2014 Senegal has adopted the use of parenteral artesunate as a first-line treatment in the management of severe malaria cases, in accordance with WHO recommendations. To operationalize this guideline, the Senegal NMCP in collaboration with partners, conducted an assessment of the use of this drug by care providers, prior to scaling up at the national level. Seven hospitals, including four pediatric structures, were selected in the regions of Dakar, Kolda, Tambacounda, Kaolack and Touba for this purpose. Between October 2015 and February 2016, we conducted a prospective, observational, open and multicenter study. We enrolled 508 patients who were admitted for severe malaria with diagnostic confirmation by RDT or microscopy and all of whom received parenteral artesunate upon hospitalization. Follow-up continued for 28 days after hospitalization (day 0) and assessment criteria were clinical, biological and parasitological, including time to clearance of fever and parasitemia, correction of anemia and occurrence of adverse reactions. Of the 508 patients, 394 (77%) were under 15 years of age, 468 (92%) recovered fully between day 1 and day 3, with a mean temperature drop from 38.7 °C to 37.1 °C, a mean parasitic density drop from 9737 to 413p/μL; The mean hemoglobin concentration increased from 8.06/dl on day 0 to 10.58/dl on day 28. The average length of hospitalization was 3.14 days and no major adverse events were recorded during the follow-up period. These results confirm the efficacy and good tolerance of injectable Artesunate in the treatment of severe cases of malaria and highlighted the ease of use, as reported by the care providers. This ease of use will be a critical characteristic to support scale up at the national level in all health centers and hospitals.

## 1561

### PREDICTING OPTIMAL DIHYDROARTEMISININ-PIPERAQUINE DOSING TO PREVENT MALARIA DURING PREGNANCY FOR UGANDAN WOMEN RECEIVING ANTIRETROVIRAL THERAPY

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A recent randomized trial in Tororo, Uganda compared 3 doses of dihydroartemisinin-piperazine (DP) given once a month plus daily trimethoprim-sulfamethoxazole (TS) to daily TS alone for malaria chemoprevention during pregnancy among HIV-infected women receiving efavirenz based antiretroviral therapy. Intensive pharmacokinetic (PK)

evaluation of piperazine (PQ) showed that pregnancy plus efavirenz use reduced PQ exposure by 62% compared to HIV-uninfected non-pregnant controls. The primary outcome of placental malaria was too low to allow for an assessment of comparative efficacy due to the implementation of indoor residual spraying of insecticide. The aim of this analysis was to use population PK data from the trial to determine the DP dose and frequency needed for HIV-infected women receiving efavirenz to achieve protective PQ trough concentrations. 82 women contributed PQ troughs at 28 days, and 35 contributed longitudinal PK data. Nonlinear mixed effects modeling was used. Target protective PQ trough concentrations based on prior studies were 5.5 ng/mL (95% protection for HIV-negative pregnant women), 13.9 ng/mL (99% protection for pregnant women), and 20 ng/mL (99% protection for Thai adults). Simulations of different DP dosing schemes (3 dose regimen monthly, 1 dose weekly, 2 doses weekly, and 3 doses every 2 weeks) were modeled for 1,000 individuals. Modeled population clearance of PQ was 5196 L/day (relative standard error 6%), with between subject variability of 30% (RSE 12%). Only 2% of women maintained a concentration >5.5 ng/mL for >95% of the time with monthly DP (none reached higher PQ trough targets). With 1 dose weekly DP, 30% of women achieved a PQ concentration >5.5 ng/mL for 80% of the time and 52% were above the PQ target 50% of the time. 2 doses weekly provided the highest coverage (80% of women had a PQ concentration >5.5 ng/mL for 80% of the time; 97% were above the PQ target 50% of the time). Due to rapid PQ clearance, intermittent preventive treatment during pregnancy with monthly DP may not effectively protect pregnant women receiving efavirenz against malaria. Alternative dosing strategies should be evaluated.

## 1562

### IMPACT OF DIFFERENT MALARIA CHEMOPREVENTION REGIMENS FOR PREGNANT UGANDAN WOMEN ON *PLASMODIUM FALCIPARUM* DRUG RESISTANCE-MEDIATING POLYMORPHISMS

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In a recent randomized controlled trial of intermittent preventive therapy in pregnancy among 300 women in Tororo, Uganda, dihydroartemisinin-piperazine given every 4 weeks (DP4w) or every 8 weeks (DP8w) was superior to sulfadoxine-pyrimethamine every 8 weeks (SP8w). We now report how the different regimens impacted on *Plasmodium falciparum* genetic polymorphisms associated with sensitivity to antimalarial drugs. Blood samples collected monthly from asymptomatic women were tested for *P. falciparum* using a highly sensitive loop mediated isothermal amplification (LAMP) assay. 374 samples positive by LAMP and 33 episodes of symptomatic malaria (diagnosed by blood smear) were genotyped for polymorphisms in genes encoding putative drug transporters (*pfcr1* and *pfmdr1*) and folate enzymes (*pfdfhr* and *pfhdps*). 219 samples were isolated before chemoprevention was initiated; after initiation 122 samples were from the SP8w arm, 37 the DP8w arm, and 29 the DP4w arm. Considering transporter genes, treatment with DP was associated with reduced prevalence ratios (PRs) for wild type alleles at *pfmdr1* N86Y, *pfmdr1* Y184F, and *pfcr1* K76T compared to samples collected before treatment was initiated (PRs: 0.45 95% CI:[0.32-0.63], p<0.001; 0.17 [0.06-0.46], p<0.001; and, 0.47 [0.24-0.94], p=0.03, respectively) or to samples from the SP8w arm (PRs: 0.49 [0.34-0.70], p<0.001; 0.15 [0.06-0.41], p<0.001; and, 0.50 [0.25 – 1.01], p=0.054, respectively). Treatment with DP4w was associated with a reduced prevalence of wild type *pfmdr1* N86Y compared to DP8w (PR: 0.28 [0.11-0.72], p=0.009). Increased prevalences were detected for wild type *pfmdr1* D1246Y in the DP arms

compared to the SP arm (PR: 1.20 [1.06-1.36],  $p=0.004$ ). Considering folate enzyme genes, treatment with SP was associated with increased prevalences for mutant alleles at *pfdhfr* I51N and *pfdhps* K540E compared to the DP arms (PRs: 1.26 [1.10-1.45],  $p=0.001$ ; and, 1.29 [1.07-1.55],  $p=0.009$ , respectively). These findings suggest that IPTp with SP or DP selects parasite genotypes associated with decreased sensitivity to these regimens.

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### CHARACTERIZATION OF ARTEMISININ RESISTANCE IN THREE CONTINENTS: A MULTICENTER TRIAL IN PERU, KENYA AND THAILAND IN PATIENTS WITH UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA

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Artemisinin-resistant (ART-R) *Plasmodium falciparum* poses a significant threat to global malaria elimination efforts. To examine the extent of ART-R across three continents, we conducted a prospective clinical study in Iquitos, Peru (P), Kisumu, Kenya (K), and Sangklaburi, Thailand (T) in patients treated with standard doses of artesunate (AS), followed by mefloquine (MQ) at 72 hours. The primary endpoint was comparison of microscopically determined parasite clearance half-life (PC1/2) for the 72-hour period of unopposed AS. Secondary endpoints included clinical outcome and determination of Kelch propeller gene (K13) ART-R mutations. *P. falciparum* patients aged 5-65 years were enrolled with 1000-200,000 p/μl and received directly observed AS (4 mg/kg) at 0, 24, and 48h, followed by 15 mg/kg MQ at 72 h, and 10 mg/kg MQ at 84-96 h. Microscopy was performed every 4h for the first 12h after first AS dose and every 6h until 72h or two consecutive negative smears. K13 mutations were correlated with subjects with PC1/2 ≥ 5h. In K, P, and T, respectively, 59, 45, and 47 subjects were evaluable for determination of PC1/2. In P and K all K13 genotypes were wild type (WT), thereby excluding ART-R. In T, however, 34 subjects (72%) were WT and 13 (28%) harbored K13 mutations (MT) including G449A (n=1), N537I (6), P553L (1), R561H (3), and C580Y (2). The median PC1/2 of the WT-K13-T group (3.27h [IQR 2.62, 4.20]) was longer than the P (1.91h [IQR 1.71, 2.23],  $p<.0001$ ) and K (2.35h [IQR: 1.78, 2.68],  $p<.0001$ ) sites. The T K13MT group was longer (5.50h [IQR 4.94, 6.20]) than the T K13 WT group ( $p=.015$ ) and included five subjects with PC1/2 ≥ 5h; four of these (9% of total evaluable) had WHO "resistance confirmed" mutations (R561H and C580Y). All subjects achieved 42-day cure. To our knowledge, this study is the first to assess ART-R by comparing PC1/2s in Africa, Southeast Asia and South America. In T, the finding that 9% of subjects satisfy WHO criteria establishes this area as ART-R "endemic". K13 MT ART-R appears to be confined to the T site, but PC1/2s in T WT infections are still longer than in K or P, underscoring the need to identify potential alternative mechanisms of ART-R.

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### INVESTIGATION OF MOLECULAR MARKERS OF RESISTANCE IN RECURRENT PARASITES DURING A THERAPEUTIC EFFICACY STUDY CONDUCTED BETWEEN 2013 AND 2015 IN DIORO, MALI

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Artemether-lumefantrine (AL) and artesunate-amodiaquine have been the first-line recommendations for the treatment of uncomplicated malaria in Mali since 2006. Because of the recently reported delays in parasite clearance after artemisinin combination therapy (ACT) in Southeast Asia and because malaria treatment options are limited, it is imperative to monitor the efficacy of currently utilized ACTs and the prevalence of the single nucleotide polymorphisms associated with resistance to artemisinin and its partner drugs. Because of those concerns, we recently performed a prospective therapeutic efficacy study (TES) to evaluate the efficacy of AL for uncomplicated *P. falciparum* malaria in Dioro, Mali. In this study, 229 patients were enrolled, treated on days 1-3 and followed subsequently on days 2, 3, 7, 14, 21, 28, 35 and 42. Fourteen of these 229 subjects (6%) presented with recurrent parasitemias after day 28. Two of those 14 recurrences (one on day 23, the other on day 42) were classified as recrudescences by microsatellite analysis, yielding a 99% adequate clinical and parasitological response rate. Mutations in the *P. falciparum* K13 (*PfK13*) gene and *Pfmdr-1* gene were examined in all *P. falciparum* positive samples using Sanger sequencing. Analysis of *PfK13* sequence data revealed one sample with a synonymous mutation at codon 496. No other mutations were observed within the kelch 13 propeller domain. Based on analysis of the *Pfmdr-1* gene, there was a high frequency of the N86 and D1246 wildtype alleles: 13% of samples sequenced had the 86Y mutation, one had the 1246Y mutation and 62% had the 184F mutation. One of the two recrudescence samples had the NFD haplotype (codons 86, 184 and 1246), the other had the NYD haplotype. In a setting where the efficacy of AL treatment is high and there was a high frequency of the *Pfmdr-1* N86 allele, which has been associated with lumefantrine resistance, the treatment failure rate was low (1%). Continued monitoring for clinical efficacy and molecular markers associated with treatment failure is essential to provide effective guidance for evidence-based guidelines for malaria treatment.

## BASELINE MOLECULAR DATA BEFORE SCALING-UP OF SEASONAL MALARIA CHEMOPREVENTION IN SEVEN COUNTRIES ACROSS THE SAHEL

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It is essential that national SMC programmes incorporate a drug sensitivity monitoring component, using standardised methods. Through the ACCESS-SMC project, we assessed the prevalence of molecular markers associated with resistance to sulfadoxine-pyrimethamine and amodiaquine, using standardised methods in seven countries, before the scale-up of SMC and, as part of the same project, will assess prevalence again in the same sites, after two years at scale. To measure clinical efficacy, case control studies have also been conducted and blood samples from the cases used for analysis of the prevalence of molecular markers of resistance in relation to time since SMC treatment, to understand factors associated with early breakthrough infection. Baseline surveys were conducted in January and February of 2016, in Burkina Faso, Chad, Gambia, Guinea, Mali, Niger and Nigeria, in areas that had not yet started SMC. Each survey included two age groups, children under 5 years of age, and a group of older children and adults, aged 10 to 30 years, to permit comparison before and after scale up in a group who would not be exposed to SMC drugs, for assessment of the extent of changes in the circulating parasite population. The sample size, 2200 in each age group in each country, provides adequate power to detect important changes in frequencies of the molecular markers between baseline in 2016, when the surveys are repeated in January 2018 and at future intervals. Probability sampling was used to select participants in the community; a short questionnaire was completed to record personal identifiers and the age, date of birth, gender, date of interview, residence location and other details. Finger-prick blood samples were taken onto filter paper and dried. Building on preliminary data presented at ASTMH in 2016, we will present the prevalence of asymptomatic *P. falciparum* carriage and final estimates of the prevalence of mutations at *pfcr*, *pfmdr1*, *pfdhfr* and *pfdhps* known to impact on the efficacy of AQ or SP, and analysis of frequencies of these markers in clinical cases.

## UNDERSTANDING AND OPTIMIZING OPERATIONAL SEASONAL MALARIA CHEMOPREVENTION THROUGH DATA ANALYSIS AND MODELING: THE EXAMPLE OF BURKINA FASO

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In 2015, the seasonal malaria chemoprevention (SMC) campaign in Burkina Faso aimed to significantly reduce the incidence of disease in children below 5 years of age. During the wet season, children were given a full dosage regimen of sulphadoxine-pyrimethamine plus amodiaquine at one-month intervals allowing for up to 4 rounds of treatment per child depending on coverage. At the end of the 2015 campaign, the National Malaria Control Program uncovered widespread heterogeneity in SMC

effectiveness in several intervention areas ranging from 0-50% reduction in clinical incidence compared with previous transmission seasons; these heterogeneities continued into the 2016 campaign, allowing classification of intervention areas into low- or high-performing categories. In contrast, control areas where SMC campaigns were not rolled out showed no reduction in clinical incidence, underscoring the true impact of SMC campaigns in high-performing areas and motivating optimization of SMC in low-performing areas. We combined statistical and modeling frameworks to investigate key determinants of campaign performance. Two cohorts of 500 children each from 10 villages in both low- and high-performing areas were followed up from 2015 throughout 2016, and data was collected on SMC coverage per round as well as malaria incidence per season and village. In a generalized mixed-effect regression analysis, geolocation (accessibility), distance to the nearest health facility, LLIN coverage, and health-seeking behavior were found to be significant predictors of greater reduction in the observed clinical incidence in high-performing areas (50%) as compared to low-performing areas (0-10%) given similar SMC coverage patterns. We simulated SMC campaigns in an agent-based mechanistic model calibrated to local malaria transmission, including suboptimal adherence to dosing regimens and SP/AQ resistance, and find that delivering the first two rounds of SMC treatment results in greater impact on incidence reduction than zero-round or late-round delivery.

## INVESTIGATING THE MUTATIONAL PATHWAYS TO RESISTANCE FOR CLINICALLY-RELEVANT *PLASMODIUM FALCIPARUM* DIHYDROOROTATE DEHYDROGENASE INHIBITORS

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Widespread drug resistance to frontline antimalarials is an ever-increasing threat. Strategies to prevent the emergence of resistance are urgently needed, particularly for next generation antimalarials progressing through the development pipeline. In developing these strategies, it is critical that we understand the evolutionary pathways to resistance for novel inhibitors. We have begun to characterize the progression of resistance over time with the triazolopyrimidine-based DHODH inhibitor DSM267, a close analog of the clinical candidate, DSM265. Here we present initial results from these studies, showing that progressive *in vitro* selection with DSM267 can result in parasites that are over 200-fold resistant to the compound. We also find that these selected lines are cross-resistant to diverse classes of DHODH inhibitors. Sanger sequencing of the *dhodh* locus revealed that while these highly-resistant parasite lines have mutations in *dhodh*, these mutations do not fully explain the progression of resistance. This work builds on our previous work using an *in vitro* selection approach to identify mutations conferring resistance to distinct *Plasmodium falciparum* dihydroorotate dehydrogenase (PfDHODH) inhibitors. Intriguingly, when screening both wild-type and mutant lines against a panel of inhibitors, we identified examples of compounds with increased activity against DHODH mutants relative to wild-type parental strains. We demonstrated that combinations of "mutant-type" and "wild-type" compounds effectively suppressed the emergence of resistance. We have since completed a follow-up screen of libraries from the Tres Cantos Open Lab Foundation and identified additional compounds with mutant-type or equipotent activities. Cross-resistance profiling of candidate compounds against a panel of DHODH mutants revealed compound combinations active against the full range of resistance mutations identified thus far, providing support for the feasibility of targeting resistance.

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**ELUCIDATING THE ROLE OF EIK1 IN NON-GENETIC RESISTANCE TO HALOFUGINONE**

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Aminoacyl tRNA synthetases (aaRSs) are attractive targets for chemotherapeutic intervention in malaria. aaRSs are a class of enzymes that catalyze the linkage of a tRNA with its cognate amino acid and are essential for protein translation in all species. They are also highly conserved across protozoan parasites, and thus represent an ideal target for broad-spectrum drug development. Amino acid starvation or inhibition of aaRSs leads to the accumulation of uncharged tRNA, which binds and activates the stress kinase GCN2. Activated GCN2 phosphorylates the regulatory subunit eIF2 $\alpha$  of the ribosome, which blocks general protein translation and subsequently triggers the Amino Acid Response (AAR). We have previously shown that shown that halofuginone (HFG), a potent inhibitor of the cytoplasmic prolyl tRNA synthetase, induces phosphorylation of eIF2 $\alpha$  in *P. falciparum* parasites, which is generally considered as sensitive biomarker of the AAR activation in the context of amino acid starvation. In investigating the antimalarial activity of HFG, we identified an unprecedented mechanism of drug resistance via the upregulation of free intracellular proline. We sought to understand whether the activation of the AAR is required to provide rapid drug tolerance to HFG via upregulation of proline. Here, we present our results elucidating the requirement of eIK1, the *P. falciparum* homolog of GCN2, for the halofuginone-induced resistance phenotype. It has previously been shown that amino acid starvation does not induce the phosphorylation of eIF2 $\alpha$  in eIK1<sup>-/-</sup> blood stage parasites. Using this knockout line, we investigated whether eIK1 activity is essential for induced HFG resistance. We found that HFG-treated wildtype and eIK1 knockout parasites acquired phenotypic resistance in response to drug treatment with a concomitant increase in intraparasitic proline levels. These results suggest that the modulation of proline homeostasis in response to drug treatment is independent of the AAR.

## 1569

**PHARMACODYNAMIC META-ANALYSIS OF HUMAN PLASMODIUM FALCIPARUM MONOTHERAPY DRUG TRIALS**

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A hallmark of uncomplicated and severe malaria is a high microbial load where a 5% parasitemia represents 200 million *Plasmodium* parasites per millil of blood or almost a trillion parasites for the 6 L of adult blood volume. A rapid pharmacodynamics kill rate averts death and theoretically hinders drug resistance. Here we explored the recent 4 decades of published *P. falciparum* monotherapy drug trials on more than 16,000 patients from 330 trials for which we could extract pharmacodynamic data related to parasite reduction or clearance rates. The artemisinin class represented 36%, methanol-quinolines 27%, 4-amino quinolines 20%, antifolates 10% and antibiotics 7% of the useable trials. The ratio of parasites reduced in 48 hours and the time to clear 50% of the initial parasitemia (Parasite Clearance50) were the more robust comparable metrics because either value can be used to reliably predict the other. Other metrics like parasite clearance times (90 or 100%), cure rates, lag times or number reduction have clinical significance and are widely reported but provide little insight into pharmacodynamic kill rates. The critical first 48 hour parasite reduction ratio was upward of 5,100 for artesunate, 1,100 for quinine, 3,700 for chloroquine, 840 for sulfadoxine-pyrimethamine, 100 for atovaquone-proguanil and 2 for clindamycin and

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azithromycin. Interestingly, pyronaridine had a 5,700 reduction ratio. The two antibiotics after a three day lag then had a maximum reduction ratio of 5,700 The artemisinins tended to have a smaller lag phase than the quinolines. We plan to compare the monotherapy *P. falciparum* data to drug combination pharmacodynamic data as well to murine malaria.

## 1570

**DETECTION OF PLASMODIA SPP. INFECTION BY MERIDIAN ILLUMIGENE® MALARIA COMPARED TO REFERENCE MICROSCOPY AND REAL-TIME PCR**

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Malaria is one of the leading causes of infectious diseases in returning travelers from the tropics. Diagnosis of malaria is typically performed by Giemsa-stained thick and thin peripheral blood examination. The latter method can be time-consuming, labor-intensive, and require continuous proficiency in a non-endemic area where specimen volumes may be low. Molecular testing has repeatedly demonstrated increased analytical sensitivity. The favored molecular method to date has been polymerase chain reaction (PCR) however this method requires significant capital investment and molecular specialist knowledge. Loop-mediated isothermal amplification (LAMP) is an alternative molecular method which is rapid, sensitive, and requires less capital equipment and technologist training. We conducted a retrospective study comparing a commercial LAMP kit (Meridian illumigene® Malaria) versus reference microscopy on frozen, archived blood specimens (n=139) from returning travelers suspected of having malaria reflecting all human malaria species. The LAMP assay primers are able to detect *Plasmodium falciparum*, *P. vivax*, *P. ovale*, *P. malariae*, and *P. knowlesi*. Discordant results were resolved by using a laboratory developed, ultra-sensitive real-time PCR method. Our results demonstrated that Meridian illumigene® Malaria was 100% sensitive (both M kit and MP kit) and 98.4% (M kit) and 96.7% specific (MP kit) compared to reference microscopy after resolving discrepancies with a validated real-time PCR method and repeat testing for invalid runs. The extraction method followed by run time for the Meridian illumigene® Malaria takes approximately 60 minutes (M kit) with a sample throughput of 10 specimens. No centrifugation is required. The LAMP instrument provided with the Meridian illumigene® Malaria makes an automatic call to the genus level only. Implementation of this assay may be useful in ruling out malaria infection without the need for repeat examination with faster turn-around times and labor cost savings, but does not replace the need for microscopy to confirm positives by speciation and determine parasitemia levels.

## 1571

**MALARIA AND DENGUE INFECTIONS AMONG PATIENTS ATTENDING TERTIARY CARE AND HEALTH CARE CENTERS IN AND AROUND MANGALURU, INDIA: A PROSPECTIVE STUDY**

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Malaria is one of the leading causes of acute febrile illness in India. The prevalence of dengue and malaria infections has not been investigated and very few cases have been reported. To study the association of malaria and dengue among patients with febrile illness. This is a cross-sectional study of patients with febrile illness carried out at a coastal belt of India. The study was conducted after the approval of Institutional Ethics Committee, KSHEMA. This study was carried out in a tertiary care centre and public health centres in and around Mangalore. All blood samples confirmed with either malaria or dengue were included in this study. Malaria and dengue diagnosis was done on the basis of Conventional and molecular methods. Followed by dengue was done by using dengue-specific NS1 antigen kit and real time PCR. The collected information were summarized

by the descriptive Statistics. In our study, 121 samples were confirmed with malaria by conventional and real time PCR reaction. *P. vivax* (*P.v*) was 89%, *P. falciparum* (*P.f*) 0.83% and mixed infection was 10%. 25 were positive for Dengue by NS1 antigen kit method and further confirmed by Dengue real-time polymerase chain reaction (RT-PCR). None of the samples were positive for both malaria and dengue coinfections. To compare the differences in biochemical parameters such as Total bilirubin, SGOT, SGPT and platelet count between Mixed infection and *P.v* independent sample 't' test was used. Since the p value is <0.05 for total bilirubin, there was a difference between mixed infection and infection with *P.v* and it was clinically correlated with the patient outcome. The p value for SGOT/SGPT and platelet count was >0.05, there was no difference between mixed infection and infection with *P.v* Since there was no case of co-infection with dengue and Malaria, association was not performed. In endemic areas for dengue and malaria, jaundice (comorbid patients) and spontaneous bleeding (in malaria patients) should raise the suspicion of co-infection. careful monitoring of the patient is required for reducing the complications which may result in morbidity and mortality of the patients.

## 1572

### PERFORMANCE OF STANDARD AND HIGH SENSITIVITY MALARIA RAPID DIAGNOSTIC TESTS FOR THE DETECTION OF ASYMPTOMATIC PLASMODIUM FALCIPARUM INFECTIONS

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In all malaria endemic settings and even more so in areas approaching elimination, asymptomatic malaria infections significantly contribute to the ongoing disease transmission and represent an infectious reservoir that needs to be identified and treated. Most of these asymptomatic infections are characterized by low parasitaemia and represent a diagnostic challenge for classical tests, such as light microscopy or standard rapid diagnostic tests, because of the limited analytical sensitivity of these assays. Improved RDTs with enhanced analytical sensitivity are being developed and could contribute to solve this issue. The aim of this work was to evaluate the performance of RDTs with different analytical sensitivity levels for the detection of *Plasmodium falciparum* asymptomatic infections. A large number of specimens collected from asymptomatic adult volunteers, infected or not by *P. falciparum* as determined by nested PCR, were evaluated with various RDTs based on the detection of the histidine-rich protein 2 (HRP2). The positivity and false positivity rates of the RDTs are reported and analysed taking into account the HRP2 concentrations of the specimens which were measured using a quantitative ELISA method. The use of standard malaria RDTs for active case detection in screen-and-treat campaigns has been shown to be ineffective, largely because of the limited sensitivity of this diagnostic tool. The advent of improved RDTs might overturn this assumption and enable screen-and-treat interventions to be positioned as a suitable complementary strategy in the elimination toolbox. With this study, we provide a first evaluation of the added-value of malaria RDTs with enhanced analytical sensitivity for asymptomatic infections detection and discuss the use of such test for active case detection.

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### TRACKING HEMOZOIN LEVELS IN SYMPTOMATIC PATIENTS POST TREATMENT USING MAGNETO-OPTICAL DETECTION, MOD

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Hemozoin is a by-product of malaria digestion of haemoglobin. This partially magnetic substance can be used as a biomarker for the presence of malaria in the blood of patients. Still in question is the length of time hemozoin remains detectable in the blood stream post-treatment using modern day drugs and detection methods. The lone previous examination of this question showed that chloroquine treatment eliminated hemozoin from the blood stream by Day 9 on average by microscopy. The current study utilized a novel rapid malaria detection method (Magneto-Optical Detection, MOD) which measures the levels of transmitted light passing through blood samples in the presence and absence of magnetic fields to which the hemozoin particles respond. Patients reporting to Chulaimbo Hospital in western Kenya were monitored before and after their treatment with artemisinin-based drugs by MOD as well as microscopy and RDTs to determine how long after treatment hemozoin and other markers of malaria were detectable. This information helps to define the utility of the new MOD technology by defining the window in which patients can be malaria negative but hemozoin positive.

## 1574

### LIMIT OF DETECTION OF MAGNETO-OPTICAL DETECTION, MOD, ON SAMPLES OF P. VIVAX AND P. FALCIPARUM

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Detection of the presence of malaria parasites in patient samples primarily relies upon the use of RDT cards or microscopy-based methods. Microscopy testing time is 30-60 minutes per sample and requires skilled readers. RDT testing time is 15-20 minutes, and sensitivity is lower in some species and variants. There is a need for a malaria diagnostic technique to rapidly and accurately diagnose across all species including those with HRP2 deletions. We have designed an inexpensive, rapid (one minute) malaria detection device called MOD (Magneto-Optical Detection) that indicates the presence of hemozoin, a by-product of parasite digestion of hemoglobin. Polarized light passing through a blood sample is used to diagnose malaria. When the partially magnetic malaria hemozoin is present in a blood sample, it aligns with a magnetic field decreasing the amount of polarized light able to pass through it. In this study, a dilution series of samples of *P. falciparum* and *P. vivax* strains was tested using MOD, RDT, and microscopy using PCR as the gold standard. MOD's limit of detection was shown to be under 10 parasites/uL for both species -- an improvement over microscopy and RDTs. Our long term goal is to translate this MOD technology into a robust, one-minute, \$1 per test device, which can be used in malaria-endemic regions to enable rapid malaria diagnosis at the point-of-care for all species of malaria.

## 1575

### DISTRICT-BASED SUPERVISION AND MENTORSHIP PROGRAM FOR IMPROVING THE QUALITY OF MALARIA RAPID DIAGNOSTIC TESTING IN UGANDA 2014- 2016

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Following the World Health Organization recommendation on parasite-based diagnosis for malaria, many endemic countries have scaled-up the use of Rapid Diagnostic Tests (RDTs). While RDTs have increased parasite confirmation, the quality of testing at point of care may be poor. We present findings of the effect of a locally designed supervision and mentorship program on improving the quality of RDT testing at point of care in Uganda. Between 2014 and 2016, we conducted support supervision and mentorship for a total of 4,300 health workers (nurses and laboratory assistants) that were initially trained to perform malaria RDT testing in 21 targeted districts. As the main intervention, each health worker received supervision and mentorship at least twice a year for 3 years. A checklist based on manufacturer's test procedure was used to assess health workers' competency in performing the test at quarterly interval. Re-training was provided where deficiencies in test performance were identified. At each visit the individual health worker's performance was assessed and summarized according to the steps correctly or incorrectly performed. Data was analyzed to determine differences in proportions of health workers that were able to perform the test procedures correctly before and after the intervention. Overall, 4,300 health workers received the initial malaria RDT training. The attrition rate was 18% (774) over the 3-year period of assessment. The proportion of health workers that were able to perform Malaria RDT correctly according to the manufacturer's instructions improved from 68% [CI: 66-79] in 2014 to 94% [CI: 88-96] in 2016,  $p < 0.02$ . Compared to nurses, laboratory assistants were more likely to adhere to the recommended RDT procedure [OR= 3.2, CI: 1.9-4.6]. Regardless of the type of health worker, better improvement was associated with provision of 3 or more supervision and mentorship visits [OR=4.8, CI:3.1-5.4]. Post-training supervision and mentorship improves health workers' competency in Malaria Rapid Diagnostic testing and is vital in maintaining competency levels.

## 1576

### SIMULTANEOUS DETECTION OF FOUR HUMAN MALARIA SPECIES FROM WHOLE BLOOD, GIEMSA STAINED SLIDES AND DRIED BLOOD SPOTS ON FILTER PAPER

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Malarial disease is found worldwide with important public health implications. In spite of extensive eradication efforts, there is still a high mortality rate, especially in children. Identification of malarial infections to the species level is most important in endemic regions of Africa, Asia and the Americas. The 'gold standard' in malaria diagnostics is still microscopy but this method is not sensitive at low levels of parasitemia or with mixed infections when one species predominates. Reading slides at very low parasitemia is a challenge even with highly trained and experienced microscopists and leads to false negative results. More sensitive methods are required as cases of low parasitemia occur which allows for continued transmission of the malarial parasite. Blood spots on filter paper have been

used as an alternative to obtaining blood samples from patients and are simpler to obtain, transport and process for diagnostics. We developed a multiplex real-time PCR assay that can identify the most common malarial species that infect humans. The real-time PCR assay is performed on DNA extracted from whole-blood specimens and can simultaneously detect *Plasmodium falciparum*, *P. vivax*, *P. malariae*, *P. ovale curtisi* and *P. ovale wallikeri*. The gene target is the 18S rRNA gene and the limit of detection is 23 gene copies per PCR reaction. The assay identifies mixed infections in patient samples and was shown to be 100% specific when tested against a panel of pathogens that are found in blood. This method is rapid, accurate, inexpensive and very sensitive for detection of *Plasmodium* species in patient samples. In addition to whole blood, the assay can be used with DNA extracted from dried blood scraped off of Giemsa-stained slides. This is especially useful in cases where whole blood samples are difficult to obtain and store, or when only blood smears are available. This assay was also applied on DNA extracted from filter paper using a quick and simple procedure to release malaria DNA from dried blood spots. This method has proven to be a very robust, accurate, cost effective and sensitive method for identification of malaria infections at the species level.

## 1577

### INTRODUCING MALARIA RAPID DIAGNOSTIC TESTS INTO NON-FORMAL PRIVATE SECTOR OUTLETS IN MYANMAR: PRE-POST RESULTS FROM CROSS SECTIONAL STUDIES

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The Myanmar government is committed to achieving malaria elimination by 2030. To do so, every case must be identified and appropriately treated. 50% of febrile patients in Myanmar seek treatment in the private sector, including at general stores, drug shops and mobile vendors. Non-formal providers are often the only source of care in remote communities where malaria burden is highest. In 2014 only 10% of antimalarial-stocking non-formal outlets stocked malaria rapid diagnostic tests (RDTs). Increasing access to RDTs and promoting rationale treatment have been shown to improve targeting and case management in other settings. Between August 2015 and November 2016 8,728 non-formal providers were trained on a rolling basis and provided RDTs for free. Following training providers received quarterly provider behavior change (PBC) visits. Baseline and endline mystery client surveys were conducted in August 2014 (540 sites) and November 2016 (430 sites) at randomly sampled providers to assess RDT use and medicine prescribing patterns. Mystery clients played the role of a person with fever and were accompanied by the researcher acting as a friend. The researcher completed a questionnaire on leaving the outlet. Data analysis accounted for the survey design. Between 2014 and 2016 the proportion of mystery clients tested by RDT increased from 0.9% to 10.8%. All clients tested negative for malaria. Any use of medical treatment was unchanged over time (67% in 2014, 68% in 2016) but antimalarial treatment practices changed greatly over time. The proportion of clients receiving a quality-assured ACT declined from 17% to 1% and banned oral artemisinin monotherapy use declined from 7.8% to 0.6%. In comparison, antibiotic use remained relatively stable at 21.7% in 2014 and 25.6% in 2016. While training and PBC appears to have influenced prescription practices, the uptake of RDTs has been slower. By better understanding the levers of confidence, incentives and skills in relation to RDT use, programming and PBC activities could be strengthened to accelerate RDT uptake among non-formal outlets to enhance their contribution to the national malaria elimination goal.

### MOLECULAR RE-EXAMINATION OF FALSE-NEGATIVE HISTIDINE-RICH PROTEIN 2 (HRP2)-BASED RAPID DIAGNOSTIC TESTS (RDTs) FOR MALARIA

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Previous studies in Mali have found high frequencies of false-negative HRP2-based RDTs in subjects with *Plasmodium falciparum* malaria (chills, fever, headache and other symptoms plus a positive thick smear for asexual *P. falciparum* parasites). In addition, the frequencies of false-negative RDT results were higher in the dry season (when parasite densities are low) than in the rainy season (when parasite densities are higher). The first aim of these studies was to add a nested *hrp2* PCR to the HRP2-based RDT to test for both the *hrp2* gene and its protein product; the second was to add real-time PCR for the conserved *P. falciparum* 18S rRNA gene to confirm the validity of the microscopy results and the third was to consider how these results could or should affect current treatment recommendations for symptomatic subjects with negative HRP2-based RDTs. Nested *hrp2* PCR and HRP2-based RDTs: Positive *hrp2* PCRs were more frequent with samples that had positive HRP2-based RDTs; negative *hrp2* PCRs were more frequent with samples that had negative HRP2-based RDTs (30/43 vs. 60/67,  $p < 0.001$ ). Microscopy and real-time PCR for *P. falciparum* 18S rRNA: Positive real-time PCR results for *P. falciparum* 18S rRNA were more frequent among samples with  $\geq 1,000$  asexual parasites per  $\mu\text{l}$  by microscopy than among samples with  $< 1,000$  parasites per  $\mu\text{l}$  (26/31 vs. 13/79,  $p < 0.0001$ ). These results support the treatment of symptomatic subjects with positive HRP2-based RDTs (in whom parasite counts are  $\geq 1,000$  per  $\mu\text{l}$ ). However, they do not resolve the interpretation of negative HRP2-based RDTs in subjects with  $< 1,000$  parasites per  $\mu\text{l}$  because there are more false-negative test results with the nested *hrp2* PCR, the HRP2-based RDT and the real-time PCR for the 18S rRNA gene of *P. falciparum* at parasite densities  $< 1,000$  per  $\mu\text{l}$ .

### A HIGHLY SENSITIVE MULTIPLEXED BEAD-BASED IMMUNOASSAY FOR POTENTIAL MALARIA DIAGNOSTICS

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Rapid, accurate and early detection and diagnosis of malaria infection is essential for effective disease management and treatment of the disease. Traditional methods such as rapid diagnostic tests and microscopy often lack the sensitivity and specificity needed for quick disease diagnosis and management; in addition approaches like microscopy often require expert readers to obtain accurate results. Here we present results from novel bead-based immunoassay developed on an affordable miniaturized flow cytometry platform, the Muse® Cell Analyzer. The assay allows for multiplexed, high sensitivity confirmation of malarial antigens (*Plasmodium falciparum* HRP2, *Pf* LDH and *P. Vivax* LDH) and also typing of *Falciparum* and *Vivax* infection from the same sample. In addition the platform can also provide parasitemia/parasitemia density information. Our assays demonstrate high sensitivity detection with pg/mL sensitivity in detection of the 3 key antigens as well as a wide dynamic range of detection. Results will be presented from studies performed with spiked protein standards in blood as well as cultured *P. Falciparum* strains and patient samples. In

a study performed with the Infectious Diseases Research Collaboration, UCSF, and Stanford, frozen whole blood samples were obtained from a cohort of asymptomatic pregnant women from Uganda and samples were first characterized by microscopy to determine presence of *falciparum* infection and degree of parasite density. Samples were next characterized by bead based immunoassay on the Muse® Cell Analyzer and identified as positive and negative for *falciparum* Malaria. The Muse bead-based immunoassay demonstrated great sensitivity in the detection of positive samples and could clearly identify samples with low parasitemia levels. Dilution of samples also enabled determining limit of detection for these assays. The availability of a simple, rapid and highly sensitive test for identification and typing of malaria on an affordable, easy to use platform on which other pathogens can also be identified can greatly amplify the capability for effective and timely malaria diagnostics.

### WHO IS MORE LIKELY TO PERFORM MALARIA RAPID DIAGNOSTIC TESTS IN THE NON-FORMAL SECTOR IN MYANMAR?

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Non-formal providers are a major source of care for febrile cases in remote communities in Myanmar where malaria burden is highest. However in 2015 malaria rapid diagnostic tests (RDTs) were available in only 13% of antimalarial-stocking non-formal private sector outlets. Subsequently, between August 2015 and June 2016, 5,335 non-formal providers were trained by Population Services International (PSI) to conduct RDTs, treat malaria cases, and report results. The intervention specifically targeted general retail stores (GRS), itinerant drug vendors (IDVs), and drug shops. RDTs were supplied for free. To better understand how these providers can contribute to the national goal of malaria elimination, PSI performed a statistical analysis of the outlet characteristics associated with high RDT testing performance. All outlets trained by June 2016 were included in the analysis, which used routine data on RDT testing rates and supportive supervision visits (SSVs) from July to December 2016. RDT performance was simplified to whether or not an outlet performed at least 5 RDTs per month. Bivariate (Chi square) and multivariate analysis explored the correlation between RDT performance and variables like type of outlet, number of SSVs received, and malaria endemicity of the outlet location. Compared to GRS, IDV are 5 times more likely, and drug shops are 2.3 times more likely, to test at least 5 RDTs per month. Outlets in the highest endemic townships are 1.74 times more likely to test at least 5 RDTs per month compared to those in low risk areas. Outlets that received more than 3 SSVs over 6 months were 13.9 times more likely to test at least 5 RDTs per month than outlets that received fewer. Unsurprisingly, malaria endemicity seems to be a factor in the number of RDTs performed by non-formal providers. The strong positive correlation between SSVs and RDT testing suggests that support and monitoring systems for non-formal providers can improve their performance. The factors underlying different types of providers' RDT performance merit further exploration in order to design a tailored approach for maximum impact and surveillance coverage.

### THE IMPORTANCE OF EXTERNAL QUALITY ASSESSMENT IN FOCUSING IMPLEMENTATION OF QUALITY IMPROVEMENT PROGRAMS ON MALARIA MICROSCOPY IN TANZANIAN MILITARY HEALTH FACILITIES

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Quality malaria microscopy remains the gold standard method for confirmation of malaria parasites in human blood. Innovative strategies to increase access to quality malaria microscopy are now fastened to support case management and research. To ensure quality of malaria microscopy quality control (QC) in selected military health facilities, external quality assessment (EQA) and quality improvement programs (QI) have been collaboratively implemented between Tanzania Peoples Defence Forces (TPDF) and Walter Reed Army Institute of Research (WRAIR). The key objective is to build capacity and readiness of the laboratories under the program to accurately diagnose malaria for patient care and clinical research activities. An assessment is made after all testing sites are provided with high quality equipment, reagents and supplies to perform malaria microscopy. The trend of malaria positivity in the sites is monitored routinely. EQA is conducted quarterly through site evaluations using microscopy check list, randomized slide rechecking and proficiency testing for the technical staffs working in the laboratories. For the purpose of measuring and maintaining competency, at each quarterly supportive supervision cycle every testing staff at the site is given 20 (9 negatives and 11 low density positives) standardized, expert validated blood slides to read as part of EQA. The score of individual technician have been aggregated to provide an average score of a specific site laboratory. We also compared average laboratory performance in EQA and the positivity level at the site and its implications. The results show a relationship between the level of malaria at the site and performance in EQA. The best performing laboratories are also the ones having high malaria positivity rates with few exceptions contributed to lack of training and competency of individual technicians. Additionally, false negative rate was found to be higher among study sites with low malaria whose laboratory EQA performance was also low. The routine exposure to seeing malaria may be advantageous to continued skills.

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### THE USE OF FIONET™ TECHNOLOGY IN MALARIA SURVEILLANCE AND EXTERNAL QUALITY CONTROL OF RAPID DIAGNOSTIC TESTS IN MILITARY HEALTH FACILITIES IN TANZANIA

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In Tanzania, an estimated 16-18 million cases of malaria occur each year, resulting in more than 100,000 deaths. Malaria surveillance is useful for gathering data for evaluation of control programs that are ongoing. Although a recent innovation, malaria rapid diagnostic tests (mRDT) have become a viable alternative diagnostic tool for malaria surveillance, however, external quality control (EQC) is a challenge. Walter Reed Army Institute of Research (WRAIR) has partnered with the Tanzanian Peoples Defense Forces (TPDF) to implement a passive malaria surveillance and mRDT EQC strategy using Fionet™ technology. Fionet™ is a digital health system that improves field performance and oversight of mRDT. The system captures RDT images which can be remotely reviewed for EQC, it provides operational quality control measures for the laboratory technician in real-time, it compiles the data and sends data in-real time on a centralized portal that is reviewed by Managers for immediate use. Malaria RDT data and images were monitored regularly at eight military camps. About 82% of 95,247 mRDT records were uploaded over a four year period. Nearly 33% was uploaded in < 1 hour and 80% of all data was

uploaded within 24 hours. Annual average malaria positivity rates (MPR) for all sites increased from 22.9% (2013) to 29.3% (2014) to 37.1% (2015) and then declined to 22.8% (2016). The incorrect interpretation of mRDTs by peripheral healthcare users dropped from an initial 5.5% to 3.7% in the first six months, to 2.5% in the first year and 3.1%, 3.4%, and 2.9% in subsequent years. The preparation problems dropped from 2.5% to 1% after six months and to almost 0% in subsequent years. Fionet™ has improved diagnostic capacity, real-time malaria data tracking and reporting and proved as valuable tool for mRDT EQC.

## 1583

### CROSS-SECTIONAL ANALYSIS IN YOUNG NON-PREGNANT AND PREGNANT WOMEN IN BURKINA FASO OF ASSOCIATIONS BETWEEN BIOMARKERS OF IRON STATUS AND EFFECT MODIFICATION BY INFLAMMATION AND PLASMODIUM FALCIPARUM INFECTION

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Iron deficiency is a major cause of anaemia and accurate biomarkers are needed to estimate iron status and its contribution to anaemia. No previous comparative analyses of iron biomarker correlations in non-pregnant and pregnant women or the comparative effect modification by inflammation or malaria. In young non-pregnant and pregnant women living under endemic malaria transmission estimation of iron biomarker correlations, body iron stores, iron deficiency prevalence using single and multiple biomarker definitions, and effect modification by inflammation by C reactive protein and *Plasmodium falciparum* parasitaemia. The data were derived from a randomised controlled trial of periconceptional weekly iron supplementation among young mostly adolescent nulliparous non-pregnant women and primigravidae living within the Nanoro Demographic Surveillance area, Burkina Faso. Data points were at the end assessment survey for non-pregnant women (N=973), and at the first scheduled antenatal visit for nulliparae (N=314). Plasma ferritin, serum transferrin receptor, and C-reactive protein were measured in duplicate by ELISA, zinc protoporphyrin by fluorometry, and haematological indices by automated analyser. *P.falciparum* slide positivity and CRP cut-offs were used to define inflammation categories. At ANC1 69.7% were anemic ( $\leq 11\text{g/dL}$ ), and 53.7% parasitaemic, and at FIN 46.7% were anemic and 41.7% parasitaemic. Anemia prevalence was higher in non-pregnant ( $p < 0.01$ ) and pregnant women ( $p < 0.001$ ) with malaria. Prevalence of iron deficiency (sTfR/Log10ferritin  $> 5.6$ ) did not differ in pregnant or non-pregnant women after adjustment for CRP values ( $< 5$  or  $< 10\ \mu\text{g/ml}$ ), with or without *P.falciparum* parasitemia. High ZPP ( $> 85\ \mu\text{mol/mol}$  heme) was more frequent in non-pregnant or pregnant women (both  $P < 0.01$ ) in the presence of inflammation. In pregnant or non-pregnant women living under endemic malaria transmission the sTfR/log10Ferritin ratio  $> 5.6$  was not significantly affected by inflammation or concurrent malaria infection, and may be the preferred biomarker for estimating iron deficiency prevalence in areas with high infection pressure.

## 1584

### EVALUATION OF PERFORMANCE OF DEKI READER OF MALARIA RAPID DIAGNOSTIC TEST IN RURAL MILITARY HEALTH FACILITIES IN TANZANIA

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Malaria Rapid diagnostic Tests (mRDTs) are currently advocated and used as adjunct to microscopy in malaria diagnosis. However, at very low parasitaemia (<100p/μl) the test line on mRDT is expected to be weak and consequently affecting visual interpretation of mRDT test results. Fio Corporation's Deki Reader (DR) performs automated interpretation of RDTs, including multiple brands of mRDTs. The accuracy of the DR was compared to visual interpretation by 2 expert laboratory Technicians who were blinded of DR results in two Military facilities in Tanzania. Finger prick blood samples were collected from 1,293 consecutive outpatients with fever and tested for malaria using SD Bioline mRDT and microscopy, which was the gold standard. The parasitemia geometric mean was 3,398 per microliter. The sensitivity of mRDTs interpreted by DR was 94.1% while the sensitivity of manually interpreted mRDTs was 93.5%. The specificity of mRDTs interpreted by DR was 71.8% and that of human was 72.0%. Positive Predictive Value (PPV) of mRDTs by DR and Human was 75.8% and 75.4% respectively. The Negative Predictive Value (NPV) of mRDT by DR was 92.8% and by human was 92.4%. There was no significance difference in sensitivity, specificity, PPV, NPV and accuracy of mRDT interpreted by DR compared to expert human interpretation. The performance of DR in interpreting mRDTs was found to be similar to interpretation by expert laboratory technicians. The application of the DR in remote settings will ensure accurate mRDT diagnostic are available at point-of-care.

## 1585

### DEVELOPMENT OF A MULTIPLEX ASSAY FOR SIMULTANEOUS QUANTIFICATION OF *PLASMODIUM VIVAX* AND *P. FALCIPARUM* INFECTION

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Malaria is a vector-borne disease caused by *Plasmodium* parasites that threatens many lives in endemic countries. While there has been an outstanding gain from clinical case management coupled with vector control programs, which has resulted in a significant reduction for malaria burden, low-density and asymptomatic malaria infections represent a serious challenge in malaria elimination. Sensitive detection tests are needed for identifying submicroscopic malaria infection and tracking changes in malaria transmission intensity. Relating parasitemia to antigens commonly used in rapid diagnostic tests for malaria will greatly assist the development of more sensitive tests that capture submicroscopic malaria infections. We developed a multiplex assay to simultaneously detect and quantify antigens from two parasite species, *P. vivax*-specific lactate dehydrogenase (LDH), all *Plasmodium* spp. LDH, and *P. falciparum*-specific histidine-rich protein 2 (HRP2) and the biomarker C-reactive protein (CRP) from human blood specimens. The study on interference between *P. falciparum* and *P. vivax* demonstrated that the detection for each target was not influenced by other *Plasmodium* antigens, indicating the strong robustness of this multiplex assay. Further testing of clinical samples

containing either *P. vivax* or *P. falciparum* showed that differentiating between parasites was concordant with expected results on the basis of the gold standard qRT-PCR method while detecting *Plasmodium* antigen at sub-ng/mL levels. This multiplex platform offers a robust, sensitive, and specific detection tool, through which it is possible to describe the dynamics of infection with *P. vivax* and *P. falciparum* in terms of antigenemia in near-elimination settings where two parasite infections coexist.

## 1586

### COMPARISON OF COMMERCIALY AVAILABLE MOBILE MEDICAL APPLICATIONS (MMAS) FOR INTERPRETING MALARIA RAPID DIAGNOSTIC TEST (RDT) RESULTS

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The World Health Organization (WHO) recommends diagnosis by microscopy or malaria rapid diagnostic test (RDT) in all patients with suspected malaria before treatment is administered. Recently, mobile medical applications or MMAs (popularly known as "readers") which have the capability to interpret RDT test results have entered the market. However, limited evidence is available on MMA accuracy. To evaluate MMA accuracy, a laboratory evaluation was conducted by comparing RDT result interpretation by MMAs to those read and interpreted by trained end-users. Five different MMAs were evaluated on six different RDT products from three leading manufacturers using cultured *Plasmodium falciparum* (Pf) samples at dilutions ranging from 20-1000 parasites(p)/μL and negative blood samples. The RDTs were performed in a laboratory by a trained operator who interpreted the RDT results with the human eye. A second operator blinded to the human eye result then used the MMAs to interpret the RDT results, within the RDT manufacturer specified reading time. Sensitivity (Se) and specificity (Sp) were calculated using mixed models. Overall, the human eye had a higher Se than MMAs (89% vs 42-77%). For the Pf test line of Pf-only and combination Pf/Pan-pLDH RDTs, the trained human eye had a significantly higher Se than MMAs (74% vs 47%) at 20 p/μL. However, at 200, 500 and 1000 p/μL, three MMAs had a Se that was comparable to the human eye (97%). For RDTs that detect the *Plasmodium* spp. (Pan-pLDH-only and combination Pf/pan-pLDH RDTs), the human eye had a higher Se than MMAs (79% vs 56%). Sp of the human eye and MMAs was around 99%. Analyses on correlation between RDT band intensity at various sample concentrations and MMA Se will be performed and available for presentation. Overall, the study found that the trained human eye can more accurately interpret RDT results compared to commercially available MMAs. However, MMAs perform as well as the human eye at higher sample concentrations. There is a need for further research to understand the field applicability of MMAs.

## 1587

### CAUSAL CHEMOPROPHYLACTIC ACTIVITY OF PRIMAQUINE - QUINOXALINE HYBRIDS

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There is an urgent need of new chemotherapeutic agents for malaria treatment due to parasite resistance. The development of new drugs to prevent infection and/or development of liver forms of *Plasmodium*

*spp.* is a research priority. The aim of this study is to evaluate the *in vivo* causal chemoprophylactic effect of primaquine - quinoxaline hybrids in the *Plasmodium yoelii* mouse model. Five primaquine-quinoxaline hybrids were synthesized: QX6, QX7, QX8, QX9 and QX10, their structure was identified and confirmed by spectroscopic methods. The hybrids were administered on days -1, 0, +1 and +2 to Balb/c mice and those were challenged on day 0 with intravenous injection of 5,000 sporozoites of non-lethal strain *P. yoelii* 17XNL to test for causal chemoprophylactic activity. Mice weighing 20±2 g were randomly allotted to five groups and were treated orally with 100 mg/kg of hybrids, diluted in dimethylsulfoxide (DMSO). Tail-blood samples were collected from day 4 to 14 post-inoculation and parasites were quantified using microscopic examination of Giemsa-stained smears and flow cytometry. The group of mice treated with QX6 showed a significant reduction in *Plasmodium yoelii* hepatic forms (inhibition percentage on day 4 of 95.97% with respect to the untreated group) and the parasitemia pre patent period was extended by 24 hours over controls. QX6 was less toxic than the licensed drug primaquine at a dose of 500 mg/ml (acute oral toxicity test), however it was also less active than primaquine (100% inhibition). Different therapeutic schemes and pharmacokinetic parameters should be evaluated in order to continue studies with this primaquine - quinoxaline hybrid. QX6 represents a promising source of new anti-malarial agents.

## 1588

### ACTIVITY OF THE HISTONE DEACETYLASE (HDAC) INHIBITOR AR-42 IN A MURINE MALARIA MODEL

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Histone deacetylase (HDAC) enzymes are involved in the reversible acetylation of histone and non-histone proteins, regulating chromatin structure, gene expression and other important cellular processes. HDACs are validated drug targets for some types of cancer, with some HDAC inhibitors now clinically approved. HDAC inhibitors are also of interest as chemical probes and drug leads for other diseases, including infectious diseases such as malaria. In previous work we have shown that clinical anti-cancer HDAC inhibitors have potent *in vitro* activities against *Plasmodium falciparum* parasites (IC<sub>50</sub> 10-200nM) and cause hyperacetylation of parasite histone proteins. However, these compounds generally have low selectivity for malaria parasites versus human cells and poor *in vivo* efficacy in murine malaria models. Here we show that the next generation hydroxamic acid based HDAC inhibitor AR-42 has low nM *in vitro* activity against *P. falciparum* and greater than 50-fold selectivity for human cells versus *P. falciparum*. *Ex vivo* activity of AR-42 against *P. berghei* and *P. chabaudi* infected mouse erythrocytes was confirmed and *in vivo* efficacy evaluated in a murine model of *P. berghei* infection. Twice-daily oral administration of 25 mg/kg AR-42 attenuated parasitemia in *P. berghei*-infected BALB/c mice, curing 10 of 12 mice. Further studies are currently underway to investigate the potential of analogues of this compound as drug leads for malaria.

## 1589

### GAMETOCYCIDAL AND CURATIVE LIVER AND BLOOD STAGE ANTIMALARIAL ACTIVITY OF CETHROMYCIN

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Prophylaxis of malaria in travelers to malaria endemic countries is limited to atovaquone/proguanil, doxycycline, mefloquine and rarely primaquine. We investigated the liver stage activity of cethromycin in the *P. berghei* liver stage model initiated by mosquito bites. Cethromycin was identified by a quantum-based computational approach sifting

through millions of molecules for liver stage malaria activity. Cethromycin is a molecular hybrid of a nonsubstituted quinoline nucleus joined at position 3 to an erythromycin scaffold. Here we demonstrate complete cure of *Plasmodium berghei* infection by single oral dose of 60 mg/kg in mice which is equivalent to the 5 mg/kg human dose of 300 mg a day used in bacterial pneumonia studies. Both quinoline and erythromycin alone at 120 mg/kg for two doses as well as control mice resulted in patent blood stage parasitemia in all mice. Cethromycin at 30 mg/kg was also curative as well as single oral 60 mg/kg given before mosquito infection. Immunofluorescence imaging of cethromycin treated, *in vitro* hepatocyte infected cells shows complete ablation of the apicoplast. Regarding blood stage activity, cethromycin at 60 mg/kg daily for 7 days cured in a high parasitemic *P. berghei* mouse model. While mosquito membrane feeding of *P. falciparum* gametocytes incubated with 20 microM cethromycin demonstrated no decrease in oocyst numbers, oral dosing in mice showed significant decrease in both prevalence and mean number of oocysts suggesting an active metabolite similar to primaquine. Liver pharmacokinetic studies in mice are ongoing. Cethromycin has been evaluated for efficacy against bacterial pneumonia in more than 5,000 patients with good safety profiles. Cethromycin has potential for rapid clinical development for casual malaria prophylaxis and possibly radical cure of dormant liver *P. vivax* or *P. ovale*.

## 1590

### DRUG INTERACTIVITY STUDIES TO DEFINE SYNERGISTIC ANTI-MALARIAL COMBINATORIAL REGIMES FOR EMETINE DIHYDROCHLORIDE

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The emergence and spread of artemisinin resistance to *Plasmodium falciparum* in Southeast Asia poses a serious threat to ongoing malaria control efforts. Unless new approaches are deployed rapidly, the health and economic burden related to the disease in tropical countries is certain to worsen. The development of treatments through drug repositioning may offer novel candidates permitting new combinatorial regimes with existing anti-malarials. The approach could present a much needed viable, accelerated route to expand the dwindling antimalarial therapeutic repertoire. Drug repositioning screens previously carried out in our laboratory reported the potent antimalarial efficacy (IC<sub>50</sub> 47nM for *P. falciparum* K1 strain) of the anti-amoebic drug Emetine dihydrochloride hydrate. We present here the preliminary data from a study designed to define the combinatorial therapeutic potential of emetine with a panel of antimalarial drugs, in a bid to minimise non-target effects previously experienced with the use of the drug in amoebiasis. The rational discovery of novel synergistic drug combinations can be accelerated by predictions of combination effects through experimental studies. All combinations were analysed using the optimised CalcuSyn fixed-ratio method validated using the atovaquone-proguanil combination. Following a screen of current antimalarial compounds, our preliminary data identified AN16 as the combinatorial partner drug displaying maximum synergistic interactivity with emetine dihydrochloride. The isobologram plot and the combination index (CI) generated by the CalcuSyn software demonstrated that the interaction between emetine and AN16 is synergistic at IC<sub>50</sub>, 1C75 and IC90 levels. The MTT cytotoxicity results indicated that the emetine-AN16 combination has a better selectivity index in comparison to emetine alone. The results support further *in vivo* investigation of the utility of emetine-AN16 combination as an alternative antimalarial treatment for drug resistance malaria.

## TAFENOQUINE IS NOT NEUROTOXIC FOLLOWING SUPERTHERAPEUTIC DOSING IN RATS

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Tafenoquine is an 8-aminoquinoline antimalarial being developed for treatment of *Plasmodium vivax* relapse and malaria prophylaxis. The clinical, behavioral, and neurohistopathologic changes induced by different dose levels of tafenoquine were evaluated following single super-therapeutic dose administration in adult rats (doses used were 125, 250 and 500 mg/kg). Toxicokinetic data were generated to allow extrapolation to clinical exposures. At the highest dose, two animals were found dead and animals showed clinical signs of toxicity and reduced body weight 7 to 8 days after dosing. Significant decreases in motor activity were observed on more than one occasion at doses more than 9-fold higher than the clinical exposure. No other statistically significant changes in other functional or neurobehavioral endpoints were noted at any dose. No neurohistopathological changes were noted at any dose. Changes in hematological and clinical pathology endpoints were observed at the lowest dose level tested in a dose range study. Our data show that the principal target organs for tafenoquine-related adverse events in rats are the liver and blood, with sparing of the central nervous system and absence of demonstrable neurotoxicity at exposures achievable in humans.

## PYRAZINE, A NOVEL CLASS OF ORALLY ACTIVE ANTIMALARIAL. MAKING PROGRESS TOWARDS HIGH QUALITY MOLECULES

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Malaria remains one of the most devastating diseases of the developing world, killing 500K people annually. Although several therapies are available for preventing and treating malaria, most antimalarials drugs face decreased efficacy due to the emergence of resistant parasites. Consequently, there is an urgent need to replace those drugs by novel therapies that offer significant advantages over the current standard of care. Over the last years, the malaria community has been successful in identifying a range of novel drugs. As part of this global effort, GSK published TCAMS2 (Tres Cantos Anti-Malarial Set) which comprised 13,533 hits derived from phenotypic screening of 2 million compounds in the corporate collection. Different tools have been used to prioritize this set of compounds in terms of having the best chance of being converted into differentiated antimalarial drugs. As result of this work, a novel chemical series was identified containing a pyrazine scaffold. This series combines a good oral efficacy characterized by a rapid parasite clearance in the *Plasmodium falciparum* mouse model with a low propensity to select resistance. The overall properties of the series constituted a promising profile justifying further development. This communication will describe the progression on this new chemical class from the initial hit to the clinical candidate with emphasis on the different strategies used to address key issues encountered in the lead optimization program. These efforts have culminated in the discovery of a compound combining an excellent *in vivo* potency with a very good developability profile.

## PRIMAQUINE - 1,4-DI-N-OXIDE QUINOXALINE HYBRIDS: POTENTIAL TISSUE SCHIZONTOCIDE ACTIVITY IN MALARIA

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Malaria is one of the world's most important tropical parasitic diseases. Exoerythrocytic stage has been poorly characterized. In the context of malaria elimination, this parasite stage has been postulated as a therapeutic target. Currently, primaquine is the approved treatment for this stage. However, loss of susceptibility to primaquine in around 18% treatments has been reported. For this reason, it is necessary to search for compounds with tissue schizontocide activity. Synthesis of five new primaquine and 1,4-di-N-oxide quinoxaline hybrid compounds (PQ-QdNO) was done. To evaluate the *in vitro* activity, infection of cell lines (HepG2, HepG2-CD81) and primary culture of human's hepatocytes with *P. berghei*, *P. yoelii* and *P. falciparum* sporozoites, respectively, was assayed by immunofluorescence against *Plasmodium* Hsp70 after 2 or 7 days of treatment with PQ-QdNO. Cytotoxicity analyses of hybrids at cell lines and human's hepatocytes was measured by MTT assay. Generation of oxidative stress at Hepa 1-6 cell line was evaluated by flow cytometry using 2',7'-dichlorodihydrofluorescein diacetate. PQ-QdNO compounds showed a median toxic concentration (TC50) >109µM. Compound PQ-QdNO **7** (*P. yoelii* IC50=5.71µM, *P. berghei* IC50= 1.14µM and *P. falciparum* IC50= 4.34µM) was identified as the most active and selective (*P. yoelii* IS= 34, *P. berghei* IS= 136 and *P. falciparum* IS= 45) hybrid compound against hepatic stage of *Plasmodium*. PQ was used as a control (*P. yoelii* IC50=2.13µM, IS=32.7; *P. berghei* IC50= 3.65µM, IS=32.8 and *P. falciparum* IC50= 0,53µM, IS=101.8). Add, PQ-QdNO **7** at IC10 induced oxidative stress levels three folds higher than PQ after 24h post exposition. These preliminary data suggest that PQ-QdNO hybrid compounds can remain as a valid approach to develop new compounds with activity on *Plasmodium* liver stage.

## RESISTANCE SELECTION APPROACH TO IDENTIFY AND VALIDATE TARGETS FOR ANTIMALARIAL DRUG DISCOVERY

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The emergence and spread of drug resistance to current antimalarial therapies remains a pressing concern, escalating the need for compounds that demonstrate novel modes of action and prevent the development

of drug-resistance. As part of the Malaria Drug Accelerator (MalDA) consortium efforts, we have adopted a chemogenomic approach to identify the targets of the most prominent compounds from chemically diverse libraries. Study compounds were selected based on availability, purity, potency in a multi-drug resistant isolate, and lack of known mechanism of action towards the mitochondrion or folate biosynthesis. To further eliminate overlap with known targets, we performed cross-resistance testing against a panel of drug resistant parasite lines with well-characterized mutations in diverse targets. Here we present studies of two molecules: MMV019721 and MMV084864. *In vitro* resistant lines were generated by single-step selection (MMV019721) and step-wise methodologies (MMV084864) and whole genome sequencing employed to identify genetic variants contributing to the resistance phenotype. In the case of MMV019721 selected lines, variants were identified in a putative acetyl-coA synthetase, representing a potentially novel drug target in the parasite. Studies to validate this target are underway. Selections with MMV084864 resulted in high levels of drug tolerance and a dramatically altered dose-response profile. Whole genome sequencing identified large regions of chromosomal amplifications which also were observed in selections with compounds of diverse chemotypes. These data suggest that these mutations are likely broader resistance mechanisms rather than the drug target. While these results demonstrate that multiple chemical classes are rendered less active by common resistance mechanisms, they also suggest that there are a limited number of pathways in the parasite that contribute to drug-resistance. Drug-development strategies that counteract these common escape mechanisms may therefore become invaluable in extending the usable lifetime of future therapies.

## 1595

### CHROMOBACTERIUM CSP\_P MEDIATES ITS ANTIMALARIAL ACTIVITY THROUGH SECRETION OF THE HDAC INHIBITOR ROMIDEPSIN

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The limited diversity within the chemical scaffolds of the current clinically available antimalarial drugs, together with the increase of incidence of drug resistance, make it urgent to identify new compounds with novel modes of action that can treat the disease. We have previously shown that a *Chromobacterium* isolate, *Csp\_P*, exerts entomopathogenic activity against both adult and larval stages of malaria and dengue vector mosquitoes, along with *in vivo* and *in vitro* anti-*Plasmodium* and anti-dengue activities. To assess the nature of the *Csp\_P*-produced anti-*Plasmodium* factors, chemical partition was conducted recurring to bioassay-guided fractionation, where different fractions were assayed for activity against asexual stages of *Plasmodium*. It was determined that antimalarial activity was preserved following initial liquid-liquid extraction with *n*-butanol. The isolated compounds were further partitioned by reversed-phase HPLC followed by size-exclusion HPLC. Higher resolution UPLC and ES/MS data were then collected and revealed that the most active fraction contained a depsipeptide (a cyclic peptide ester) notable for an unusual intramolecular disulfide bond, romidepsin. A pure sample of this FDA-approved HDAC inhibitor allowed us to independently verify this finding. Genomic comparisons between *Csp\_P* and multiple species within the *Chromobacterium* genus further indicate a correlation between presence of the gene cluster responsible for romidepsin production and effective antimalarial activity. Taken together, our data indicate that romidepsin mediates the antimalarial activity of *Csp\_P* and further work is underway to evaluate the potential of its development as an antimalarial, both against mosquito and human stages of the disease. Even though there are described side effects for a romidepsin regimen in humans, this molecule shows great promise as a lead compound given the structural novelty and the unique mode of action when compared to the currently used antimalarials.

## 1596

### DEVELOPING LONG-TERM MALARIAL CHEMOPROPHYLACTIC COMPOUND RELEASING IMPLANTS

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Despite the successful development of chemoprophylactic compounds and multi-drug combination therapies, malaria infection remains a crucial health threat to U.S. Soldiers in malaria endemic areas. The successful prevention of malaria infection is highly dependent on compliance with a prescribed chemoprophylaxis regimen. The Experimental Therapeutics (ET) Branch at Walter Reed Army Institute of Research (WRAIR) is the U.S. Army's premier research program for the development of anti-malarial prophylaxis drugs. A current effort of ET, in scientific collaboration with the Southwest Research Institute and Titan Pharmaceuticals, is to develop long-term release implantable anti-malarial drug matrices. These implants provide continuous drug release with a non-fluctuating drug levels over an extended period from two to six months, and could potentially relieve deployed service members from adherence to a daily oral drug dosing schedule. EVA (ethylene-vinyl acetate) implants that contain piperazine, an compound effective against blood stage parasites, was tested in a mouse model with *Plasmodium berghei* to characterize the pharmacokinetics (PK) profile and long-term prophylactic efficacy *in vivo*. The piperazine formulated implant study showed the PK profile exhibited slow drug release for six weeks while maintaining stable plasma levels. Furthermore, the piperazine implants after longer than eight weeks of implantation demonstrated sufficient suppression in early blood stage malaria and complete protection malaria in mice. The development of long-acting prophylactic implants with greater potency and safety is a novel approach, and one that could greatly improve compliance of deployed Soldiers in malaria endemic regions. These preliminary findings with piperazine allow us to pursue a series of long-acting implants that include more regulatorily attractive FDA-approved anti-malarial drugs, atovaquone/proguanil (Malarone®) and doxycycline, for follow-on *in vivo* preclinical studies.

## 1597

### PROVEBLUE, METHYLENE BLUE, AS AN ANTIMALARIAL DRUG

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In 2011, the World Health Organization recommended artesunate as the first-line treatment for severe malaria. In recent years, several studies have reported clinical failures or at least extended parasite clearance times in Asia. There is an urgent need to discover partners for combination with artemisinin derivatives. Proveblue (PVB) (international patent no. PCT/FR/2007/001193), which is a methylene blue preparation that complies with the European Pharmacopoeia and contains limited organic impurities and heavy metals of recognized toxicity, was demonstrated to possess *in vitro* antimalarial activity against *Plasmodium falciparum* strains that were resistant to various other antimalarials and against African isolates. PVB exhibited noticeable *in vitro* high synergistic effects associated with dihydroartemisinin, the active metabolite of artemisinin derivatives. Treatment with 1 to 10 mg/kg of weight of PVB for five days significantly reduced or prevented cerebral malaria in mice. Additionally, the PVB *in vitro* activity was not associated with validated gene polymorphisms involved in antimalarial resistance like *pfcr*, *pfmdr1*, *pfdhfr*, *pf dhps*, *K13*, *plasmepsin 2* or potential genes coding proteins like PfMDR6, PfMDR2,

PfNHE-1, E3 ubiquitin protein ligase or exonuclease. These results confirm the therapeutic potential of Proveblue, which could be integrated into the pipeline of new antimalarial combination therapies.

## 1598

### NOVEL LIVER STAGE ACTIVE ANTIMALARIALS

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We have previously reported the discovery of a novel antimalarial acridone chemotype that displays efficacy against sporozoite-induced *Plasmodium* infection in addition to efficacy against blood stage parasites. We have been successful in producing extremely potent new lead candidates with pico molar IC50 values against MDR resistant parasites, as well as full protection of liver stage infection at comparable dosage with primaquine. Details of the design, chemistry, structure-activity relationships (SAR), safety, metabolic studies, and mechanism of action will be presented.

## 1599

### NATURAL PRODUCT INSPIRED NOVEL ANTIMALARIALS

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Natural products have been at the forefront of most approaches to combat many infectious diseases, particularly malaria. Continued discovery of new natural products, their chemical modifications, and synthesis of drugs inspired by natural product have helped to combat the inevitable development of resistance against existing antiparasitic agents. Prodiginines (PGs) and tambjamines (TAs) belong to a family of intriguing pyrrolopyrromethene (PPM) natural products, isolated from bacterial and marine sources. These PPM natural products have a unique structural composition with a wide range of biological properties, including antimalarial activity. Recently, we have developed a series of chemical entities inspired by the PGs and TAs and discovered structures with curative efficacy against *Plasmodium* infection. The mechanisms of action are unknown for these PPM products and the unique structure suggests potential to discover a new drug target to combat malaria parasites. Our data to date on the antimalarial activity of the tambjamines is very exciting and indicate a new structural class of antimalarials that meets the following key criteria: 1) orally efficacious, 2) curative in a single dose, 3) long duration of cover, 4) multistage efficacy with potent activities against liver stage and blood stage parasites, as well as inhibition of gamete formation, 5) fast-acting drug candidate, 6) synthetically accessible with a low-cost of production, 7) active against MDR *Plasmodium falciparum* strains, 8) high potency against clinical isolates, 9) non-genotoxic, 10) unique chemotype as compared to existing antimalarials, and 11) possibly operate by a novel mechanism(s) of action. Continued studies of the lead optimization, multiple stage screening, pharmacology, safety and mode(s) of action studies of the lead tambjamines will permit us to expand a range of novel tambjamines for full preclinical antimalarial studies. The ultimate objective our future work is to develop a novel antimalarial drug to be used in a combination therapy to prevent and treat malaria thus contribute an important tool to help eradicate the disease on a worldwide scale.

## 1600

### OPTIMIZING THE IN VIVO PHARMACODYNAMICS OF THE PLASMODIUM FALCIPARUM APICOPLAST INHIBITORS FOSMIDOMYCIN AND CLINDAMYCIN

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Reports from Southeast Asia continue to document the decreasing efficacy of artemisinin-based combination therapies, thereby emphasizing the need for novel antimalarials. The antibiotics fosmidomycin and clindamycin are apicoplast inhibitors with relatively short half-lives of approximately 1-2 hours. In clinical trials, this combination has resulted in suboptimal 28-day cure rates. For bacterial infections, fosmidomycin and clindamycin are dosed 4x daily; however, as antimalarials, they are often dosed 2x daily. We have hypothesized that increasing the frequency of daily dosing as well as extending the treatment duration beyond 3 days would improve suboptimal dosing regimens. We have adapted a murine model of malaria, which utilizes a luciferase-reporter *Plasmodium berghei* at high parasitemia, to study the parasite killing *in vivo*. Unlike other drug efficacy assays, which assess cytostatic activity, our assay more accurately mimics high parasitemia infections and cytotoxic drug activity in the field. This model has previously been used to demonstrate the importance of lengthening the artesunate dosing duration to span 3 life cycles in order to make existing artemisinin-based treatments more efficacious. In this study, we have shown the same total dose of fosmidomycin or clindamycin achieves enhanced parasite killing when administered every 6 hours versus when administered in a single dose. For combination therapy, we have shown that noncurative dosing regimens are improved to curative dosing regimens with a lower overall dose. This increased efficacy was achieved by increasing the dosing schedule to 4x daily (vs. 2x) and extending the treatment duration to 4 days (vs. 2 days). Additionally, to support our *in vivo* results, pulse drug studies in *P. falciparum* are currently underway.

## 1601

### IDENTIFYING HEXAHYDROQUINOLINES AS NEW ANTIMALARIALS WITH POTENT BLOOD STAGE AND TRANSMISSION-BLOCKING ACTIVITY

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Novel antimalarial compounds with dual therapeutic and transmission-blocking activity are considered valuable partner drugs for combination therapies, especially in high-burden countries where malaria transmission is high. However, current drugs are either relatively ineffective against the transmissible form of the malaria parasite or risk inducing severe toxicity. Here, we report the identification and characterization of hexahydroquinolines (HHQs) that show low nanomolar potency against both the pathogenic and transmissible intra-erythrocytic forms of the malaria parasite *Plasmodium falciparum*. This activity translates into potent transmission-blocking potential, as shown by *in vitro* male gamete formation assays and reduced oocyst infection and prevalence in *Anopheles* mosquitoes. *In vivo* studies confirmed the potential of HHQs to block transmission of *P. berghei*. Resistance selections and CRISPR-Cas9

based gene editing have identified the ABC transporter PfMDR1 in the action and resistance mechanism of HHQs. Heme fractionation assays suggest a mode of action that inhibits endocytosis of hemoglobin without directly affecting heme metabolism in the digestive vacuole. Furthermore, parasites resistant to HHQs displayed increased susceptibility to several first-line antimalarial drugs, confirming that HHQs have a different mode of action than other antimalarials drugs for which PfMDR1 is known to confer resistance. This work hereby evokes therapeutic strategies that combine opposing selective pressures on this parasite transporter.

## 1602

### MIGRATION AS A DETERMINANT OF MALARIA IN SURINAME: CHALLENGES IN REACHING ELIMINATION

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Migration is the most important determinant of malaria in Suriname. The populations most at risk are mobile migrant - small scale gold miners (MM). There is a continuous movement of MM within the Guyana Shield in search of gold. This study describes the role of imported malaria in Suriname (2006 - 2015), based on malaria surveillance data. Suriname is reaching elimination. Cases have decreased from 15,967 in 2001 to 373 in 2015 (indigenous and import). As indigenous cases decreased, the proportion of imported cases increased, from 6.8% in 2006 to 79.4% in 2015. The majority of imported malaria in Suriname is related to MM that work French Guiana (territory of France) and buy supplies in Suriname. Imported malaria from other countries is negligible. Between 2006 and 2015 the proportion of French Guianese cases ranged from 6.6% (272 cases) to 73.5% (274 cases) of the total number of cases. The top 3 *foci* producing cases in French Guiana, as observed in Surinamese import data, have been, Sophie, Eau Claire and Tadeu - mining sites -, accounting for 72.2% of the total imported cases from French Guiana. The *Plasmodium falciparum* to non- *P. falciparum* ratio for the French cases was 0.45 for 2015. The results show that Brazilian migrants moving between Suriname and French Guiana account for most cases diagnosed in Suriname, 94 %. Malaria is acquired mostly in the center of the interior of French Guiana and is not the result of border area transmission. While malaria has almost been eliminated from Suriname, there is a continuous risk of re-introduction of malaria as a consequence of cross-border movement and the resulting importation from French Guiana. To sustain the achieved success and to reach elimination in Suriname regional cooperation with the French health services is essential. In addition, it is of eminent importance that France adequately addresses the problem of malaria among the MM on its territory where they have no access to malaria services in small scale mining areas. The Suriname model to deal with malaria among MM - training peers (Malaria Service Deliverers) to provide supervised malaria diagnosis and treatment in remote areas - has been very successful.

## 1603

### FORMATIVE ASSESSMENT TO UNDERSTAND AND TARGET HIGH-RISK POPULATIONS FOR MALARIA INFECTION, CHAMPASAK PROVINCE, LAO PDR

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In the Greater Mekong Subregion, mobile and migrant populations (MMs) are considered most at risk for malaria due to their occupational environments and limited access to health care. Despite a growing emphasis on targeting MMs, regional data on MMP subpopulations and their unique risk profiles is lacking. A formative assessment set out to identify MMs in Champasak Province, Southern Laos, and characterize

their occupations, seasonality of work, migratory patterns, health-seeking behaviors, and social networks. This study utilized focus group discussions, key informant interviews, retrospective data collection, mapping and observational activities. Sixteen focus groups and 28 in-depth interviews were carried out in four districts including a range of stakeholders: Lao and foreign MMs (forestry, agriculture, industrial projects); provincial, district, facility and community malaria staff; labor, agriculture, and forestry department officials, and owners of venues known to serve MMs (restaurants, karaoke bars). Discussion themes included MMP demographics, occupations, migration patterns, health-seeking practices, social networks, and acceptability of targeted malaria interventions. A recent government decree preventing the sale of timber has led to a large reduction in logging, with subsequent closure of sawmills across Champasak. Most MMs appear to be village-based Lao nationals engaged in seasonally shifting forest and agricultural work. Key livelihood activities include rice cultivation, forest-based hunting and gathering, work on plantations (coffee, rubber), and short-term employment at small-scale factories. MMs reported seeking care at public health facilities, and in general bednet usage appeared to be high. Key findings from this study suggest that Champasak MMs are generally village-based and fairly stationary, suggesting that these populations may be more accessible than previously believed. Any interventions targeting MMs should be tailored to respond to context-specific exposures, behaviors, and access points, which require better defining and understanding of local MMs.

## 1604

### DHIS2 TRACKER DASHBOARD AS A TOOL TO CATALYZE DATA USE IN THE MALARIA ELIMINATION SETTING OF ZIMBABWE

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With a goal to eliminate malaria from 9 out of the 20 elimination targeting districts by 2020, Zimbabwe has re-oriented its individual level malaria surveillance system through a phased approach since 2012; first with a transition from paper to electronic data collection using Personal Digital Assistant devices in 2014, then to using an android-based DHIS2 Tracker Capture platform in 2016. To allow for timely decision-making using the high resolution data collected, the National Malaria Control Programme set up a DHIS2-based dashboard to automatically compute key epidemiological indicators. District managers from all elimination districts met to select indicators and design preferred visualizations for the dashboard, based on the review of existing surveillance data and understanding of required decisions. User-specific indicators of interest and preferred visualizations of data, for example in the form of a map versus a chart, informed the design of the dashboard. At routine surveillance trainings in early 2017, selected users had the opportunity to interact with the dashboard. Feedback from the initial reviews of the DHIS2 platform will inform future improvements and related trainings on the dashboard which will continue to be deployed at routine district level meetings. Key lessons from the design process included iterative inclusion of user feedback into dashboard design and provision of additional frequent refresher trainings through opportunistic avenues. Quarterly post-training assessments for detailed dashboard evaluation, which will include follow-up key informant interviews and additional analysis of data inputs, will be carried out to assess the overall impact of dashboard in June 2017.

## THE PERFORMANCE OF G6PD RAPID DIAGNOSTIC TESTS IN CAMBODIA AND IMPLICATIONS FOR PRIMAQUINE THERAPY

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Primaquine (PQ) is the only available drug that can provide radical cure of Vivax malaria and thus effectively eliminate the hidden reservoir of *Plasmodium vivax* and reduce the risk of relapse. The national malaria program in Cambodia has recommended that G6PD normal patients be treated with PQ since 2012. However, PQ deployment has been delayed due to unavailability of suitable point-of-care G6PD testing capabilities as well as concerns with potential hemolysis in deficient patients misclassified as G6PD normal. We have evaluated the performance of a fluorescent spot test (FST) and two rapid detection tests (RDTs), CareStart™ and Binax™, in 213 patients with uncomplicated *P. falciparum* or mixed malaria infection in order to assess their performance to detect G6PD enzymatic activities of <10%, <30%, and <60% of the population-adjusted male median, established by spectrophotometry quantitative test (Trinity Biotech, Ireland). The adjusted male median (100% of activity) was 8.28 U/gHb or 8.16 and 8.425, on DO and week 2 following treatment, respectively. At 30% G6PD activity, sensitivity for the FST, CareStart and Binax tests was 100% for male and female volunteers, with a deficient predictive value (DPV) of 96.88% (83.78-99.92%) and a normal predictive value (NPV) of 100 for all three tests performed at the time of acute malaria symptoms (n=29; 14.65% G6PD deficient). At 60% G6PD activity (n=38; 19% G6PD deficient), the sensitivity for FST, CareStart and Binax was 74% (95% CI: 58.83, 86.48), with a DPV of 100% and a NPV of 94.18% (89.83-97.06%). We have shown that G6PD RDTs consistently identified patients with <30% activity in a controlled setting, providing some reassurance that deployment of the RDTs should identify those most at risk for hemolytic effects of primaquine therapy. However, training of local staff in the use and interpretation as well as monitoring for misclassifications or hemolytic events despite testing will be required. Pharmacovigilance measures should be in place to detect future hemolytic risk attending PQ doses for radical cure from possible testing errors.

## IMPACT OF INDOOR RESIDUAL SPRAYING WITH PIRIMIPHOS-METHYL IN THE CONTEXT OF A COMPREHENSIVE MALARIA ELIMINATION STRATEGY IN SOUTHERN PROVINCE ZAMBIA

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Annual rounds of indoor residual spraying with organophosphate insecticide pirimiphos-methyl (IRS-PMCS) were implemented as part of a

comprehensive malaria elimination strategy in Southern Province Zambia from 2014-2016. The impact of mass drug administration (MDA) with dihydroartemisinin-piperazine was also tested as part of the strategy using a community randomized controlled trial in 60 health facility catchment areas during this time. The entire study area received standard of care of long-lasting insecticidal nets (LLINs), IRS-PM CS and improved access to case management over this period. Household LLIN coverage ranged from 73-82% from 2014-2016, but pyrethroid resistance has been documented. Household IRS-PMCS coverage increased from 0% in 2013, 14% in 2014, to 47% in 2016. Child malaria parasite prevalence by RDTs declined across the study area from 31% in 2014 to 4% after 4 MDA rounds in 2016, including in control areas. A spatiotemporal model will be used to assess the dose-response relationship between IRS-PMCS exposure and malaria parasite prevalence and confirmed case incidence between 2011-2017, accounting for LLIN coverage, treatment seeking, mass treatment, community case management, household factors and climate variability. Data from 11 rounds of household-level mass treatment campaigns conducted in the study area since 2011 will be incorporated, including a total of 277,179 households and 1,412,632 individual data points. Interactions between IRS-PMCS and other interventions will also be explored. Results from this study will be critical in understanding the impact of IRS using next-generation chemicals as part of a comprehensive elimination strategy, as well as the role IRS-PMCS played in reducing transmission in areas that did not receive MDA.

## MOVEMENT PATTERNS ASSOCIATED WITH MALARIA RISK DERIVED FROM OUTPATIENT REGISTER BOOKS IN AMHARA REGION, ETHIOPIA

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The malaria burden in Ethiopia has steadily decreased over the last several decades. As a result, the country aims to achieve subnational malaria elimination in 200 districts by 2020. However, population movement and importation of malaria infections from high- to low-risk areas is a major challenge to achieving zero malaria transmission. We assessed the movement patterns in Amhara Region, Ethiopia, using data from 133 health posts in low-to-moderate transmission areas. In 2014-16, a total of 71,818 outpatients were tested with a rapid diagnostic test (RDT) and travel history in the previous month was recorded. Additional data were extracted from 9,044 RDT-positive cases (with or without travel history) and RDT-negatives with travel history. Among the 8,163 RDT-positive patients, 74% were male and 18% had travel history; 41% of infections were due to *Plasmodium vivax*, 36% were due to *P. falciparum*, and 23% were due to mixed infections. *P. falciparum* (including mixed) infections were more frequent among travelers than non-travelers. The percentage of cases due to *P. vivax*-only decreased with age both in travelers and non-travelers, whereas *P. falciparum* (or mixed) increased with age. The mean age of RDT-positive cases with travel history was significantly higher (25.1 years [SD 10.7]) than those without travel history (20.6 years [SD 14.8]). Among the 2,371 cases with travel history, 84% were males, 80% were 15-44 years old, and 62% were RDT-positive. Patients with travel history had a 6.1 times greater risk of having malaria compared to patients without travel history. Of the travelers, 69% traveled within the Amhara Region, while 31% traveled to other regions such as Tigray (19%), Oromia (6%), Benishangul-Gumuz (5%), and Afar (3%). Nearly all destinations were high-risk areas located in the western lowlands and the Nile valley, and comprised large agricultural farms that attract a high influx

of seasonal laborers. These laborers have a high risk of becoming infected at the farms, and specific strategies are needed to reduce the risk of importing malaria back to their homes in low-transmission areas.

## 1608

### **COST AND EFFECTIVENESS ANALYSIS OF MALARIA CONTROL IN SENEGAL: "THEORETICAL" SINGLE INTERVENTIONS VS. "ACTUAL" PACKAGES OF INTERVENTIONS**

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Malaria prevalence in Senegal varies greatly by district. Senegal's National Malaria Control Program (PNLP) therefore implements control interventions as packages based on prevalence. However, data are only available by single intervention, posing a challenge for the PNLP as it prioritizes packages/interventions during strategic planning for malaria pre-elimination. A broader review of the literature showed that in general, most malaria cost and effectiveness data is based on trials of single interventions. To help address this gap, USAID's Health Finance and Governance (HFG) project worked with the PNLP to design and implement a retrospective study that estimated costs and effectiveness of Senegal's malaria control packages. Cost per DALY (Disability Adjusted Life Years) averted were estimated for the six packages: 1) Scale Up For Impact (or SUFI, which includes bednets, intermittent preventive treatment, rapid diagnostic tests, and artemisinin combination therapies), 2) SUFI + FSAT (focal screen and treat + active surveillance) or MSAT (mass screen and treat + active surveillance), 3) SUFI + interior residual spraying (IRS), 4) SUFI + seasonal malaria chemoprophylaxis (SMC), 5) SUFI + SMC + IRS, and 6) SUFI + SMC + PécadomPlus (active home management). There was wide variation of cost per DALY averted across packages: \$70-\$667. Total and unit costs of single interventions within packages were also estimated. Non-facility-based treatment interventions (\$6-17 per person tested/treated) cost more than facility-based (~\$1). SUFI-only had the lowest unit cost (\$0.54 per person tested/treated), while the SUFI+SMC+IRS package had the highest unit cost (\$4.55 per person). Senegal seems to have effectively distributed packages to target the high burden areas with a wide range of interventions, though greater efficiency gains are still possible. For example, SMC seemed to make IRS more cost effective in the same district (\$274 vs. \$373). This study may assist PNLP and other control programs with the detailed data needed for sustainability planning and domestic resource mobilization needed to eliminate malaria.

## 1609

### **MALARIA PARASITEMIA AND SEROLOGICAL PREVALENCE IN NEAR-ZERO TRANSMISSION SETTINGS IN ETHIOPIA**

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Ethiopia has made enormous progress in the fight against malaria, and is close to elimination in certain regions. Passive incidence of malaria cases is the only metric available in near-zero transmission settings, and other methods to assess potential interruption of transmission at the sub-national level are needed. A study was conducted in Amhara National Regional State in December 2015-February 2016 to estimate prevalence of *Plasmodium* infection by rapid diagnostic test (RDT), microscopy and polymerase chain reaction (PCR), sero-prevalence of anti-AMA-1 and

anti-MSP-1 antibodies, and serological conversion rate. Four health post catchment areas with zero confirmed, locally-acquired malaria cases in the last two years were selected. A community-based cross-sectional study was conducted in the study area in 1,649 randomly selected households, in which all consenting children aged 1-9 years and a subsample of randomly selected individuals aged  $\geq 10$  years old were included. A total of 1,272 individuals were included: 456 aged 1-4 years old, 383 aged 5-9 years old, and 433  $\geq 10$  years old. To assess whether an operationally easier convenience survey could be used instead, a survey in a convenience sample of 404 children 1-4 years old recruited at health facilities and during community health outreach activities in the same areas was also conducted, and prevalence of infection and sero-prevalence results will be compared to the community survey results. In the community survey, there was only one *P. falciparum* infection by both RDT and microscopy. In the convenience sample, no infections were found. Samples are being assayed for PCR and serology, and results will be available by mid-2017. Serological conversion rates will also be estimated for the community survey.

## 1610

### **OPTIMIZING HIGHLY FOCAL MASS DRUG ADMINISTRATION TARGETS FOR MALARIA ELIMINATION ACCELERATION OVER NETWORKED POPULATIONS: THE CASE OF HAITI**

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With malaria prevalence of ~1%, Haiti is committed to malaria elimination by 2020. The Malaria Zero alliance, planning and implementing elimination activities in the country, has proposed an intervention program with emphasis on strengthening surveillance systems to increase case management (CM) rates throughout the nation; targeted reactive case detection (RCD); and highly focal mass drug administration (fMDA), with only limited vector control interventions. What are the appropriate scale, coverage, and locations of fMDA and targeted RCD deployment in the context of heightened CM to ensure malaria elimination in the country? Is there a requisite passive CM rate that needs to be sustained throughout Haiti? Are these interventions sufficient to achieve elimination sans vector control? We set up a dynamic, spatial, agent-based mathematical model comprising a network of 500m x 500m grid cells spanning the country, capturing multiscale human migration and health-seeking patterns. Heterogeneity in vectorial capacity due to significant small-scale variation in topography leads to high-transmission pockets occasionally seeding short outbreaks in lower-transmission areas. Furthermore, high-transmission pockets are not necessarily proximal to health facilities: a number of treated cases register in health facilities relatively far from infection origins. We caution that reliance on clinical case counts from health facilities alone may lead to inaccurate estimates of transmission pocket locations, rendering highly focal (village scale) MDA and RCD ineffective in the elimination context. Heightened passive CM rates (50%-70%) maintained throughout endemic areas like Grand'Anse have the potential to achieve elimination, but much later than the 2020 target. Including well-connected high-transmission pockets and nearby settlements in fMDA campaigns, in addition to the heightened CM rates, accelerates elimination towards the 2020 target.

## 1611

### **POPULATION GENETIC DIVERSITY OF SAMPLES FROM THE 2012 AND 2015 MALARIA INDICATOR SURVEYS, ZAMBIA**

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To assess progress in malaria burden reduction, biennial malaria indicator surveys (MIS) have been implemented in Zambia since 2006. MIS data show high spatial and temporal heterogeneity in parasite prevalence by rapid diagnostic test, with some areas now approaching elimination, while others display historical resurgence. The variation in transmission intensity may be reflected in the parasite population genetics. As malaria prevalence decreases, the amount of genetic crossing between different parasite strains is expected to decrease. With reduced crossing, population genetic diversity as measured by complexity of infection (COI) should reduce. By selecting samples from both high and low transmission strata across both space and time, this hypothesis can be tested. Parasite DNA was extracted from dried blood spots (DBS) and a 24-point single nucleotide polymorphism barcode was determined by Taqman genotyping. Sample analysis has been completed for 57 and 21 RDT-positive DBS with genotyping results at 15 or more of the SNPs from the 2012 and 2015 MIS, respectively. From 2012, lower burden (10% prevalence by RDT) Southern Province has 36% monogenomic infections vs 10% and 11% in the higher burden provinces of Eastern (51% prevalence by RDT) and Luapula (56% prevalence by RDT) provinces, respectively. Similarly, the average percentage of mixed calls (i.e., those indicating a polygenomic infection with both major and minor alleles present) in Southern at 21% was lower than Eastern and Luapula at 28% and 29%, respectively. Once the remaining samples have been genotyped, COI will be determined using COIL software. Spatial and temporal differences in the COI will be assessed with a chi-square test, comparing complex (multiple genotypes) or simple (single genotype) infections across region (high prevalence compared to low prevalence) and time (2012 compared to 2015). The association between COI and other measures of exposure (e.g., travel, age, sex) will also be investigated using logistic regression, wherein a complex infection is predicted from hypothesized factors. Spatial clustering of the genotype populations will also be assessed.

## 1612

### ADAPTING REACTIVE CASE DETECTION FOR MALARIA IN FOREST WORKERS IN ACEH, INDONESIA

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Reactive case detection (RACD) is conducted by many malaria elimination programs to detect and treat infected individuals living in close proximity to an index case originally presenting to a health facility or community health worker. In settings where risk of malaria infection is primarily due to occupational exposures such as forest work, there may be minimal spatial clustering of infections, which limits the utility of conventional RACD approaches. Recent RACD studies in Aceh, Indonesia found limited clustering of additional infections around the households of index cases, identified forest work as a risk factor for infection among those screened by RACD, and found *Plasmodium knowlesi* to be a common source of infection. Based on these findings, a novel RACD strategy is being tested, whereby an index case that meets pre-determined socio-behavioral risk factors will trigger follow-up testing at forest work sites (i.e. small mines and plantations) and other venues, and amongst socially-networked groups of individuals reporting recent forest travel. Beginning in April 2017, all confirmed malaria cases presenting to health facilities in Aceh Jaya and Aceh Besar districts will be screened for risk factors related to forest work, and a total of at least 1,183 individuals tested as part of this “socio-behavioral RACD” strategy using LAMP and RDTs. A similar number of individuals will be tested by household RACD, and the prevalence of secondary infections found during each activity compared using LAMP

and PCR, in addition to comparisons of geographic and demographic coverage. Spatial and occupational risk factors will be incorporated into the analysis, along with cost data and entomological assessments conducted at high-risk work sites; results of the first six months of data collection will be presented. RACD targeted based upon risk factors such as recent forest work can provide a method for Indonesia's national malaria program to treat the highest-risk populations to help achieve its elimination goals, and has the potential to be adapted to other malaria elimination contexts.

## 1613

### FIRST-YEAR RESULTS FROM THE COMMUNITY-LED RESPONSES FOR ELIMINATION (CORE) TRIAL ASSESSING THE EFFECTIVENESS OF REACTIVE FOCAL DRUG ADMINISTRATION COMPARED TO REACTIVE FOCAL TEST AND TREAT IN REDUCING *PLASMODIUM FALCIPARUM* INFECTION PREVALENCE AND INCIDENCE IN AN ELIMINATION SETTING IN SOUTHERN PROVINCE, ZAMBIA

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Zambia has seen impressive reductions in malaria transmission, such that the government has set a target of eliminating the disease by 2021. Part of Zambia's success has been the implementation of community-based reactive focal test and treat using rapid diagnostic tests and artemether-lumefantrine. To continue to accelerate toward zero, the use of a more aggressive approach—reactive focal drug administration using longer-acting dihydroartemisinin-piperaquine—is being explored through the Community-led Responses for Elimination (CoRE) community randomized controlled trial. This trial started enrolling in March 2016 and is being implemented in 16 health facility catchment areas in four districts in Southern Province, Zambia—an area of low malaria transmission and high coverage with vector control. Briefly, reactive responses, triggered by a confirmed malaria case at any government health facility, are performed by community health workers (CHWs) within seven days of the index case confirmation date, out to a radius of 140m from the index case household. Drug compliance is monitored over a 3-day visit. As of May 2017, one year's worth of field and laboratory data from the trial will be available, and we will conduct preliminary analyses assessing differences between the two arms of two separate outcomes: 1) PCR positivity during reactions, and 2) health facility trends in case incidence. A subset of the trial population will have dried blood spots (DBS) collected at days 1, 30, and 90 after a reactive response. Samples are analyzed by PET-PCR to determine the sub-patent carriage rate, and reinfection rates will be evaluated in the two study arms. Analyses of the parasite genotypes will be conducted to determine spatial distribution and the association between infection complexity and other risk factors (e.g., age or travel). Finally, the frequency of newly detected infections between the two arms will be examined to look for preliminary evidence of the effect of the different reactive case detection and management approaches.

## 1614

### PROGRAMMATIC MASS DRUG ADMINISTRATION IN SOUTHERN PROVINCE, ZAMBIA: AN EVALUATION OF IMPACT AND POSSIBLE SPILL-OVER EFFECTS USING DHIS2 MALARIA CASE INCIDENCE DATA

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The Zambia National Malaria Elimination Centre began rolling out programmatic mass drug administration (MDA) with dihydroartemisinin-piperazine (DHAP) in combination with high coverage vector control and intensive community-case management and surveillance in late 2016 as part of an updated national malaria elimination strategy. This follows a two-year trial of MDA which demonstrated significant benefit in malaria burden reduction in areas receiving this full package of interventions. We present two related analyses of the programmatic MDA outcomes. The first analysis will examine confirmed case incidence in 50 health facility catchment areas (HFCAs). The first group is 25 HFCAs that received MDA during 2016-2017 but were not previously included in the trial and are at least 25km from previous trial study areas. These 25 catchments are among the highest burden areas within the province with a 2016-2017 malaria season incidence of 61 cases per 1,000 population and were selected for the MDA this year due to their high incidence. The second group is 25 HFCAs selected from neighbouring Western Province using propensity score matching to provide a comparison group. This analysis will use a negative binomial model with a difference-in-difference estimator. The exposure of interest will be the MDA treatment with control variables for environmental factors and concurrent malaria control interventions. The second analysis will assess the possibility of spill-over effects of MDA by comparing changes in malaria incidence among HFCAs that are not receiving MDA (but are geographically contiguous to MDA areas) to more distant catchments. The possibility of a spill-over effect was observed during the trial and this new area of treatment provides a larger geographical area upon which to test the relationship between proximity to treated areas and changes in incidence. This analysis will use a dose-response framework to assess the effect of distance to nearest MDA areas on malaria incidence among a subset of the catchments within Southern Province.

## 1615

### MONITORING POPULATION AND TRANSMISSION DYNAMICS OF MALARIA VECTORS ALONG LAKE KARIBA OF SOUTHERN ZAMBIA: IMPLICATIONS FOR THE MALARIA CONTROL AND ELIMINATION PROGRAM

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Indoor residual spraying (IRS) and long-lasting insecticidal nets (LLINs) remain the key interventions against malaria vectors in Zambia. However,

the impact of IRS with pirimiphos methyl (PM; brand name Actellic-CS) on malaria vector populations and transmission remains confined to areas targeted for malaria elimination in Southern Zambia. Indoor host-seeking mosquitoes were sampled using CDC light traps and pyrethrum spray catch in 12 study sites where a mass drug administration (MDA) trial using dihydroartemisinin-piperazine was implemented in 2014-2016. Houses in the study areas were also targeted for IRS with PM during the trial. The sporozoite infection rates of malaria vectors were determined by ELISA while sibling species of *An. gambiae* s.l. and *An. funestus* group were identified by PCR. After two rounds of IRS, of all Anopheline species collected, the proportion that were *An. funestus* s.s. mosquitoes was reduced from 95.6% (n=6,302) in 2014 to 12.2% (n=2,334) in 2016, while the average number of *An. funestus* s.s. collected per month per house decreased significantly from 31.63 in 2014 to 3.58 in 2016. In contrast, of all Anopheline species collected, the proportion of *An. arabiensis* increased from 2.4% in 2014 to 81.0% in 2016 and average number per house of *An. arabiensis* increased substantially from 0.79 in 2014 to 14.52 in 2016. The percentage of sampled mosquitoes testing positive for *Plasmodium falciparum* (Pf) sporozoites in *An. funestus* s.s. decreased from 1.75% in 2014 to 0.25% in 2016 while only one positive was detected in populations of *An. arabiensis* in 2014 and no sporozoite positives were found in 2015 or 2016. These data suggest that IRS with PM suppresses the more endophilic *An. funestus* s.s. populations but not the largely exophilic *An. arabiensis* in Southern Zambia. The reduction in *An. funestus* s.s. populations and sporozoite rates further suggests the additive impact of malaria interventions (IRS and MDA) on the malaria parasite reservoir in the study areas. The persistence of *An. arabiensis* could challenge malaria elimination efforts and require additional outdoor vector control interventions.

## 1616

### THE ECONOMIC AND EDUCATIONAL IMPACTS OF A MALARIA ELIMINATION CAMPAIGN IN MOZAMBIQUE

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The elimination of malaria can bring benefits to a society which go well beyond improvements in health. Two areas often cited are education and worker productivity. But these hypothetical benefits have previously only been analyzed retrospectively and at the ecological level, casting doubt on elimination's causal role in societal improvements. The ongoing malaria elimination campaign (MALTEM) in southern Mozambique offers the ideal setting for a retrospective and person-level analysis of the economic and educational effect of malaria elimination. Prospective, observational data collection was carried out on a sample of schoolchildren between 6 and 12 years old, as well as adults working in sugarcane factories from both the malaria elimination intervention area (Magude) as well as a control area (Manhiça). Data were collected for the period one year prior, during, and one year after the initiation of mass drug administration (MDA) in Magude. Difference-in-difference was performed along with regression analysis, allowing for the control of potential confounding factors, such as the demographic, socio-economic, morbidity and mobility status of the population. Preliminary results are expected in June of 2017. The results are still undetermined as the project is currently in progress. However, the results from this study will be crucial to inform policymakers as well as the international community on the magnitude of the economic gains as well as the efficacy of investing resources in moving from a state of controlled malaria to malaria elimination. This research forms part of a more general goal to quantify the economic costs of malaria, and the potential corresponding benefits of its eradication. The data for this project are among the most granular and detailed for any project of similar scope in Sub-Saharan Africa. The findings of this project will inform not only policy-makers's public health decisions, but also those of private industry leaders.

## REACTIVE CASE DETECTION IN A SETTING OF DECLINING TRANSMISSION, SOUTHERN PROVINCE, ZAMBIA

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Reactive case detection (RCD) is a malaria surveillance and treatment strategy that has been rolled out in southern Zambia since 2011. RCD is triggered when a patient seeking care at a health facility or by a community health worker is confirmed by a rapid diagnostic test or other laboratory diagnostic test to have malaria (an index case). The investigation tracks index cases back to their corresponding households and neighborhoods, where either all individuals in the immediate area are treated, or all individuals are tested and those who test positive for malaria are treated. The success of this approach rests on the assumption that infections are clustered and that tracking passively detected index cases will find the most infections. We review progress to date with RCD activities and lessons learned for scaling up RCD throughout Zambia.

## REACTIVE CASE DETECTION IN TRANSITION TO PROGRAMMATIC SURVEILLANCE, NORTHERN SENEGAL

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As more countries move toward malaria elimination, new strategies need to be implemented in low transmission settings to deal with residual or imported cases before elimination is reached. In northern Senegal, malaria case investigation (CI) and reactive case detection (RCD) are currently being implemented. Case investigation is the characterization of all passively detected malaria cases (sociodemographic characteristics, symptoms, history of travel, use of malaria control tools, etc.), which is followed by RCD to actively search for more infections and/or administer antimalarial treatment at the index case household and neighboring households. The success of this approach rests on the assumption that infections are clustered and that tracking passively detected index cases will find the most infections. However, many questions remain about the effectiveness and operationalization of these strategies and available data from the field should inform policies. Since 2012, Senegal has been implementing CI with RCD in the north, where malaria transmission is the lowest in the country. It initially started in Richard Toll District, where RCD was done using focal test and treat in the index case household and five closest neighboring compounds, changing to focal screen test and treat (only testing with a rapid diagnostic test those with risk factors), and finally to focal drug administration where all eligible individuals in the index case household are treated. Since 2016, RCD has been implemented by the National Malaria Control Program in 10 districts in northern Senegal covering a population of about 1.7 million, with over 90% of passively detected cases investigated. The coverage of the intervention, operational description, and feasibility will be presented. To evaluate whether CI with RCD had an impact on malaria incidence in the intervention areas,

an impact evaluation will be conducted using a quasi-experimental design with a comparison group from areas that have not received the intervention.

## FACTORS ASSOCIATED WITH INTERMITTENT PREVENTIVE TREATMENT OF MALARIA DURING PREGNANCY IN MALI

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Intermittent preventive treatment in pregnancy (IPTp) with three or more doses of sulfadoxine-pyrimethamine (SP) is recommended by WHO to prevent malaria in pregnant women living in high risk areas. According to Mali's 2015 malaria indicator survey the prevalence of malaria was 34.6%. The high risk of malaria among pregnant women and children led government to provide SP free of charge during ANC visits. The government recommends to take three doses least during pregnancy. The proportion of women taking three or more doses of sulfadoxine pyrimethamine to prevent malaria during pregnancy (IPTp 3+) remains low. According to the 2015 Mali MIS, only 37% of women with a live birth in the past two years received IPTp 3+. To investigate factors associated with this low coverage, we carried out a secondary analysis of data from the Mali 2015 Malaria Indicator Survey. A multiple, logistic regression was used to analyze the factors associated with IPTp3+ among the 2382 interviewed women with a live last birth preceding the survey. Factors associated with higher IPTp 3+ after adjustment of potential confounding factors included: the households who had radio as source information (OR=0.71 95% CI [0.53-0.95]), and reside in Segou's region (AOR=0.56 95% CI [0.35; 0.90]). However women aged below 20 years ago was associated in lower IPTp 3+ (AOR=1.43 95% CI [1.03; 1.98]). The place of residence urban or rural, educational level, and wealth index were not associated with IPTp3+. This study identifies that media accessibility and Segou's region residence of women were a factor associated with IPTp3+.

## IMPACT OF STRATIFICATION METHODS USED TO TARGET INDOOR RESIDUAL SPRAYING IN BIKO ISLAND, EQUATORIAL GUINEA

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Targeting of interventions to areas most at risk is common for malaria control programmes, however, the methods used to determine stratification vary and their impact has not been well evaluated. In Bioko Island, Equatorial Guinea, blanket Indoor Residual Spraying (IRS) was policy up until 2014 when an evaluation revealed lower than expected coverage (57%), in part due to high refusal rates. In 2015, the Bioko Island Malaria Control Programme (BIMCP) decided to target communities for IRS utilising data collected from surveys undertaken in 2014. Stratification was based on community-level parasite prevalence, quality of housing, risk of case importation and previous IRS coverage (as a proxy for acceptability in the community). A risk score (R) and a risk-acceptability score (Ra) were derived for each community. In relation to the budget, the top 30% of communities with the highest R and Ra values were selected for IRS- targeting, with over 80% coverage achieved in these areas in 2015. Areas no longer targeted with IRS received a top of bed nets. Results from an island-wide prevalence survey in 2015 prior to spraying were compared to data from the same communities in 2016 to evaluate the impact of the targeted IRS. The data suggests that *P. falciparum* prevalence was significantly reduced in areas that had been targeted in the most

recent spray round. However, areas that were no longer part of the spray campaign maintained similar levels of prevalence between 2015 and 2016 suggesting that current control interventions in these areas are not currently sufficient to further reduce transmission although did prevent any apparent resurgence of transmission. Understanding the impact of stratification is key for future IRS targeting and will help the programme design more efficient strategies for malaria control.

## 1621

### VARIATION IN AMBIENT TEMPERATURE DRIVES THE SEASONALITY OF MALARIA PARASITISM IN WILD CHIMPANZEE RESERVOIRS ACROSS EQUATORIAL AFRICA

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African great apes harbor diverse assemblages of malaria parasites, including the progenitors of *Plasmodium falciparum* and *P. vivax*, which adapted to the human population following ancient cross-species transmission events. Vectors of ape parasites exhibit promiscuous biting preferences, presenting a public health imperative to identify human populations at risk of exposure for follow-up assessment of zoonotic potential and molecular barriers to transmission in epidemiologically relevant settings. To elucidate spatiotemporal variation in ape malaria parasitism, we model the ecological niche of ape malaria parasites in wild chimpanzee hosts by analyzing the relationship between environmental variables and fecal parasite rates. Analysis of 878 samples collected longitudinally from a cohort of 54 chimpanzees in western Uganda indicates that high prevalence (31.5%), early age of onset, and seasonality of infection characterize ape malaria epidemiology. These findings are consistent with an elevated magnitude of ongoing transmission within the reservoir, suggesting that humans living on the borders of many equatorial African forests are likely to be exposed to zoonotic parasites. Analysis of 2436 samples collected at 55 sampling sites across equatorial Africa recapitulate these findings and support the hypothesis that mean ambient temperature drives spatiotemporal variation in parasite prevalence. However, chimpanzee parasitism peaked at a lower mean ambient temperature (~24.5°C) than the commonly referenced temperature optimum of *P. falciparum* (~30°C). Forest cover was positively correlated with parasite prevalence, consistent with the finding that forest-dwelling Anopheline species constitute the primary vectors of ape parasites. These results suggest that adaptation of ape parasites to non-forest vectors may be a critical prerequisite for widespread emergence in human populations. We extrapolate these findings across equatorial Africa to generate a hypothetical exposure risk map, offering a tool for the prediction of exposure hotspots that can be mobilized to design zoonotic disease surveillance efforts.

## 1622

### DIFFERING PATTERNS OF PROTECTIVE ASSOCIATIONS FOR ANTIBODIES TO SURFACE ANTIGENS OF *PLASMODIUM FALCIPARUM*-INFECTED ERYTHROCYTES AND MEROZOITES IN IMMUNITY TO MALARIA IN CHILDREN

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Acquired antibodies play an important role in immunity to *Plasmodium falciparum* malaria and are typically directed towards surface antigens expressed by blood-stage parasites, such as merozoites and infected erythrocytes (IEs). The importance of specific IE surface antigens as immune targets remains unclear, especially in populations outside Africa, and a lack of tools has hampered the study of individual antigens. We evaluated antibodies and protective associations in two cohorts of older and younger children in Papua New Guinea (PNG). We used genetically-modified *P. falciparum* with reduced PfEMP1 expression to evaluate the importance of IE surface antigens and a *P. falciparum* isolate with a virulent phenotype defined by expression of specific PfEMP1 variants. We found that PfEMP1 was the dominant target of antibodies to the IE surface, with limited reactivity to other antigens. Antibodies were associated with increasing age and active parasitemia, and were higher among children exposed to a higher force-of-infection which was determined using molecular detection and genotyping. Antibodies to IE surface antigens were consistently associated with reduced risk of malaria in both younger and older children's cohorts. However, protective associations for antibodies to merozoite surface antigens was only observed in older children. This suggests that antibodies to IE surface antigens, particularly PfEMP1 variants linked with virulent phenotypes, play an earlier role in acquired immunity to malaria, whereas greater exposure is required to develop protective antibodies to merozoite antigens. These findings have implications for vaccine design, and serosurveillance of malaria transmission and immunity, especially in the current era of declining malaria transmission.

## 1623

### SEASONAL MALARIA CHEMOPREVENTION IN ANKILILOAKA, MADAGASCAR

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Malaria remains a major public health problem in Madagascar. From 2013 to 2015, an increasing malaria morbidity (6.8% to 12.5%) and malaria mortality (8.4% to 11.9%) was recorded. In 2015, malaria outbreak was mostly observed in South-West of the country, notably in commune of Ankiloaka, district of Toliara II. As a response to this situation, 10,000 long lasting insecticidal nets was distributed especially in this commune. Also, a strengthening of awareness-raising was carried out. Despite this, a resurgence of the number of cases of malaria was observed with 7 times more cases in November 2015 than in September 2015 (234 to 1566). Given this situation, the search of new prevention strategy is necessary to reduce the burden of this disease. The seasonal Malaria Chemo-prevention (SMC) is one of the strategies proposed by WHO to prevent morbidity and mortality related to malaria, particularly in areas where malaria transmission is highly seasonal. Indeed, SMC is able to reduce the incidence of malaria by 82% and severe cases by 77%. SMC was carried from December 2015 to February 2016 in the commune of Ankiloaka. SMC was administered in children 6 months to 14 years old given the dynamics of malaria epidemiology in Madagascar and where

children between 5 and 14 years old are mostly affected by the disease. Also, given that the Amodiaquine is available only as part of ASAQ in Madagascar, Artemisinin Combined Therapy (ACT) was used for 3 days of directly observed treatment. A total of 1686 children was recruited with 13,16% of lost follow up. As a result, we observed a decrease of 72% of malaria prevalence between pre-and post-intervention. A scaling up and an evaluation of cost efficacy of this prevention strategy will be recommended.

## 1624

### THE EFFECT OF HOUSING IMPROVEMENTS ON MALARIA IN AFRICA, 2000-2015

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Improving housing is a promising strategy for sustainable malaria control. As Africa undergoes rapid economic and social change, it is vital to understand continent-wide changes in housing quality and their impact on malaria, to inform effective inter-sectoral malaria strategies. Here we provide the first formal quantification of changes in housing quality across sub-Saharan Africa (SSA) from 2000 to 2015 and of the effect of these changes on malaria endemicity. Data on housing quality were abstracted from 131 unique Demographic and Health and Malaria Indicator Surveys comprising over 1.1 million households, and combined within a Bayesian geostatistical framework to estimate changes in housing quality between 2000 and 2015. These estimates were linked with a large database of malaria prevalence surveys, environmental, sociodemographic, and intervention factors to estimate the effect of housing quality on malaria endemicity in SSA during 2000-2015. We will present data, trends, and maps on changes in house quality across SSA, evaluate the impact on malaria endemicity, and discuss the potential of improved housing as an intervention against malaria in a range of transmission settings across SSA. Alongside the current suite of interventions, improvements in housing quality linked to socioeconomic development may be an important strategy to support long-term and sustainable malaria control, elimination and the prevention of re-emergence.

## 1625

### UNDERSTANDING MIGRANT BEHAVIORS AND MALARIA RISK IN AYEYARWADDY REGION, MYANMAR

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Myanmar reported less than 60,000 malaria cases in 2016, and with the growing threat of artemisinin resistance, is committed to eliminating malaria by 2030. Ayeyarwaddy is the third most populated region in Myanmar and accounts for nearly 10% of total malaria. Mobile and migrant populations (MMPs) in Ayeyarwaddy are considered at greater risk for malaria, but little is known about their malaria knowledge and behavior. A cross-sectional cluster survey was conducted in September 2016 in Ngaputaw township, the area that accounts for 44% of total malaria confirmed cases reported in Ayeyarwaddy. MMPs aged 15 years-old or above were interviewed in 48 village clusters conveniently sampled along the road network. Data were collected about malaria knowledge, prevention measures and access to care through face-to-face interviews conducted by healthcare workers. Among the 230 MMPs interviewed, 70% reported that malaria can be transmitted through a mosquito bite,

and 74% agreed with the statement that there is more risk to contract malaria when working at night. Sleeping under a mosquito net was mentioned as the preferred method for preventing malaria among 90% of the respondents, and 60% reported having at least one net. In relation to case management, 29% of respondents believed they had malaria at least once in the last 6 months, and of those, 95% reported they received a blood test. Additionally, 78% of respondents seeking any type of healthcare sought it in a public facility, and only 1% reported seeking care from village health volunteers (VHVs). MMPs demonstrated relatively good malaria knowledge and prevention practices amongst affected communities. Low utilization of VHVs may be due to the convenient sampling method along the road network. Their role in improving access to malaria diagnosis and treatment among MMPs should be further investigated.

## 1626

### MALARIA DISASTER IN VENEZUELA: TIME FOR ACTIONS

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Venezuela was the first nation in the world to be certified by the WHO for eradicating malaria in 70% of its territory in 1961. From 2008 onwards, the number of malaria cases started to increase slowly and, by 2013, progressive malaria epidemics have been affecting the country. The national surveillance system has been reporting confirmed cases weekly since the 1940s, a backbone of the malaria national program. The Ministry of Health (MOH) prohibited the publication of official epidemiological bulletins -including malaria indicators- in late-2014. We reconstructed the national malaria epidemiological data (confirmed cases and deaths) from national sources (weekly reports, key informant interviews, field data and PAHO/WHO). We estimated the number of malaria cases - adjusting for relapses, recrudescences, under-reporting and self-medication- and deaths by 2016. Officially, the MOH reported to PAHO/WHO 136402 and 240631 cases in 2015 and 2016, respectively. However, our findings show 212787 and 437097 cases for the same years. Comparing annual malaria cases between 2000 vs. 2016, there is an increase from 30234 to 240631 cases (officially, 795%) and adjusted cases with an increase of 1446%. Similarly, the number of malaria-related deaths rose from 24 in 2000 to 150 in 2016. Venezuela contributed to nearly half of the malaria cases in the Americas in 2016; it is spreading to its neighbor countries and the national government has not shown effective response to address the situation. Several factors have been contributing to this malaria disaster including a) severe shortages of drugs, insecticides, supplies, etc.; b) massive internal migration to illegal gold mining areas; c) limited financial and logistic support to control activities in the field; d) inadequate prioritization of interventions; and, e) collapse of health services. Effective actions including support with cost-effective interventions are urgently needed by international malaria partners in order to manage the malaria epidemic in Venezuela. An international multisectoral task force is needed to address this regional malaria disaster.

## 1627

### EFFECT OF MALARIA IN THE FIRST TRIMESTER OF PREGNANCY ON FETAL GROWTH: A PRE-CONCEPTIONAL COHORT STUDY IN BENIN

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In Africa, preventive drug strategies against malaria in pregnancy are recommended from the 2nd trimester, and bed nets are rarely distributed before the 1st antenatal care visit at 4 months of pregnancy. Therefore, women remain insufficiently, or not, protected during the 1st trimester. For the first time, we assessed the consequences of malaria in the first trimester of pregnancy on birth outcomes using a specifically-designed study. From June 2014 to March 2017, South Benin, 1214 women of child bearing age were recruited and followed up monthly until 411 became pregnant. Pregnant women were then followed up from 5-6 weeks of gestation until delivery. Microscopic malarial infections were detected monthly. Five ultrasound scans were performed for the datation of pregnancy and fetal growth assessment. Path analysis was used to assess the direct effect of malaria in the 1st trimester (i.e., not mediated by malaria in the 2nd or 3rd trimester) on small-birthweight-for-gestational age (SGA) according to Schmiegelow's charts. This preliminary analysis was based on the first 180 deliveries. The prevalence of SGA was 11.9%. Malaria in the 1st, 2nd and 3rd trimester of pregnancy accounted for 24.7%, 18.8% and 17.7%, respectively. After adjustment for malaria in the 2nd and 3rd trimester and other potential confounders, we found a marginally significant direct effect of malaria in the 1st trimester on SGA (aORa=2.34 [0.86-6.38]; P=0.10). We did not evidence any effect of malaria in the 2nd or 3rd trimester. Using a multivariate logistic regression model, women with repeated infections in the 1st trimester—and not thereafter—had the highest risk of SGA compared to uninfected women (aORa=6.86 [1.24-38.00], P=0.03). These preliminary results suggest an independent effect of malaria in the 1st trimester on fetal growth, which should be confirmed on the full sample. If confirmed, they may argue in favour of implementing additional preventive measures starting before or during the 1st trimester of pregnancy.

## 1628

### FACTORS ASSOCIATED WITH SUBMICROSCOPIC MALARIA PARASITE CARRIAGE IN SICK CHILDREN AGED 6 - 59 MONTHS OLD IN URBAN AND PERI-URBAN FACILITIES IN BLANTYRE, MALAWI

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The extent and role of submicroscopic malaria (SM) in *Plasmodium* transmission and clinical disease in urban settings remain unclear. SM contributes to pathogen transmission in sub-Saharan Africa urban settings that experience relatively low incidence, and therefore being able to identify human reservoirs is critical for successful malaria control and elimination. This study assessed factors associated with submicroscopic *Plasmodium* parasite carriage in outpatients presenting to urban and peri-urban facilities in Blantyre, Malawi. Children 6-59 months old attending sick child clinics were enrolled from four urban and two peri-urban facilities from April 2012-October 2015. Children were tested for malaria by both microscopy and real time PCR (rt-PCR). SM was defined as a positive PCR test that was negative by microscopy in any sick child. To evaluate factors associated with SM carriage, we compared all children who had SM in the study to a computer-generated, random sub-sample of children who were *Plasmodium* negative by both microscopy and rt-PCR in the ratio of 1:2. Of the 3,348 children enrolled, 69 (2.1%; 95% CI: 1.6%-2.5%) had SM. Almost all children (98.6%) with SM were not prescribed antimalarial drugs by the attending clinician on the day of clinic visit. There were no statistically significant differences in SM prevalence between urban and peri-urban areas (2.5 vs. 1.8%, p=0.2); between patients with or without history of fever (0.3% vs. 3.9%, p=0.7); between patients whose

temperature was  $\geq 37.5^{\circ}\text{C}$  vs.  $< 37.5^{\circ}\text{C}$  (1.5% vs. 2.4%, p=0.1), nor; between rainy and dry seasons (1.7% vs. 2.4%, p=0.2). In multivariate analyses, residing in an urban site was associated with increased odds of SM, while age (24-59 months) and fever ( $\geq 37.5^{\circ}\text{C}$ ) were associated with decreased odds of SM. Our results show that SM parasite carriage is occurring in sick, under-five children in both urban and peri-urban settings in Malawi. Further studies are needed to investigate the effect of untreated SM parasitemia on illness outcomes in these children, the burden of SM parasite carriage in older age groups and its significance in malaria transmission in urban settings.

## 1629

### DECLINE OF MULTIPLE INFECTIONS OF *PLASMODIUM FALCIPARUM* FROM 2007 TO 2012 AND DIFFERENCE IN MULTIPLE INFECTIONS BETWEEN HUMANS AND MOSQUITOES IN WESTERN KENYA

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With vector control and efficacious interventions implemented over 15 years, malaria prevalence declined dramatically from approximately 80% in 1996 to 26% in 2008 in an intensely studied rural area in western Kenya, but has stagnated at around 40% since 2009. Our previous studies have reported that overall rates of multiple infection and genetic diversity of *P. falciparum* in human blood did not change from 1996 (baseline) to 2001 and 2007 following intensive malaria control interventions in the same area. An additional follow up study was performed on blood samples collected in a cross-sectional survey in 2012. To explore if the lack of change in the rate of multiple parasite infections in humans was related to vector-parasite interactions, we also genotyped midgut parasites from mosquitoes collected from within the study area in 2012. In total, 258 blood smear-positive samples and 61 oocyst-positive mosquitoes were genotyped employing eight microsatellite markers. The results from blood were compared to our previously reported data in 2007. Differences in rates of multiple infections in humans and mosquitoes were also assessed. Preliminary results show that mean allele counts (MAC) declined from 2.76 (2007) to 1.64 in 2012 (p<0.001), while there was no significant change in the overall proportion of multiple alleles (MA) between 2007 (87.7%) and 2012 (83.3%) in human samples. In addition, overall MAC was significantly higher in human blood (1.64) than in mosquito midguts (1.48) (p<0.001), but no differences were seen in overall MA between human blood and mosquito midgut (p=0.084). For individual *loci*, *adl* and *taa60*, both MAC and MA were significantly higher in human blood compared to mosquito midguts while for *pfpk2* and *p195*, MAC was significantly higher in human blood. No differences in MAC or MA were detected at any other *loci*. The decline in mean allele counts in 2012 may indicate reduced multiple infections within-host. Results from this study also suggest a higher level of multiple infections in humans compared to mosquitoes. Further analysis of genetic diversity and population structure of parasites in the two hosts is needed on these samples.

### AGE-SPECIFIC CHANGES IN THE INCIDENCE OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA: SEASONAL MALARIA CHEMOPREVENTION (SMC) IN AN AREA WITH INTENSE TRANSMISSION: DANGASSA, MALI

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The community of Dangassa in Mali has experienced intense malaria transmission during the past 2-3 decades with peak Entomologic Inoculation Rates (EIRs) >10 infectious bites per person per month in October and November and an annual Incidence of uncomplicated malaria that approaches 1.0/person/year for the persons at greatest risk (children 5-14 years of age). Because of interest in the impact of Seasonal Malaria Chemoprevention (SMC) on the incidence of human disease, data from longitudinal cohort-based studies in Dangassa that began in 2012 have been examined to compare the incidence of uncomplicated malaria in 2013-2014 before the implementation of SMC to its incidence during the implementation of SMC in 2015-2016. From 2013-2014 to 2015-2016, the average incidence of uncomplicated malaria in Dangassa rose 9% from 477 to 518 cases of uncomplicated malaria/1,000 persons/year. Across age groups, the only decrease and the lowest increase observed were among children 0-4 and 5-9 years of age. These children were also the only persons in the community who received amodiaquine and sulfadoxine-pyrimethamine 3-4 times during 2015 (children 0-4 years of age) and 2016 (children both 0-4 and 5-9 years of age). Unlike previous reports on SMC, in Dangassa there was no evidence that SMC for children 0-9 years of age provided any benefits to the other (older) members of community. In those groups (persons 10+ years of age), the annual incidence of malaria rose from 2013-2014 (before SMC) to 2015-2016 (during SMC). The average increase in incidence across those age groups was 69% and ranged from 15% in persons 30-39 years of age to 173% in persons 10-14 years of age. These results are consistent with the patient data for this study, which indicate compliance with the amodiaquine + sulfadoxine-pyrimethamine regimen was 85% and with the modest (30%) reduction in disease observed in children 0-4 years of age. They suggest that strategies such as SMC may be less efficacious in areas such as Dangassa where malaria transmission is intense.

### EPIDEMIOLOGICAL TRANSITION OF MALARIA IN GABON: DATA FROM A SENTINEL SITE (MELEN 2010-2016)

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Between 2005 and 2008, a decline of malaria prevalence has been observed in Gabon followed by a rebound of the infection rates in 2011. This change in malaria burden related to a slowdown of malaria strategies. Such variation requires a monitoring of malaria prevalence evolution, in order to better define the control strategies. Thus, the aim of the present study was to assess the prevalence of malaria among febrile patients in a sentinel site between 2010 and 2016. Cross-sectional surveys were carried between 2010 and 2016 in a sentinel site for malaria surveillance, located at the Regional Hospital of Estuaire in Gabon. Parasitemia and malaria parasites species were determined. Febrile patients were screened for malaria based on microscopy. Body temperature, history of fever, age, and sex were collected. Any antimalarial drug intake before consultation was also reported. A total of 6,723 febrile patients were enrolled, 58,7% (n=3388) were less than five years. The sex ratio was of 1.08. Overall, 65.4% (n=3821) of the patients used a bed net. Moreover, among patients,

69.9% (n=3744) had a fever that lasted more than 24 hours. History of fever associated to self-medication with anti-malarial drug was found in 50.8% (n=1179) of the study population. The prevalence of malaria was 33.8% (n=2270) essentially at *Plasmodium falciparum* and 33.2% (n=1270) of the patients used bed net was malaria infection. The evolution of malaria prevalence showed 3 phases: the first phase corresponded to the lowest prevalence with 29.4% in 2010 and 27.2% in 2011. The second phase was marked by an increase of malaria between 2012 to 2015 with a peak in 2012 with 40.7% and in 2013 39.6%; 2014 36.3%. During the last phase in 2015 and in 2016 the frequency of malaria infection declined: 32.7% and 33.9% respectively (p<0.01). The present study underlines a variation of malaria prevalence in a sentinel site in Gabon over a period of six years. This data suggest the need of a review of national guidelines of malaria control strategies.

### REGIONAL BURDEN OF MALARIA IN PATIENTS PRESENTING WITH FEBRILE ILLNESS IN KENYAN HOSPITALS

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The prevalence of malaria infection and distribution patterns of *Plasmodium* parasites vary across the country. This can be attributed to varying climatic conditions primarily temperature and rainfall that impact both the rates of sporogonic and mosquito life cycles. This study evaluated the burden of malaria in patients who presented with acute febrile illness at 11 hospitals/clinics in Kenya. A total of 2,522 blood samples were collected and qPCR used for parasite speciation and densities determination. *Plasmodium* parasites were found in 32% (800/2522) of the AFI patients, with the following regional distribution: 38% (397/1038) for hospitals in the Lake Victoria basin, 38% (262/687) for those in Garissa, 28% (29/103) for patients attending Malindi District Hospital and 16% (112/694) for the Kisii District Hospital. In all the surveillance hospitals, about 50% of parasitemias were below the limit of detection by microscopy or RDTs. This number was highest (89%) at the Coast. Unlike in the other surveillance sites, in the Lake Victoria basin, more than 30% of the parasitemias were above pyrogenic threshold (>250,000 parasites/ $\mu$ L). Of the 800 samples with malaria parasites, only 74% (594/800) could be speciated: 73% (434/594) as mono-infections of *P. falciparum*, 7% (42/594) *P. ovale* and 2% (10/594) *P. malariae*. The remaining 18% (108/594) were mixed infections. All the four species of *Plasmodium* were identified at all surveillance sites except *P. malariae* which was not observed in the coastal region. In conclusion, this study identified a huge proportion of individuals with parasitemias below detection limit of microscopy or RDT. In the Lake Victoria basin, over 30% of the parasitemias were above pyrogenic threshold that is associated with increased risk of morbidity and mortality. Efforts are underway to establish why 26% (206/800) of *Plasmodium* positive samples could not be speciated.

### ELUCIDATING THE ROLE OF MOSQUITOES IN DRUG RESISTANT MALARIA EPIDEMIOLOGY

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Molecular surveillance is a critical tool in monitoring drug-resistant malaria, yet little is known about the spread of resistance-mediating polymorphisms through the mosquito vector. In order to gain a more comprehensive understanding of the prevalence and transmission dynamics of drug resistance at the household and village level, this research combines advanced mutation-detection methods with a novel approach: analyzing blood meals in mosquitos that recently fed on humans. Field samples

were obtained over a peak transmission month in eight households in the village of Diarkadougou-East in southwestern Burkina Faso in 2014. Cross-sectional sampling using aspirations of indoor-resting mosquitoes (n=543) was conducted at eleven time points spread over 32 days. Abdominal contents of blood fed mosquitoes were pressed onto 96-well format FTA cards. DNA was extracted and genotyped for clonal diversity using merozoite surface protein-2 (MSP-2), and drug resistance was assessed at 7 parasite polymorphisms in *pfcr1*, *pfmdr1*, *dhps*, and *dhfr*. Thus far, we have analyzed two alleles from the *pfmdr1* gene (N86Y and Y184F) using a ligase detection reaction fluorescence microsphere assay at 4 time points, and MSP-2 genotyping has been conducted using capillary electrophoresis. Notably, over half of collected *Anopheles* blood meals were positive for *Plasmodium falciparum*. Among those samples, there was a high multiplicity of infection (MOI) by MSP-2 genotyping (mean=2.5 strains, range=1-9 per sample) and a high prevalence of resistance-conferring mutations (21% of blood meals contained the 86Y and 73% contained 184F). We plan to examine all time points, and four additional mutations to investigate changes in allele prevalence/frequency over time within a household and within the village. This research will shed light on drug resistance patterns across time and a limited geographic space during peak transmission in a highly endemic region of Burkina Faso.

## 1634

### OVERNIGHT TRAVEL AND THE RISK OF MALARIA: PROSPECTIVE COHORT STUDIES AT 3 SITES IN UGANDA OF VARYING MALARIA TRANSMISSION INTENSITY

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Although malaria remains highly endemic in most of Uganda, there are areas where transmission is much lower due to urbanization or the implementation of indoor residual spraying of insecticide (IRS). People living in relatively low transmission areas may be at increased risk of malaria following travel. To evaluate associations between overnight travel (a night out of the sub-county of residence) and the incidence of malaria, we conducted cohort studies at 2 sites with relatively low transmission intensity, Walukuba (peri-urban area) and Nagongera (rural area receiving IRS); and 1 site with moderate transmission intensity, Kihhi (rural). All children aged 0.5-10 years and one adult from 100 households per site were enrolled. Data on dates of overnight travel were collected from October 2015 through June 2016. Malaria, defined as fever in the presence of parasites detected by microscopy, was diagnosed using passive surveillance at clinics available full-time to study participants. Any overnight travel was reported in 64 of 273 (23%) study participants in Walukuba, 31 of 313 (10%) in Nagongera, and 22 of 314 (7%) in Kihhi. Considering only participants who reported any overnight travel, we compared the incidence of malaria 1-60 days following overnight travel (recent travel) with that before or >60 days following overnight travel (no recent travel). In Walukuba, recent travel was associated with an almost 5-fold increase in the incidence of malaria (0.80 vs. 0.17 episodes PPY, IRR=4.78, 95% CI 1.70-13.4, p=0.003). In Nagongera, recent travel was associated with an almost 10-fold increase in the incidence of malaria (0.95 vs. 0.10 episodes PPY, IRR=9.85, 95% CI 1.87-51.9, p=0.007). In Kihhi, recent travel was associated with a non-significant 2-fold increase in the incidence of malaria (1.83 vs. 0.79 episodes PPY, IRR=2.05, 95% CI 0.73-5.75, p=0.17). In 2 relatively low transmission areas of Uganda, recent overnight travel was associated with a markedly higher incidence of malaria compared to that in non-travelers. These findings suggest the need for targeted intervention to reduce the risk of malaria during overnight travel.

## 1635

### LONGITUDINAL SURVEY OF MALARIA BURDEN TO ASSESS THE EFFECTS OF MALARIA CONTROL INTERVENTIONS IN A LOW TRANSMISSION SETTING IN SOUTHERN ZAMBIA

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Assessment of malaria control interventions is key to guiding future programs to support malaria elimination. Malaria interventions in Southern Province, Zambia, include indoor residual spraying (IRS), insecticide treated net distribution, mass drug administration and a reactive test and treat program. The impact these interventions have had coupled with rainfall on the malaria burden in this area are not well understood. Macha Research Trust in southern Zambia has been using a mobile phone short message service (SMS) surveillance system to transmit weekly malaria cases from 14 rural health centers (RHCs) in the catchment of Macha Mission Hospital, Choma, since 2012. We analyzed this SMS data to assess trends in the burden of malaria and the effect of control efforts across years and health centers. Data showed that malaria transmission in Macha was seasonal with high number of cases after the rainy season (April - July) and low number of cases in the hot dry season (August - October). Inter-annual variation in reported RDT positives was evident, with little change in number of cases in the first year (534 in 2012, test positivity rate (TPR) 7.6% and 517 in 2013, TPR 4.1%), a marked increase in 2014 (957, TPR 8.1%), reductions in 2015 (643, TPR 5.0%), followed by a large spike in 2016 (2318, TPR 12.8%). The high number of cases in both 2016 and 2014 was associated with lower and infrequent rains, hypothesized to support larvae development due to increased and more stable stagnant water. The amount of rainfall received in 2014 was 501 mm and 369 mm in 2016 compared to 750 mm, 640 mm and 578 mm in 2012, 2013 and 2015 respectively. In 2016, three RHCs with catchment areas where IRS was rolled out reported low numbers of malaria cases (52 - 63) compared to (92 - 2318) cases in RHCs without IRS. Malaria transmission in Macha appears to be largely governed by the amount and pattern of rainfall; higher and frequent rainfall of at least four days per week was associated with fewer cases. The Number of malaria cases was lower in RHCs covered by IRS, but further in-depth analysis on this is required. This shows that well timed interventions are key in lessening the malaria burden.

## 1636

### PREVENTION OF TRANSFUSIONAL MALARIA IN THE STATE OF SAO PAULO BRAZIL

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Malaria is a disease of high morbidity, but the occurrence of asymptomatic carriers with low parasitemias is a challenge for screening of blood donors in endemic and non-endemic areas. This scenario led to the implementation of epidemiological or even laboratory screening in different countries, according to the risk to which they are submitted. In Brazil there is a distinct policy for donating blood in the endemic and non-endemic areas. High-risk donors, coming from endemic areas are rejected for donation for 30 days. From 1-12 months, they are accepted if they test negative for *Plasmodium*. Cases of transfusion malaria in Brazil have been reported in the state of Sao Paulo and the asymptomatic donors were infected in areas considered as non-endemic, where sporadic autochthonous cases are reported. The aim of this study was to evaluate some epidemiological questions that can identify situations of greater

risk of infection, allowing the improvement of the transfusion safety and the screening algorithm, without compromising the bloodstock. Blood samples were collected from 6,324 from candidates that attended Fundação Pró-Sangue/Hemocentro de Sao Paulo. A questionnaire was applied to candidates in order to associate the epidemiological features with laboratorial results. IgG antibodies were surveyed in plasma samples by ELISA using *P. vivax* MSP1-19 recombinant antigen. qPCR *Plasmodium*-genus specific and thick blood smear were carried out. Out of 6,324 donors, 58 (0.9%) were positive for IgG anti-PvMSP1-19. None sample was positive in qPCR or in microscopy. Among the 58 samples positive by serology, 86.2% were from donors with displacements to the Atlantic Forest biome in São Paulo State and/or who live in regions near the Atlantic Forest. Among the 118 candidates living near the Atlantic Forest, 12.7% were positive in ELISA, pointing to the need for using laboratory assays in the screening of donors. The adoption of a questionnaire with specific questions should be applied in areas where the epidemiology of malaria discloses a particular scenario, which do not means less risk for transfusion malaria.

## 1637

### LESSONS FROM TWO DECADES OF MALARIA SURVEILLANCE IN RHODE ISLAND

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In the United States, malaria is primarily imported by travelers to endemic areas and immigrants or refugees from those regions. The Centers for Disease Control and Prevention report 1500-2000 cases per year nationally. However, there is a paucity of comprehensive malaria surveillance data at the state level. This study was a retrospective chart review of all patients with a confirmed diagnosis of malaria treated at the two largest academic referral hospitals in Rhode Island from 1997-2015. We identified patients using multiple databases including clinical surveillance software (Theradoc), malaria ICD9/10 discharge codes and cases reported to the RI Department of Health (DOH) and compared trends in annual incidence between these data and RI specific CDC data. There were 192 documented cases of malaria in RI during a 19-year period. None of the available databases was complete with more than 40 patients missing from each one. Of 161 cases with relevant information, 97(51%) were visiting friends or relatives (VFR), 24(13%) were immigrants, 17(9%) were refugees, 18(9%) were business travelers, 10(5%) were tourists, and 6(3%) were related to research/education. 9 individuals had >1 reason for traveling. Most cases were from Sub-Saharan Africa (80%). Appropriate prophylaxis was prescribed in 41 cases (21%) but only 8 patients completed the course. The most common type of malaria was *Plasmodium falciparum* (77%), *P. vivax* (9%), *P. ovale* (3%), *P. malariae* (2%) and 9% were unknown. No mixed infections were reported. 33 (21%) cases required ICU admission. 28(15%) patients had severe manifestations including cerebral malaria (n=18), pulmonary edema (9), and severe anemia (7). No deaths were reported. Almost one-fifth of patients diagnosed with malaria in RI over 2 decades had life-threatening disease requiring intensive care. We identified multiple missed opportunities for malaria prophylaxis, patient education and DOH notification. The public health establishment should address these deficiencies at national and state level to improve malaria prevention and surveillance.

## 1638

### A MALARIA INDICATOR SURVEY IN A MALARIA ELIMINATION AREA OF PHU YEN PROVINCE, COASTAL VIETNAM

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Indicators for malaria prevention should reflect the most important interventions for achieving malaria elimination in low endemic areas. We aimed to assess malaria prevention indicators in an elimination setting against Vietnam's malaria elimination strategic targets. We conducted a malaria indicator survey. Census data from 2015 revealed a total of 4668 households among 18 villages in Phu Yen Province. These data were used to select 20% of the households. GPS coordinates were captured where each interview was conducted. Data were collected using a smartphone application (KLL Collect®) and uploaded in real-time into an online database (ONA®). Logistic regression models were used to calculate prevalence odds ratios (PORs) and 95% confidence interval (CI) to assess differences among those who slept in forests and on farms. A total of 4411 people were interviewed, of which 1074 were respondents and 3137 were their members. Among 1074 respondents, 472 slept in forest and 92 slept on a farm, 132 slept both sites, and 378 slept at their villages within last 12 months. Age, gender, literacy, occupation and ethnicity were significantly different among those who slept in forest versus at other sites (p<0.001). Malaria infections were higher among those who reported having slept in the forest (147 [57%]) versus those who slept on a farm (18 [42%]). Ownership and use of all net types among forest-goers was significantly lower than those who reported having slept on a farm or in their village. Huts without walls in the forest were significantly prominent at forest goers' sleeping site (POR=10.3; 95% CI 4.67–22.7). All respondents who slept in a forest requested standby malaria drugs and one-third of them self-treated without blood testing. The proportion of respondents who knew about malaria prevention was 34% (82) without a subjective measure. Data about intervention coverage at the actual transmission site and net preference need to be routinely captured. Targeting preventive interventions where transmission occurs is critical. If malaria elimination is to be achieved, control activities should be scaled up in forest and forest-fringe communities.

1639

### COMMUNITY-BASED SCHEDULED SCREENING AND TREATMENT OF MALARIA DURING PREGNANCY PROVIDES ADDITIONAL PROTECTION AGAINST FEBRILE ILLNESSES DURING THE FIRST YEAR OF LIFE IN A BIRTH COHORT STUDY IN BURKINA FASO

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In order to prevent the adverse consequences of malaria in pregnancy (MiP), a community-based scheduled screening and treatment of malaria in combination with intermittent preventive treatment with sulfadoxine-pyrimethamine (CSST+IPTp-SP) has been proposed as potential alternative to the standard IPTp-SP. In this study, we investigated the effect of CSST+IPTp-SP on the risk of malaria and non-malaria fevers (NMFs) during the first year of infant's life. Between June 2014 and October 2016, a birth cohort study (n=734) was conducted in Nanoro, a high seasonal malaria transmission area in Burkina Faso. Clinical malaria episodes were recorded by passive case detection from birth until 12 months of age. In addition, 4 cross-sectional surveys were carried out at 3, 6, 9 and 12 months to detect asymptomatic infections. Multivariate models were used to assess the effect of CSST+IPTp-SP strategy on time to first clinical malaria episode, incidence of malaria episodes, asymptomatic infections and incidence of all NMFs. The overall incidence of clinical malaria was 1.03 episode per child/year whereas incidence of all NMFs was 1.18 episode per child/year. Prevalence of asymptomatic malaria was 17.7% (120/678), 20.1% (127/631), 18.4% (108/587) and 30.9% (200/648) at 3, 6, 9 and 12 months, respectively. Compared to the standard IPTp-SP, the CSST+IPTp-SP strategy significantly increased time from birth to the first clinical malaria episode amongst infants born during malaria low transmission season (HR=0.74, 95% CI= 0.55-0.99, P= 0.047), and reduced the incidence of NMFs in infants born during malaria high transmission season (adjusted IRR= 0.80, 95%CI= 0.65-0.98, P= 0.036). In addition, CSST+IPTp-SP strategy tends to increase chance of having an asymptomatic infection that did not develop into a clinical episode (adjusted RRR= 1.61, 95%CI= 0.93-2.77, P=0.087). Our findings indicate that MiP preventive treatments may provide additional protection against both malaria and NMFs for infants during the first year of life.

1640

### HOST AGE AND PLASMODIUM FALCIPARUM MULTICLONALITY ARE ASSOCIATED WITH GAMETOCYTE PREVALENCE: A 1-YEAR LONGITUDINAL COHORT STUDY

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Since *P. falciparum* (Pf) transmission from humans to mosquitoes relies exclusively on gametocyte infection of mosquitoes, several malaria transmission control strategies aim to disrupt this step of the life cycle. Thus, a better understanding of which individuals constitute the primary gametocyte reservoir within an endemic population, and the temporal dynamics of gametocyte carriage, especially in seasonal transmission settings, will not only support the effective implementation of current transmission control programs but also inform the design of more targeted strategies. From June 2013, we conducted a 1-year longitudinal cohort

study to assess the year-long dynamics of Pf gametocyte carriage in Kenieroba, a village with intense seasonal malaria transmission in Mali. We recruited a cohort of 500 individuals ranging 1-65 years in age, measured gametocyte prevalence every month for 1 year using Pfs25-specific qRT-PCR, and analyzed the effects of host age and multiclonality of Pf infection on gametocyte prevalence for 1 year. During this period, most Pf infections in this population were accompanied by gametocytemia (51-89% of Pf-positive individuals). We observed no effect of seasonality (wet vs. dry) on gametocyte prevalence among Pf carriers, while gametocyte prevalence was significantly affected by age (P = 0.023). Although gametocyte prevalence among >30-year-old Pf carriers appeared relatively lower than in younger age groups, a multiple comparison test detected no statistically significant difference between age groups. Interestingly, we found that Pf infections with gametocytemia had higher multiclonality than Pf infections without gametocytemia. Taken together, our results demonstrate that transmission control programs should target a relatively large section of this population in order to significantly impact transmission. Furthermore, our results indicate that the occurrence of multiple Pf genotypes in an infection, a common feature of Pf infections in high transmission areas, is associated with gametocyte prevalence.

1641

### SCALING IRRIGATION AND MALARIA RISK IN MALAWI

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Irrigation systems are expanding across Africa to improve food security, particularly given the changing climate and fragility of food security systems. While the expansion of irrigated agriculture has demonstrated a 2-4-fold boost in crop productivity, the agrarian transformation of the landscape for irrigated agriculture is associated with increasing malaria vulnerability for those residing within close proximity to irrigated agricultural schemes. We present a critical examination of the dynamic changes in irrigated agriculture on malaria vulnerability across Malawi. Malawi is a small-holder agrarian society; the government of Malawi established a major program, the Green Belt Initiative (GBI), a long-term program aimed at land use modifications for the development of extensive small and large-scale irrigation. Through the GBI, nearly 1 million hectares of land have been offered to local and international investors for irrigated agriculture. Yet, while Malawians continue to face challenges directly related to food insecurity, the country is simultaneously holoendemic for falciparum malaria. Using the Bwanje Valley Irrigation Scheme as a case study, this work critically examines the impact of spatial structure on malaria risk across scales. Through a combination of time-series remotely sensed land cover data, the proposed GBI sites, and historical malaria prevalence data from the Demographic Health Survey, these spatial relationships offer predictive power for hypothesizing mosquito vulnerability, exposure risk, and malaria transmission driven by irrigated agriculture across Malawi.

1642

### CONFIRMATION OF MALARIA DIAGNOSIS IN AFRICAN COUNTRIES WITH THE HIGHEST BURDEN

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The increased access to an accurate diagnosis of malaria improves the quality of care, and ensures a rational use of antimalarial medicines. In early 2010, WHO recommended that every suspected malaria case be confirmed by microscopy or a rapid diagnostic test (RDT) prior to treatment. Also since 2010 the World Malaria Report (WMR) 2010

includes the reported number of cases positive for microscopy and/or RDT positive. In order to assess the reported compliance of countries with this policy, we have extracted from the Annex 4H of the WMR 2016 the number of cases, cases confirmed by microscopy and cases confirmed by RDT in 2000, 2005, 2010 and 2015 of those 13 countries which reported 80% of those approximately 129 million malaria cases of 2015 reported by the WHO-Africa Region. Unfortunately, as in previous years, the WMR 16 does not identify the number of cases which were confirmed by both microscopy and RDT; having observed that for these countries and during the years indicated: a) the percentage of cases confirmed by microscopy was never higher than 13%, and b) the sum of cases confirmed by both methods is never higher than the total of cases reported, in this report we will use "confirmed" as the addition of those confirmed for each method. In the countries included in the analysis, the percentage of cases confirmed with respect to the total reported was 1, 9, 16 and 66% in the years 2000, 2005, 2010 and 201, evidencing a remarkable increase in the access to lab confirmation since the WHO policy was set. However inter-annual increases higher than 50% in 2014 and 2015 were observed in six countries which in some cases coincided with a decrease in the total number of cases reported, raising concerns about the inclusion of those cases not confirmed in the country reports. We plan to collect additional information from these countries to assess if changes in the national strategies, availability of resources and projections of the total number of cases, to assess the reliability of the data reported in the WMR.

## 1643

### FIRST TO BED, LAST TO BE BITTEN

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The governments of Haiti and the Dominican Republic have a binational agreement to work towards the elimination of malaria on the island of Hispaniola by the year 2020. This study aims to examine individual behaviors, like ITN ownership and time to bed, matched with malaria seroconversion status. In 2013, cross-section survey data ( $n = 377$ ) were collected in community, clinic and school settings from the Ouest and Sud-Est departments of Haiti. Descriptive statistics of survey responses were presented with sample sizes and frequencies. A logistic regression model was constructed to examine associations between bed-time and malaria, as measured by Apical Membrane Antigen (AMA-1) and Merozoite Surface Protein (MSP-1) AMA and MSP antibodies. The majority of survey participants (87%) knew that mosquitoes transmit malaria and owned bed nets (68%). Approximately half of the sample (47.5%) used in-home insecticides. The most common sources of malaria-related education came from clinics, the radio or school. After adjusting for city location, socioeconomic status indicators, age and knowledge of someone with malaria, individuals who indicated going to bed between 18:00-20:00 were 80% less likely to have been exposed to malaria than those who went to bed after 22:00 (OR = 0.20, 95% CI: 0.07-0.52), which is in line with suspected feeding behaviors of *Anopheles albimanus*. The reported frequencies of malaria-related knowledge and behaviors in these departments can be used as a reference point for comparison in future studies or interventions in these departments. This study adds to the limited research on the impact of mosquito biting behavior in Haiti and its link to malaria exposure based on bed time. Future research is needed to investigate vector competence and feeding behavior of *A. albimanus*, to better understand peak biting hours and host seeking preferences.

## 1644

### IMPACT OF MASS NET DISTRIBUTIONS ON MALARIA PREVALENCE, ANEMIA AND INTERVENTION COVERAGE IN ABIA AND PLATEAU STATES, NIGERIA

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Nigeria accounts for more than 25% of malaria cases globally. As part of the National Plan for malaria elimination, mass distribution of free long-lasting insecticidal nets (LLINs) began in 2009. Abia state, in South East zone, distributed 710,530 nets in 2012; Plateau state, in North Central zone, distributed 1.5 million nets in 2010 and 2.1 million in 2015. Modified malaria indicator surveys were conducted in 2010 (pre-distribution) and September 2015 to assess the impact of mass net distribution on malaria and anemia prevalence. At least 955 households and 4297 individuals per state were sampled in each survey. LLIN ownership increased significantly in both states between 2010 and 2015: the proportion of households owning at least 1 net rose from 10.1% to 58.7% in Abia ( $p < 0.001$ ) and from 35.1% to 85.5% in Plateau ( $p < 0.001$ ). Full net access (1 net/2 persons) also increased in Abia from 1.4% in 2010 to 37.3% in 2015 ( $p < 0.001$ ) and in Plateau from 6.3% to 50.0% ( $p < 0.001$ ). Despite those increases, coverage in both states was less than the national target of 80%. Reported net use the previous night in all individuals increased significantly in Abia from 3.4% to 24.2% ( $p < 0.001$ ) and in Plateau from 14.7% to 65.6% ( $p < 0.001$ ). Net use in children under 5 and pregnant women was 3-4 times higher in 2015 compared to 2010, but failed to reach the 80% goal in either state. Age-adjusted microscopy-diagnosed *Plasmodium* prevalence significantly decreased in Abia from 36.1% (95% CI: 32.3-40.1) in 2010 to 26.4% (95% CI: 20.1-31.5) in 2015. In Plateau, a non-significant increase from 36.6% (95% CI: 31.3-42.3) to 43.4% (95% CI: 39.9-46.9) occurred. Over the same period, anemia in children 10 years and younger also significantly declined in Abia from 74.7% (95% CI: 72.1-81.0) to 58.3% (95% CI: 53.9-62.6), with a non-significant reduction observed in Plateau from 57.1% (95% CI: 50.6-63.4) to 52.5% (95% CI: 44.6-60.2). In summary, significant gains in net coverage and use occurred in both states, with reductions in *Plasmodium* and anemia prevalence observed in Abia, but not Plateau. Additional measures are needed to ensure intervention coverage meets national targets.

## 1645

### GEOGRAPHIC TRENDS IN IDENTITY BY DESCENT BETWEEN MALARIA PARASITE POPULATIONS

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Molecular data are increasingly used to track routes and volumes of disease transmission. However, methods sensitive to recent and local migration events relevant to many disease elimination programs are

lacking for sexually recombining organisms such as the *P. falciparum* malaria parasite. Using data from four clinics on the Thai-Myanmar border, we demonstrated the ability of relatedness based on identity by descent to resolve recent connectivity between proximal *P. falciparum* populations, while traditional metrics ( $F_{ST}$ ) show no such relationship. Here we evaluate this approach using an independent dataset from Colombia. Preliminary results based on data from four states on the Colombian coast, sampled over 8 years, show a decrease of 0.01 in the log odds of relatedness with every kilometer and week between samples, but only when one population is excluded from the analysis. We explored factors influencing the nonconformity of the excluded population, including temporal breadth of sample collection and greater proximity to the international border, and discuss considerations involved in making inferences of population connectivity from empirical genetic data. We believe that this approach has enormous promise for resolving transmission dynamics in natural parasite populations that can be further improved using human behavioral data extracted from travel surveys or mobile phone records.

## 1646

### OPTIMIZING APPROACHES TO GENERATE WHOLE-GENOME SEQUENCE FROM NON-LEUKOCYTE DEPLETED *PLASMODIUM FALCIPARUM* CLINICAL SAMPLES

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With the advancement in high-throughput sequencing technologies, genomics is contributing to progress in understanding the evolution of drug resistance, the dynamics of disease transmission, and the rational design of malaria vaccines. However, obtaining whole genome sequence data from clinical samples is one of the major hurdles in the field of malaria genomics. In order to obtain whole genome sequence data from non-leukocyte depleted blood and dried blood spots, we optimized approaches to generate high-quality, whole genome sequence data from *P. falciparum* clinical samples with low amounts of DNA, including approaches to degrade the host DNA, selectively amplify the parasite genome, and perform whole-genome capture. We used *MspII* to degrade human host DNA, and selective whole-genome amplification to amplify the parasite DNA. Our initial results show that *MspII* is inefficient as it degrades human as well as parasite DNA. We also show that selective whole-genome amplification efficiently amplifies only the parasite DNA in the presence and absence of human DNA contamination. Also, optimizing this approach by diluting and filtering the DNA before amplification greatly enhances the amplification process, suggesting that inhibitors from the extraction could be interfering with the amplification. Indeed, we observed that diluting and filtering the DNA before amplification improves the percent of the parasite genome covered for a range of different parasitemias from 500 parasites/ $\mu$ L to 10,000 parasites/ $\mu$ L, and in non-leukocyte-depleted samples, based on initial results. We are currently exploring the use of whole-genome capture methods to generate sequence data from samples in which the proportion of host DNA makes direct sequencing prohibitively costly. These approaches will help overcome one of the major challenges in the field of *Plasmodium falciparum* genomics and may lessen the need to leukocyte deplete samples in the field.

## 1647

### NEW *PLASMODIUM FALCIPARUM* GENOME ASSEMBLIES FROM DIVERSE ENDEMIC REGIONS ENABLES THE COMPREHENSIVE GENOMIC AND GENETIC CHARACTERIZATION OF CLINICAL ISOLATES

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The *Plasmodium falciparum* 3D7 genome is the only high quality reference assembly available to the research community to guide genetic and genomic studies. The characterization of other strains is based on the identification of sequence variants relative to 3D7, and both the gene and genome structure of 3D7 are viewed as representative of the species. The lack of other reference assemblies makes it difficult to describe structural variants that occur in natural *P. falciparum* populations, and may result in the underestimation of genetic diversity in highly polymorphic genomic regions obtained through read mapping-based approaches. Similarly, read mapping approaches are also poor at characterizing members of extremely diverse multi-gene families, which are particularly important in immune evasion and malaria clinical outcomes. We have recently generated whole genome sequence data for 23 *P. falciparum* strains from Brazil, Mali, Guinea, Malawi, Myanmar, Laos, and Cambodia using a combination of Pacific Biosciences and Illumina sequencing technologies. We used the Canu assembler to generate assemblies, and then polished with Quiver and Pilon. The Canu assemblies, which consisted on average of 25 contigs each, are much less fragmented than those generated with the HGAP assembler (50 contigs per assembly). These new assemblies will allow a more thorough documentation of genome structural variation among *P. falciparum* strains, and provide new allelic references for genes that have been difficult to assemble with short-read data, such as members of multi-gene families and genes with long repetitive regions. For example, we found that the internal repetitive region in liver stage antigen 1 (LSA-1) is incorrectly collapsed in the 3D7 reference genome. Initial results also show that mapping reads from isolates collected outside of West Africa to an assembly of similar geographic origin results in a more comprehensive characterization of sequence variants than mapping to 3D7, particularly for isolates from South East Asia, exposing the limitations of SNP calling methodologies that rely only on 3D7 for characterization of clinical isolates.

## 1648

### TARGETED DE NOVO ASSEMBLY OF VAR2CSA FROM CLINICAL SAMPLES USING SHORT READ WHOLE GENOME SEQUENCE DATA

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The *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1) family of proteins, which are expressed on the surface of infected erythrocytes, has a central role in malaria pathogenesis. VAR2CSA, a member of this family, is a key ligand that mediates the sequestration of the infected erythrocytes in the placenta, leading to maternal and fetal

complications of malaria during pregnancy. The VAR2CSA protein is an antigen and a leading vaccine candidate for placental malaria. Although it is a desirable vaccine candidate, the major roadblock in the development of VAR2CSA-based vaccines is the genetic variation of the locus encoding this antigen. Despite extensive sequencing efforts, full-length *var2csa* sequence data are very sparse. In this study, we took advantage of a few existing full-length sequences, previously generated by us and others, to serve as a reference database to recruit *var2csa*-like short paired-end reads based on sequence identify. These sequence reads and their mate pairs were then used to target-assemble *var2csa* using the SPAdes assembler. Using this approach we were able to reconstruct 78 full-length *var2csa* exon 1 sequences, encoding the entire extracellular region of the protein, from 130 Illumina whole genome sequence datasets. Analysis of these sequences shows that the protein region containing the receptor-binding site is highly variable, whereas two C-terminal domains, namely DBLepam4 and DBLepam5, were significantly more conserved among geographically distant parasites, highlighting the potential for these domains to be considered in the composition of VAR2CSA-based vaccines. This pipeline is useful for the assembly of *var2csa* using Illumina short read sequence data. The pipeline also has the potential to perform targeted assembly of other *var* genes from clinical isolates, as additional reference alleles for those *loci* are obtained that can be used to recruit reads. The ability to sequence and target-assemble *var2csa* will enable the design of a diversity-covering pregnancy malaria vaccine.

## 1649

### WHOLE GENOME SEQUENCE CAPTURE TO GENERATE HIGH QUALITY GENOMIC DATA FOR *PLASMODIUM VIVAX* FROM CLINICAL ISOLATES

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*Plasmodium vivax* malaria has been refractory to malaria elimination efforts along the China-Myanmar border. Better understanding of the genomic epidemiology of *P. vivax* could inform these efforts by helping to identify sources and sinks of transmission and distinguish local from imported cases. The usefulness of direct whole genome sequencing (WGS) of clinical isolates is limited by its inability to generate high-quality genome-wide data from infections with low parasite densities and very high content of human DNA. We adapted the Roche-NimbleGen SeqCap EZ whole genome sequence capture method to leukocyte-depleted venous blood samples from 14 patients with RDT-confirmed acute *P. vivax* infection on the China-Myanmar border. Capture probes were designed based on the *P. vivax* Sal I reference genome. The genomic DNA library was sequenced with and without capture, and using Illumina HiSeq and MiSeq platforms, to compare the quality, breadth and depth of genome coverage, and the suitability of the data for genome assembly. Preliminary results show that the whole genome capture method was highly effective in capturing extremely low quantities of parasite DNA from a small amount of total DNA (1.41 µg). Leukocyte depletion eliminated most human DNA, resulting in 89.31% of the pre-capture reads being parasite origin. However, the capture method produced nearly complete enrichment with 98% of parasite DNA post-capture mapping to *P. vivax* reference genome. The breadth of genome coverage was 95%, similar pre- and post-capture, demonstrating that the specificity in this capture approach did not compromise sensitivity. With >99% of *P. vivax* coding regions recovered, this novel whole genome sequence capture application dramatically improves our ability to generate *P. vivax* WGS data from clinical samples, especially if shown to be similarly successful in non-leukocyte depleted

samples. This will empower studies of *P. vivax* genetic diversity, population structure and parasite migration patterns, potentially leading to new tools and approaches for malaria elimination in low transmission and pre-elimination settings.

## 1650

### IMPACT OF THE G6PD DEFICIENCY ON THE PREVALENCE OF MALARIA INFECTION IN SICKLE CELL PATIENTS UNDER 15 YEARS OLD LIVING IN BURKINA FASO

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Sickle Cell Disease SCD and Glucose -6-Phosphate Dehydrogenase (G6PD) confer protection against malaria. Their association would better protect against malaria. In Burkina Faso, the prevalence of these genetic abnormalities varies between 8 and 10%. This study aims to describe malaria morbidity in subjects living with sickle cell and deficit of G6PD. A cross-sectional survey was conducted in children of 6 months to 14 years, at the end of the malaria transmission season to collect malariometric parameters. A review of the medical history of affected children was done to collect sickle cell crisis and the number of blood transfusions during the last 6 months. A total of 120 subjects was enrolled, 12 subjects with G6PD plus SCD and 108 subjects with SCD only. The prevalence of malaria infection was lower in the SCD group (0%) than G6PD plus SCD group (6.5%) ( $p < 0.0001$ ). The prevalence of clinical malaria in subjects presented fever was respectively 91.7% (11/12) in SCD group and 8.3% (1/12) SCD plus G6PD group ( $p < 0.0001$ ). Mean haemoglobin level was 9.61 g/dl (IC95% [9, 33-9, 9]) vs 9,11 g/dl (IC95% [8, 10-9, 25]). The number of subject who had at least one sickle cell crisis during the last 6 months was lower in both groups. Only one subject of the SCD group had blood transfusion during the study period. The burden of malaria seems higher in the groups of subject with SCD compared to the group with SCD and G6PD. The risk of hemolysis associated with these genetic deficiencies necessitates early diagnosis of these genetic factors, for adequate care.

## 1651

### DIFFERENCE IN EXPRESSION AND POLYMORPHISM OF GENE ENCODING FOR THE RECEPTOR FOR ADVANCED GLYCATION ENDPRODUCTS (RAGE) IN FULANI AND DOGON IN MALI

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Difference of susceptibility to malaria has been documented by several studies in Fulani and Dogon living in sympatric in malaria endemic area of Mali, with different lifestyles and diet habits. The complex interactions between environment, diet, lifestyle and the modulation of the host's immune responses constitute a research area of great interest in elucidating this phenomenon. The role of the immune system dysfunction induced by nutrients-derived metabolites and the polymorphism of gene encoding for the cellular receptors for AGEs (RAGE) in susceptibility to bacterial infection is already described. But no study has yet explored their role in susceptibility to malaria. This study aimed to investigate the expression and polymorphism of RAGE gene in two sympatric populations with known difference of susceptibility to malaria. We determined by real-time quantitative PCR the expression of RAGE, and the -374 T/A and -429 T/C polymorphisms by fragment length restriction polymorphism (FLRP) in 44 Dogon and 35 Fulani. The blood samples used were collected by cross-sectional survey. RAGE was more expressed in Dogon than Fulani (0.08 vs 0.04),  $P=0.08$ . The -374A polymorphism was more frequent in Fulani

(32%) compared to Dogon (20%). These results showed that the -374A polymorphism was more frequent in naturally resistant Fulani population to malaria while RAGE gene was more expressed in Dogon susceptible to malaria. These differences were not statistically significant. The chronic exposure to dietary advanced glycation endproducts could lead to immune responses impairment and/or polymorphism with implications in malaria susceptibility. But more powerful studies are necessary to better investigate this hypothesis.

## 1652

### CHARACTERIZING *PLASMODIUM FALCIPARUM* GAMETOCYTE GENE EXPRESSION IN A COHORT OF ASYMPTOMATICALLY-INFECTED ADULTS IN WESTERN KENYA

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We hypothesize that co-infection with HIV-1 and *Plasmodium falciparum* is associated with an increase in the burden of malaria transmission due to increased gametocyte carriage. Gametocyte carriage in asymptomatic individuals varies over time, and gametocytemia is not routinely quantified during microscopic examination of blood, further complicating attempts to enumerate the prevalence of gametocyte carriage in a given population. To gain a better understanding of gametocyte prevalence, we are developing and validating a multiplex quantitative PCR (qPCR) assay to quantify expression of a panel of genes that are expressed throughout the processes of gametocytogenesis and gametocyte development. Molecular techniques such as qPCR are much more sensitive and specific than microscopy, and a multiplex approach allows simultaneous quantifying of several markers, which reduces the amount of time and volume of sample needed for analysis. As part of the assay validation process, we will quantify gametocyte expression in cultured *P. falciparum* parasites over time to evaluate kinetics. We will also explore the variation in gametocyte production between various lab strains of *P. falciparum*. After the assay has been fully validated, it will be used to quantify gametocyte carriage in a cross-sectional study of HIV-1 and malaria prevalence in asymptomatic persons in Kisumu, Kenya. Specifically, we are looking at the overall prevalence of gametocyte carriage and the relationship between HIV co-infection and gametocytemia. Ultimately, a better understanding of the contributions of HIV to malaria transmission will help focus elimination and eradication strategies to reduce gametocyte burden in populations with disproportionately high transmission potential.

## 1653

### DEVELOPMENT OF TOOLS TO VALIDATE *PLASMODIUM FALCIPARUM* GENOME ASSEMBLIES GENERATED WITH PACBIO DATA

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Malaria is one of the most widespread parasitic diseases in the world. It has been eliminated from major developed countries, but is still highly prevalent in over 100 others, albeit with different transmission intensities. *Plasmodium falciparum* malaria alone is responsible for over 400,000 deaths each year. The *P. falciparum* 3D7 genome is used as the reference for genetic and genomic studies. Studies based on *P. falciparum* whole genome sequence data have revealed important characteristics of the

genome, facilitating drug and vaccine development. All new strains, regardless of geographic origin, are characterized with respect to the 3D7 genome. Varying transmission rates and extent of migration among endemic regions contribute to different levels of genetic diversity within, as well as to genetic differentiation among, parasite populations. Relying on only one reference genome for the genetic characterization of different populations may bias the estimation of strain genetic composition and population diversity, potentially hindering the efforts to identify the genetic basis of drug resistance and the development of vaccines with regional relevance. We recently generated de novo genome assemblies for 23 *P. falciparum* strains from Brazil, Guinea, Mali, Malawi, Myanmar, Laos and Cambodia using long-read PacBio sequence data. In order for these assemblies to serve as the reference, against which strains from the same region are characterized, it is important that their accuracy be validated, to ensure that PacBio sequencing errors have been properly corrected and that no structural errors were introduced during assembly. Initial comparative analyses of genome assemblies from strains originating from West Africa, Brazil and Southeast Asia revealed considerable differences between them and the 3D7 reference genome, in both gene and genome structure. We have developed a pipeline to identify and validate these differences with respect to the 3D7 genome. We use as controls the recently published PacBio-based 3D7 genome assembly, as well as the PacBio assembly we generated for NF54, the parental isolate from which 3D7 was cloned.

## 1654

### GENETIC DIVERSITY OF *PLASMODIUM FALCIPARUM* IN ASYMPTOMATIC AND SYMPTOMATIC CHILDREN IN AN ENDEMIC MALARIA AREA IN BURKINA FASO

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In areas of high malaria transmission, it is common that infected individuals with *Plasmodium* harbor more than one genetically different parasite. Differences in the multiplicity of infection between asymptomatic and symptomatic individuals infected with *Plasmodium* in the same endemic areas have been reported and multiplicity of infection has been associated with protection against symptomatic malaria. Here, we characterized *Plasmodium falciparum* diversity in a pediatric cohort from Burkina Faso during asymptomatic and symptomatic malaria using Illumina high-throughput sequencing. Hundred fifty children from two malaria-endemic villages of Banfora health district were sampled between May and December over two consecutive years (2015 and 2016). Blood samples were collected before infection as well as during asymptomatic and symptomatic infection and genome-wide host and *P. falciparum* DNA and RNA profiles generated. Bioinformatic analysis of RNASeq data was used to generate a catalogue of genetic variants that are used for strain and clone characterization and population genetic analysis of *P. falciparum* in Burkina Faso. Using this approach, we quantified the diversity of *P. falciparum* within and between individuals during the course of infection and report the results of association tests between host phenotypes and parasite genetic variation.

## 1655

### GENETIC VARIATION IN INTERLEUKIN (IL)-3 AND IL-7 ARE ASSOCIATED WITH ALTERED ERYTHROPOIETIC RESPONSES IN CHILDREN WITH MALARIAL ANEMIA IN SIAYA, KENYA

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Severe malarial anemia (SMA, Hb  $\leq$ 5.0 g/dL) is a leading cause of morbidity and mortality in children with malaria. Using genome wide association studies (n=144, age 3-36 months) and transcriptomics (n=72), we have identified several genes associated with SMA, including interleukin (IL)-3 and 7. Expression levels for both genes were non-significantly reduced in children with SMA. Since the role of IL-3 and IL-7 in malarial anemia has not been reported, we selected genetic markers in these genes based on an allelic distribution (>10%), and determined their association with clinical outcomes in children with malarial anemia (n=1,082, age, 3-36 months). IL-3 is important for promoting erythroid development and regulating immunity, while IL-7 is involved in the function of T cells, NK cells and monocytes. The association between SMA and reduced erythropoiesis [reticulocyte production index (RPI), <2.0] were examined for markers in IL-3 (36608A/G, rs 247011; -4651A/G, rs3091336 and -8181A/G, rs246752) and IL-7 (72194C/T, rs 2583759 and -2440 A/G, rs 7007634). Although there were significantly different distributions of the IL-3 GGG haplotype (-4651G, -8181G and 36608G,  $P=0.044$ ) and the IL-7 GT haplotype (-2440G/72194T,  $P=0.030$ ), logistic regression analysis controlling for covariates of anemia, did not reveal any significant associations with SMA for either the genotypes or haplotypes. However, additional analyses revealed that the IL-3 GGG and AGA haplotypes were associated with inefficient erythropoiesis (OR=2.83, 95%CI: 1.46-5.50,  $P=0.002$  and OR=5.36, 95%CI: 1.91-15.01,  $P=0.001$ , respectively), while the IL-7 AC haplotype was protective against inefficient erythropoiesis (OR=0.54, 95%CI: 0.30-0.97,  $P=0.042$ ). Measurement of circulating IL-7 levels showed that IL-7 was significantly lower in children with SMA compared to those with non-SMA ( $P=0.002$ ). IL-3 levels are currently being determined. Taken together, results presented here suggest that the IL-3 and IL-7 variants selected for measurement are associated with altered erythropoiesis, but do not appear to have as strong of impact on susceptibility to SMA.

## 1656

### TRANSCRIPTIONAL PROFILING AND GENE CO-EXPRESSION NETWORK ANALYSIS IN MALARIA PARASITE IMPROVES UNDERSTANDING OF K13 MECHANISM IN ARTEMISININ RESISTANCE

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Malaria mortality has decreased substantially over the last 15 years due to widespread use of the key drug for treating malaria, artemisinin, but these gains are threatened by the emergence of resistance in Southeast Asia. If artemisinin resistance in *Plasmodium falciparum* spreads to Africa, as was observed for previous anti-malarial drugs, malaria deaths will increase considerably. A precise understanding of the resistance mechanism is urgently needed to forestall the potential collapse of current control efforts that rely on the efficacy of artemisinin. SNPs in Pf3D7\_1343700 (K13) have been associated with artemisinin resistance, but a detailed molecular understanding of the mechanism of resistance remains elusive. Gene co-expression network analysis can refine the molecular level understandings of complex phenotypes; in malaria parasites, transcriptional profiling of

*pfprt* and *pfmdr* mutants revealed insights about chloroquine resistance. To elucidate K13 function and its role in artemisinin resistance, we transcriptionally profiled seven piggyBac (pB) transposon mutants that clustered with a K13 pB mutant based on highly related chemogenomic signatures. These mutants, along with the isogenic background line, NF54, were profiled for transcripts across the erythrocytic cell cycle every 4 hours. Differentially expressed genes were identified and were further analyzed via gene set enrichment analysis to determine the pathways most affected by each transposon insertion. In addition, gene co-expression networks were constructed and analyzed for all of the pB lines and NF54 to identify alterations in the gene co-expression network. Our results indicate that the combination of transcriptional profiling and network analysis can provide key insights into K13's function and role in artemisinin resistance. Further refinement of these genes and interactions has the potential to point to optimal drug partners that could counteract resistance and restore artemisinin efficacy.

## 1657

### VARIATION IN THE CD40 PROMOTER IS ASSOCIATED WITH SUSCEPTIBILITY TO PLASMODIUM FALCIPARUM-INDUCED SEVERE MALARIA ANEMIA IN KENYAN CHILDREN

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The etiological basis of pediatric severe malaria anemia (SMA, Hb<5.0g/dL and any density parasitemia) is a multifactorial process. An important element in the pathogenesis of SMA is genetic susceptibility factors in immune response genes. Our global genomic association studies in a subset of children with malarial anemia identified a candidate gene, cluster of differentiation 40 (CD40) that is expressed on both immune and non-immune cells, and has been shown to enhance immunoglobulin G (IgG) class-switching, generation of B-lymphocytes, hyper-mutation, and stimulation of inflammatory responses: processes that may represent signatures of SMA. Based on the important role of CD40 in immunity, we explored genetic polymorphisms in a larger population and determined the association between variation and downstream inflammatory mediators, i.e., IL-12 and IL-17. Three polymorphic variants: [(-1C>T rs1883832, -173C>T rs752118, and -508G>A; rs1800686) were genotyped in children (n=1,118, aged 6-36mos.)] with malaria from Siaya County, western Kenya, a *P. falciparum* holoendemic transmission area. CD40 genotypes were determined using Taqman® allelic discriminations assays and inflammatory mediators were measured using the Hu Cytokine 25-plex Ab Bead Kit. Bivariate regression analysis, controlling for confounders of anemia, revealed that the CCG haplotype (CD40-1C/-173C/-508G) was associated with protection against SMA (OR 0.31, 95%CI 0.14-0.67,  $P=0.003$ ), while the TCG haplotype (CD40-1T/-173C/-508G) was associated with enhanced risk of SMA (OR; 5.24, 95% CI; 3.31-8.82,  $P<0.001$ ). Additional analysis revealed that carriage of TCG haplotype was associated with elevated levels of IL-12 ( $P=0.001$ ) and lower levels of IL-17 ( $P=0.011$ ). These results demonstrate that variation in the CD40 promoter are associated with susceptibility to SMA, and appear to influence levels of circulating IL-12 and IL-17 during *P. falciparum* infection.

## 1658

### SYSTEMS GENETIC APPROACHES TO STUDY THE TEMPORAL DYNAMICS OF HOST AND PARASITE TRANSCRIPTOMES IN MALARIAL CHILDREN IN BURKINA FASO

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Malaria is a complex infectious disease with many genetic and environmental determinants influencing host immune response to infection, the progression of the disease and its severity. To date, surveys of host transcriptional responses were used largely on cross-sectional study designs. Here we investigate several layers of biological variation in both host and parasite including whole genome sequence variation, methylation profiles and transcriptomic data from a pediatric cohort in Burkina Faso. We focus on studying the temporal dynamics of peripheral blood transcription response to *Plasmodium falciparum* infection. We investigate how infection status modulates gene expression of the host and document the nature, magnitude and significance of transcriptional changes taking place during the course of infection. Integrated genotype, gene expression analysis allowed quantification of the contribution of host genotype to response to infection. The study provides a high-resolution picture of temporal transcriptional changes in a highly malaria-endemic region.

## 1659

### GENE CO-EXPRESSION NETWORK ANALYSIS OF MALARIA PARASITE TRANSCRIPTION REFINES POTENTIAL GENE INTERACTION UNDERLYING ARTEMISININ RESISTANCE

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Malaria infects 212 million people annually and results in 429,000 deaths, primarily in children under age five. Since 2000, significant progress has been made in controlling malaria, with mortality decreasing by 62%. Spread of artemisinin resistance to Africa would have devastating consequences for malaria control. K13 has been identified as a major determinant of artemisinin resistance, however a detailed molecular understanding of K13 function and its role in the resistance mechanism remains to be defined. A clearer understanding of artemisinin resistance could indicate novel partner drugs to prolong artemisinin efficacy. Network analysis can account for gene interactions and refined views of molecular mechanisms underlying complex phenotypes. In a previous study, we used gene co-expression (transcript) networks that distinguish chloroquine resistant and sensitive progeny from a genetic cross to generate a detailed hypothesis of *pfcr* function in chloroquine resistance. Here, we apply network methods to publicly available high resolution transcriptional datasets for parasites from Southeast Asia examine the modular context of K13 and its network neighbors in both resistant and susceptible parasites. We find that K13 'wiring' relationships show differences between artemisinin resistant parasites: in sensitive parasites, the K13 module is enriched for cell cycle progression and homeostasis genes, while in resistant parasites, in addition to homeostasis, K13 expression partners are enriched for protein folding, lipid biosynthesis and metabolism, response to heat, response to stress and response to stimulus. This network analysis relies on network modules to extend our ability to predict the molecular mechanism of artemisinin resistance beyond standard differential expression analysis.

## 1660

### LEUKOCYTE-ASSOCIATED IMMUNOGLOBULIN LIKE RECEPTORS 1 (LAIR1) AND 2 (LAIR2) EXPRESSION AND POLYMORPHIC VARIATION IN CHILDREN WITH SEVERE MALARIAL ANEMIA FROM WESTERN KENYA

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Severe malarial anemia (SMA) [hemoglobin (Hb) <5.0 g/dL, with any density parasitemia] is a multifactorial complication of malaria and is common in children residing in *Plasmodium falciparum* holoendemic areas. To investigate SMA pathogenesis, we conducted a genome-wide association study (GWAS, n=144, 3-36 months) and whole genome transcriptome profiling in a subset of individuals (n=72) from Western Kenya. These investigations revealed an association between SMA and leukocyte-associated immunoglobulin like receptor (LAIR) genes. Both LAIR1 and LAIR2 are pleiotropic inhibitory receptors important in the prevention of autoimmunity. To validate our initial studies, selected markers in LAIR1 (rs2287827 A/G; rs11084333 A/G, rs6509867 C/A) and LAIR2 (rs6509880 C/T, rs4806767C/T) were measured in our larger cohort (n=1,259; age 3-36 months). Subsequent validation expression assays (TaqMan®) and inflammatory mediator analysis (Hu Cytokine 25-plex Ab Bead Kit) were also performed. Bivariate logistic regression analysis controlling for confounding factors of anemia showed that heterozygous LAIR1 (rs2287827 GA) was associated with protection against SMA (OR=0.657, 95%CI: 0.449-0.962, P=0.031), whereas the mutant LAIR2 (rs4806767 TT) increased susceptibility to SMA (OR=1.89, 95%CI: 0.977-3.66, P=0.059). Associations were also observed between carriage of intragenic and intergenic LAIR haplotypes and SMA: LAIR1 AGC (OR=2.32, 95%CI: 0.99-5.42, P=0.052), LAIR2 CC (OR=1.62, 95%CI: 1.02-2.56, P=0.039), and LAIR1/2 AAAC (OR=0.296, 95%CI: 0.093-0.944, P=0.040). In addition, carriage of LAIR2 CC haplotype was associated with increased RANTES levels (P=0.05), while the LAIR1/2 AAAC haplotype was associated with reduced levels of interleukin (IL)-1β (P=0.034) and IL-8 (P=0.025). TaqMan® LAIR expression analysis confirmed LAIR1 downregulation (-5.0 fold, P=0.017) and LAIR2 upregulation (+2.3 fold, P=0.59) in children with SMA. Findings here demonstrate that genetic variation in LAIR1/2 and altered LAIR gene expression are associated with susceptibility to SMA and immune dysregulation.

## 1661

### ASSESSING COMPLEXITY OF PLASMODIUM FALCIPARUM INFECTION IN TWO ECOLOGICAL ZONES IN GHANA USING MOLECULAR INVERSION PROBES AND NEXT GENERATION SEQUENCING

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Genetic diversity of the *plasmodium* parasite is the driving factor in the evolution of drug resistance and also affects immune regulation. Complexity of infections (COI) is also important in vaccine efficacy monitoring. Standard methods like PCR and RFLP underestimate the estimation of COI. In order to totally eliminate malaria, it is important to understand the diversity of the parasite in the population and thus a need to develop robust methods to accurately estimate complexity of infection. Children aged between 6 months to 14 years presenting with *Plasmodium falciparum* mono-infection at hospitals in two ecological zones in Ghana

were included in the study. Genetic diversity of a highly polymorphic region of Ama-1 was investigated from dried blood blot samples obtained from 500 children using the molecular inversion probes followed by next-generation sequencing (NGS). Oligonucleotide probes were designed to capture sections of the AMA1 gene and targeted regions amplified using barcoded primers. The amplicons were then sequenced using MiSeq. SeekDeep pipeline was used to analyze the sequence data to estimate haplotypes diversity and frequency. A total of 307 and 190 of samples were analyzed for AMA1 diversity and complexity of infection (COI) respectively. The most variable region of AMA1 had a total of 103 distinct haplotypes. The forest ecological zone had 61 distinct haplotypes while the coastal savanna had 78 haplotypes. The complexity of infection (COI) ranges from 1 to 7 in the forest zone and 1 to 8 in the coastal savanna zone. There was no statistical difference in the mean COI for both sites (COI= 1.56, 1.64 for Begoro and Cape Coast respectively, P value=0.692). The targeted amplicon sequencing using MIP shows higher COI values, which has not been captured before in these sites in Ghana and high number of distinct haplotypes of the vaccine candidate gene AMA1. This method would be beneficial in the search for resistance and monitoring vaccine efficacy.

## 1662

### CAS-9 BASED SEQUENCING ENRICHMENT FOR MALARIA GENOTYPING

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DNA polymorphisms in *Plasmodium falciparum*, including single nucleotide polymorphisms, microsatellite lengths, and structural variations, can be used to track transmission and drug resistance patterns. Whole genome sequencing produces large amounts of data, most of which does not provide relevant information, whereas targeted genotyping via PCR can be laborious and difficult to multiplex. Here we describe a method for isolation and enrichment of an arbitrary number of genomic regions of interest for Next Generation Sequencing (NGS) based on targeted Cas9 enrichment of multiple regions of interest called FLASH: Finding Low Abundance Sequences by Hybridization. Cas9-based targeting strategies have been successfully used to enhance pathogen detection in patient samples such as cerebral spinal fluid. Here we optimize and apply a related method to selectively enrich for targeted regions of high genetic diversity in *P. falciparum* from dried blood spot samples. This method enables detection of polymorphisms including short range haplotypes and structural variations, allowing for highly multiplexed rapid and reliable sequencing of specific regions of interest. Preliminary results demonstrate significant enrichment of target *P. falciparum* sequences from dried blood spot samples, especially when combined with selective whole genome amplification. Updated results will be presented at the meeting. This method offers the promise of rapid generation acquisition of NGS data from interchangeable sets of target regions from malaria field samples.

## 1663

### ACCURATE ASSEMBLY OF REGIONS OF COMPLEX DIVERSITY IN *PLASMODIUM FALCIPARUM* FROM SHOTGUN GENOME SEQUENCING AND ASSESSMENT OF STRAIN SPECIFIC IMMUNITY--TOWARDS OPTIMAL FORMULATION OF POLYVALENT VACCINES

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A conundrum of malaria vaccine design is that key proteins targeted by the adaptive immune response often display a high level of variation driven by selection for the ability to escape a previous immune response. It can be difficult to determine the extent of variant specific immunity or what key variants to incorporate into polyvalent vaccines. Whole genome sequencing can aid in this but one challenge to standard approaches using reference based mapping is accurately capturing the proper extent of variation. To overcome this, we have developed a novel graph-based program that uses local de novo assembly to infer long haplotypes (300-1800bp) at a region of interest. It accurately assembles highly complex regions of diversity and structural variation including repetitive regions in mixed infections. We used this algorithm to assess available *P. falciparum* whole genome sequence to determine the extent of geographic dispersion of variation within genes focusing on vaccine candidates including Circumsporozoite Surface Antigen (CSP), Apical Membrane Antigen 1 (AMA1), and Pfs48/45. We observe striking patterns of variation within these genes including strong signals of frequency dependent selection. Interestingly, the RTS,S vaccine, based on the African parasite 3D7, represents a CSP haplotype that has minimal similarity to Asian haplotypes, thus underscoring potential challenges for deploying the vaccine worldwide. A second challenge in developing polyvalent vaccines is determining the impact that a singular epitope or epitope combinations imparts to strain specificity. Traditionally this requires laborious immunologic studies to map antibody reactivity to specific variant epitopes. Leveraging targeted high-throughput sequencing, we examined serial infections within large cohorts posited on the fact that strain specific immunity will impart a decreased probability of repeat infection and/or clinical disease. Combined these methods provided improved delineation of vaccine targets and identification of key epitopes within target antigens.

## 1664

### IDENTIFICATION OF PFEMP1 EPITOPES ASSOCIATED WITH SEVERE MALARIA USING A DIVERSITY-COVERING ULTRADENSE PEPTIDE MICROARRAY

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When probed with carefully chosen serum samples, an ultra-dense peptide microarray offers the potential to identify epitopes critical for natural protection against malaria. *Plasmodium falciparum* erythrocyte membrane protein-1 (PfEMP1) antigens, which are encoded by 40 to 60 *var* genes per genome, play an important role in parasite sequestration

and host immune evasion and possess extraordinary genetic diversity. Acquired antimalarial immunity is at least partially due to antibodies directed against highly variable antigens like PfEMP1. We designed a pilot PfEMP1 peptide array to identify PfEMP1 epitopes that may underlie this antibody-mediated protection. Ninety-five thousand 16-amino acid (aa) peptides were synthesized *in situ* on an array, spanning the complete *var* repertoires of the reference genome 3D7, the Indochina strain DD2, and an Indian isolate, RAJ116, as well as three PfEMP1s from the Indian isolate IGH-CR14. In total, 151 PfEMP1s were included with 12 aa overlap, with representation of all described PfEMP1 domain cassettes. We probed this array with sera from 20 children aged 1-6 years old and 10 adults from rural Mali. For all PfEMP1 repertoires, adult sera consistently recognized more PfEMP1 peptides than pediatric sera. Peptides from PfEMP1s of Asian origin were just as likely to be recognized by Malian sera as peptides from 3D7 PfEMP1s. Adult sera reacted more intensely than pediatric sera (defined as a ratio of geometric means of the fluorescence intensities of the two groups > 5.0) to less than 10 percent of these serorecognized peptides. For Domain Cassettes 8 and 13, which have previously been associated with severe malaria, we identified a subset of serorecognized peptides. Adult sera reacted more intensely to a small minority of these peptides versus pediatric sera, with most such peptides belonging to Duffy binding-like domains, not the neighboring cysteine-rich interdomain regions. With sera from severe malaria case-control studies, this approach will facilitate further targeting of specific PfEMP1 regions to identify epitopes critical to natural immunity to severe malaria and thus inform vaccine design.

## 1665

### PHAGOCYTTIC EFFICIENCY OF BEADS COATED WITH VARIOUS MALARIAL PFEMP1 DOMAINS BY MONOCYTES/MACROPHAGES DEPENDS ON THE DOMAIN IDENTITY AND/OR BINDING AVIDITY TO MONOCYTE SURFACE RECEPTORS

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Mortalities from *Plasmodium falciparum* infection can be attributed to the ability of infected erythrocytes (IEs) to sequester to endothelium of blood vessels, uninfected erythrocytes, and cells of the immune system. Sequestration leads to blood flow problems, local and systemic immune response, and prolonged malarial infections. Sequestration is mediated by the interaction between specific PfEMP1 domains presented on the surface of IEs and endothelial cell receptors, including CD36 and ICAM-1. The receptors implicated in sequestration of IEs to endothelial cells can also be found on the surface of innate immune cells. However, the consequences of PfEMP1-immune cell receptor interactions are not well understood. Using microscopy and flow cytometry, we have demonstrated that human macrophages isolated from PBMC and THP-1 cell line monocytes phagocytize 5µm polystyrene beads (similar in size to IEs) coated with CD36-binding PfEMP1 CIDR1 domains more efficiently (up to 30%) than beads coated with irrelevant control protein. Moreover, domains with the highest (PF08\_0106) and lowest (PFD1015c) CD36 affinity had the highest and lowest phagocytic efficiency, respectively. Using THP-1 we have confirmed that interaction with CD36 receptor is important for phagocytosis, as pre-incubation of bead-immobilized PF08\_0106-CIDR1 with soluble CD36 reduces phagocytic efficiency. Similarly, in another experiment with bead-immobilized ICAM1-binding domain, we have demonstrated that the phagocytic efficiency was 58%, which was reduced by competition with soluble ICAM1 to 35%. These experiments indicate that PfEMP1 domain sequence identity and/or level of receptor avidity might be an important factor for phagocytic efficiency and will shed light on the mechanisms utilized by innate immune cells for parasite clearance.

## 1666

### MOTHER TO FETAL TRANSFER OF NATURALLY OCCURRING PLASMODIUM FALCIPARUM ANTIBODIES

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Maternal antibodies transferred from mother to child are thought to confer protection against malaria during the early months after birth. We have investigated the effectiveness of the transfer of naturally occurring IgG antibodies against *Plasmodium falciparum* antigens in cord blood from mothers living in an endemic area in the Greater Accra region in Ghana. Paired maternal cord serum samples (n=160; aged 18 years and older) were screened by Enzyme Linked Immunosorbent Assay against pre-erythrocytic *P. falciparum* Circumsporozoite (PfCSP), asexual blood stage Pf Apical Membrane Antigen (PfAMA-1), Pf Merozoite Surface Protein 10 (PfMSP-10), and sexual stage Pfg24, a novel Pf gametocyte antigen recently identified in our laboratory. These antigens were expressed as recombinant proteins in *E. coli*. The magnitude of the mean ± standard deviation of the antibody levels in descending order is as follows: PfAMA-1 (Mother: 1.32±0.76, Infant: 1.17±0.63), PfMSP-10 (Mother: 1.33±0.68, Infant: 1.13±0.63), PfCSP (Mother: 0.71±0.59, Infant: 0.45±0.55) and Pfg24 (Mother: 0.83±0.54, Infant: 0.43±0.40). Importantly, we found a strong correlation in antibody transfer from mother to child (Pearson moment correlation [range]: PfCSP (0.66[0.52 - 0.77]), PfAMA-1 (0.77[0.66 - 0.84]), PfMSP-10 (0.82[0.74 - 0.88]), and Pfg24 (0.73[0.61 - 0.82])). Rapid Detection Test, based on the HRP-2 and LDH combination and performed at the time of birth, showed the absence of parasitemia in the infants. These results establish that in areas of high transmission, at birth, infants possess the same level of antibody-mediated immunity as the mother which may form the basis of immunity against malaria during the first months after birth. IgG isotype analysis is currently being performed on paired maternal cord serum samples to determine whether certain isotypes are preferentially transferred from mother to child.

## 1667

### REDUCED HSP70 AND GLUTAMINE IN PEDIATRIC SEVERE MALARIA ANEMIA: ROLE OF HEMOZOIN IN SUPPRESSING HSP70 AND NF-KB ACTIVATION

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Severe malarial anemia [SMA, hemoglobin (Hb) <5.0 g/dL] is a leading cause of global morbidity and mortality among children residing in *Plasmodium falciparum* transmission regions. Our exploration of molecular pathways through genome-wide studies (GWAS) and transcriptomics revealed that SMA is characterized by polymorphic variation in heat shock protein (Hsp) 70 with decreased gene expression. Since the role of host Hsp70 in malaria pathogenesis is unexplored, we investigated Hsp70 and its molecular pathways in children with SMA. Generation of functional pathway networks using significant signatures from GWA and transcriptomics identified the Hsp70/TLR immune pathway as highly significant. Hsp70 is a ubiquitous chaperone that regulates Nuclear Factor-kappa B (NF-κB) signaling and production of pro-inflammatory cytokines known to be important in malaria pathogenesis (e.g., IL-1β, IL-6 and TNF-α). RT-qPCR experiments revealed that leukocytic *HSP70* transcripts were reduced in SMA relative to non-severe malaria, and intraleukocytic *P. falciparum*-derived hemozoin (Pfhz) was associated with lower *HSP70* transcripts. *HSP70* also correlated positively with reticulocyte production and Hb. *In-vitro* studies using PBMCs demonstrated that phagocytosis of hemozoin (Pfhz) causes activation of NF-κB, down-regulation of

Hsp70 expression, and dysregulation in inflammatory mediators known to influence suppression of erythropoiesis. Since glutamine (Gln) can up-regulate Hsp70 and decrease NF- $\kappa$ B activation, circulating Gln was measured in children with malaria. Reduced Gln was associated with an increased risk of developing SMA. In addition, Gln treatment of PBMCs overcame PfHz-induced suppression of HSP70 transcripts/protein, reduced NF- $\kappa$ B activation, and suppressed over-expression of cytokines. Overall findings here demonstrate that SMA is characterized by reduced intraleukocytic HSP70 and circulating Gln, and that PfHz-induced suppression of HSP70 can be reversed by Gln. Thus, Gln supplementation may offer important immunotherapeutic options for future studies in children with SMA.

## 1668

### CD4 T-CELL EXPRESSION OF IFN- $\gamma$ AND IL-17 IN PEDIATRIC MALARIAL ANEMIA

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In *Plasmodium falciparum* holoendemic transmission regions of western Kenya, life-threatening pediatric malaria manifests primarily as severe malarial anemia (SMA, Hb $\leq$ 6.0 g/dL with any density parasitemia). To determine the role that CD4+ T-cell-driven inflammatory responses have in the pathogenesis of SMA, peripheral CD4+ T-cell populations and their intracellular production of pro-inflammatory cytokines (IFN- $\gamma$  and IL-17) were characterized in children aged 12-36 months of age stratified into two groups: non-severe malarial anemia (non-SMA, Hb $\geq$ 6.0 g/dL, n=50) and SMA (n=39). In addition, circulating IFN- $\gamma$  and IL-17 were measured as part of a Cytokine 25-plex Antibody Bead Kit, Human (BioSource™ International). SMA group had higher overall proportions of circulating lymphocytes (P=0.003) and elevated proportions of lymphocytes expressing IFN- $\gamma$  (P=0.014) and comparable IL-17 (P=0.101). In addition, SMA was characterized by decreased memory-like T-cells (CD4+CD45RA-) expressing IL-17 (P=0.009) and lower mean fluorescence intensity in memory-like CD4+ T-cells for both IFN- $\gamma$  (P=0.063) and IL-17 (P=0.006). Circulating concentrations of IFN- $\gamma$  were higher in children with SMA (P=0.009), while IL-17 levels were comparable between the groups (P=0.164). Furthermore, circulating levels of IFN- $\gamma$  were negatively correlated with IL-17 levels in both groups of children (SMA: r=-0.610, P=0.007; and non-SMA: r=-0.516, P=0.001), while production of both cytokines by lymphocytes were positively correlated (SMA: r=0.349, P=0.037; and non-SMA: r=0.475, P=0.001). Taken together, these results suggest that enhanced severity of malarial anemia is associated with higher overall levels of circulating lymphocytes, enhanced intracellular production of IFN- $\gamma$  by peripheral lymphocytes and high circulating IFN- $\gamma$  levels. The observed inverse relationship between the circulating levels of IFN- $\gamma$  and IL-17, with the reduction in levels of memory-like CD4+ T cells expressing IL-17 in SMA group may suggest possible relocation of these cells in the deeper tissues for their pathological effect.

## 1669

### AUTOANTIBODIES IN MALARIA AND SYSTEMIC LUPUS ERYTHEMATOSUS

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It has long been established that people residing in malaria endemic regions have elevated levels of autoantibodies, but lower incidence of autoimmune diseases such as systemic lupus erythematosus (SLE). Autoantibodies in SLE fluctuate with disease activity and directly cause pathology such as lupus nephritis. Malaria induced autoantibodies are speculated to have a role in malaria pathogenesis, but it is unclear if the targets of malaria vs. SLE induced autoantibodies differ. Using a protein microarray platform containing 701 human genes encompassing known targets of autoantibodies, we tested plasma from healthy Kenyan adults (n=34) and children (n=38, ages 1-10years) residing in a malaria endemic area of western Kenya, plasma from US healthy adults (n=16), and plasma from US children with SLE at multiple times in their disease course (n=11 patients). We found that the greatest breadth of antigen recognition was by Kenyan adults followed by Kenyan children and US children with SLE, with US adults having the lowest recognition of human autoantibody targets. The top targets recognized by children with SLE were several SNRPs (small nuclear ribonucleoprotein polypeptides) which are diagnostic for SLE. In Kenyan adults and children, however, the top targets were several myosin light chains, RNA polymerase, insulin-like growth factor binding protein, and a membrane transport protein. Ongoing microarray analysis will compare Kenyan adult vs child targets and Kenyan children vs. US children with SLE. Additionally, preliminary studies indicated that Kenyans have a moderate frequency of B cells specific for human DNA compared to healthy controls (low) and patients with SLE (high). However, DNA-specific B cells may have a differential pattern of distribution in B cell subsets in Kenyans vs patients with SLE. These results show that the autoantibody profiles are different between healthy Kenyans and children with SLE. Further analysis will add to our knowledge of the overlap between malaria and SLE induced autoantibodies and the biological significance of autoantibodies to human malaria pathogenesis.

## 1670

### T FOLLICULAR HELPER CELL SUBSETS AND MEMORY B CELL FUNCTION IN PAPUA NEW GUINEAN CHILDREN WITH SYMPTOMATIC MALARIA AND FEBRILE NON-MALARIA ILLNESS

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The mechanisms underlying the development of acquired immunity to *Plasmodium falciparum* (Pf) malaria are not fully understood. Repeated exposure to Pf is required before protection from clinical disease develops, implying that B cell memory develops slowly and is incomplete. We examined memory B cell (MBC) recall responses and related T follicular helper cell (Tfh) subset distributions in Papua New Guinean (PNG) children with uncomplicated Pf malaria and compared these data to matching samples obtained at convalescence 9-12 weeks after treatment. MBC recall responses were evaluated by ELISPOT to quantify antibody secreting cells (ASC) following polyclonal stimulation of peripheral blood mononuclear cells. The distribution of MBC subsets and CD3+CD4+CD45RO+CXCR5+ Tfh1, 2 and 17 subsets was estimated by flow cytometry. The number of ASC observed during acute malaria was significantly less than at convalescence (respective average ASCs per 5000 plated cells 56.8 and 121.9, p=0.0018). The frequencies of MBC and quiescent and activated Tfh1, 2 and 17 subsets were similar during acute malaria and at convalescence. However, the proportion of quiescent and activated Tfh2 subsets was expanded compared with malaria naive controls. Ongoing studies are examining the specificity of MBC responses and Tfh subset distributions for Pf infection by comparison to responses of children with non-malaria febrile illness and the mechanisms underlying differences of MBC responses and cognate Tfh cell subsets.

1671

### QUEST FOR IMMUNE CORRELATES OF PROTECTION USING A STREAMLINED ANALYSIS APPROACH

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The lack of known Immune correlates of protection has been a major hurdle for the development of effective vaccines. To generate insights into protective immune mechanisms, novel readout methods and advanced technologies are urgently needed for the in-depth immunoprofiling of responses induced by protective vaccines. The work presented here applies a two-step approach to samples from a previously reported AMA-1 based vaccine that had shown promising clinical responses. The first step of the process established a broad cytokine profile of antigen-specific responses in *ex vivo* stimulated PBMCs using a multiplex testing platform (Mesoscale). These results are the basis for the design of the subsequent flow cytometric analysis; whereby, the antigen-specific cells are characterized in detail. The targets of the flow-based analyses are rare cell subpopulations. The low precursor frequency of key immune cell populations has slowed progress in this research area. To overcome this obstacle, we are enriching antigen-specific, activated cells using an automated system for magnetic bead separation and analyzing these cells based on their lineage, activation and differentiation state. Here, we report on changes in the functional subsets of AMA-1 specific follicular helper T cells. By combining state-of-the art technologies, we continue to generate unprecedented insights into vaccine-induced protective immune mechanisms studying viral and parasitic vaccines. Computational integration of the immunoprofiling, characterization of antigen-specific cells, serology, and clinical outcome after a controlled human malaria infection will be reported in an effort to identify immune correlates of protection.

1672

### MULTI-OMIC ANALYSIS OF SEVERITY OF INFECTION IN MACACA MULATTA INFECTED WITH PLASMODIUM CYNOMOLGI

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Severe anemia accounts for a large portion of the complications associated with malaria. This phenomenon is multifactorial, and the mechanisms that cause it remain elusive. A multi-omic approach comprised of immunology, transcriptomics, metabolomics, lipidomics, and proteomics was used to study five *M. mulatta* infected with *P. cynomolgi*. This experiment resulted in one death, two severe infections, and two mild infections. Particularly, we discovered that TLR3 and RIG-I pathways in macrophages and dendritic cells, conventionally linked to host antiviral responses, are upregulated in severe malaria. These pathways cross-talk, and their interaction had not been clearly implicated before in severe malaria. Associated with the activation of these pathways are changes in the effector response proteins IL1 $\beta$ , AP1, MX1, MX2, OAS1-3, and PML. Our analysis provides novel insight into the molecular and cellular basis for the development of severe malaria. This project has been funded in whole or in part with Federal funds from the National Institute of Allergy and Infectious Diseases; National Institutes of Health, Department of Health and Human Services [Contract No. HHSN272201200031C] and the National Center for Research Resources [ORIP/OD P51OD011132].

1673

### EFFECT OF MALARIA ON CXCL10 AND ANGIPOYETIN-2 IN PATIENTS FROM A COLOMBIAN ENDEMIC AREA

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Malaria is a widespread disease in the world. Data provided by WHO estimate that about 3.8 million people are at risk of contracting it. Colombia has many regions with geographical and climatic conditions that favor the proliferation of the vector and for that reason it increases the transmission of the Plasmodium, generating a serious public health problem. Córdoba is a state affected by this disease in much of its territory, reporting a large number of annual cases. Malaria is a complex disease with many genetic and environmental determinants that influence the response to infection, progression and severity of the disease. The inflammatory response generated by the infection induces the release of cytokines that lead to tissue damage and favor the activation of macrophages in order to eliminate circulating parasites. The association between complicated non-cerebral malaria, cytokine levels and angiogenic factors such as CXCL10 and ANG-2 has been poorly studied. The aim of this study is to evaluate the effect of CXCL10 and ANG-2 in patients infected with malaria. Plasma levels of the CXCL10 and ANG-2 molecules were determined by ELISA techniques. Confirmation of the Plasmodium species was performed by PCR. The 86.4% of patients hospitalized with some type of complication were infected with *P. vivax*. The levels of CXCL10 and ANG-2 were higher in the group of hospitalized patients with some type of complication compared to the group of patients with uncomplicated malaria ( $p < 0.0001$ ). There was no significant correlation between hemoglobin levels and parasitemia versus concentrations of the molecules studied. The results suggest that these molecules are potential biomarkers predictors of complicated malaria.

1674

### THE MOST EFFECTIVE FUNCTIONAL RESPONSES AGAINST PLASMODIUM FALCIPARUM MEROZOITE INVASION LIGANDS ARE MEDIATED BY IGG3 SUBCLASS ANTIBODIES

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Naturally-acquired antibodies are important for protection from symptomatic malaria. Some antibodies target merozoites and inhibit erythrocyte invasion. Field studies suggest that these antibody responses are typically skewed to IgG1 or IgG3 subclasses, and that IgG3 responses often have a stronger association with protection. However, it is unclear how their different properties impact protective immunity, and therefore which IgG subclass is required for the most effective vaccine response. We hypothesised that the different effector responses of these IgG subclasses would impact functional immunity. To assess this, we made IgG subclass-switch antibodies and compared them using *in vitro* functional assays. We focused on merozoite antigens EBA-175, Rh5 and AMA-1, as they are important invasion ligands and leading vaccine candidates. For each antigen, existing monoclonal antibodies (mAbs) that target known neutralising epitopes were selected, and used to express IgG subclass-switch mAbs; these are mAbs engineered with the same variable region, i.e. same epitope target, but different human IgG subclass backbones. Characterisation confirmed their expected size, subclass, similar N-glycosylation patterns and ability to bind their respective recombinant and native proteins. Unexpectedly, IgG3 variants showed significantly greater inhibition of merozoite invasion compared to other IgG subclasses. IgG3 variants were most effective at C1q fixation and subsequent deposition of the membrane attack complex. IgG3 also demonstrated

strongest interactions with Fcγ receptors (FcγRIIa and FcγRIIIa). These results indicate the key role of IgG3 in mediating functional immunity and support findings from field studies that show strong associations between IgG3 responses and protection. It is likely that the superior Fab flexibility of IgG3 enables more effective antigen binding leading to enhanced neutralisation and formation of IgG complexes for complement and Fcγ receptor interactions. These findings provide a strong rationale to explore the development and efficacy of vaccines that induce strong IgG3 responses.

## 1675

### MALARIA IN PREGNANCY: IMPLICATIONS OF MICROSCOPIC AND SUB-MICROSCOPIC INFECTION ON CORD BLOOD CYTOKINE RESPONSES

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Children exposed *in utero* to maternal malaria are immunologically less responsive to malarial antigens and are more susceptible to malaria. This study examined the relationship between timing of exposure to both heavy (microscopic) and light (sub-microscopic) malaria infection on fetal immunity at birth. 500 pregnant women residing in a malaria endemic area in Kenya were recruited at various stages of pregnancy and followed to delivery. Mothers received the standard 2 dose malarial prophylaxis and those found positive by blood smear were treated. Maternal malaria infection was defined as either heavy (detectable by both blood smear and PCR) or light (sub-microscopic, only detectable by PCR). Newborns' cord blood lymphocytes (CBL) were examined for malaria antigen-specific cytokine responses. Newborns were categorized by timing of maternal infection i) malaria pos. at ANC and neg. at delivery (ANC+/DEL-) (n=169 for heavy infection, n=205 for light infection); ii) malaria pos. at delivery (DEL+) (n=19 heavy infection, n=39 light infection); and iii) malaria neg. at ANC and delivery (ANC-, DEL-) (n=312 heavy infection, n=257 light infection). Newborns from ANC+/DEL- mothers showed higher stimulation index, with significance noted in heavy infection p=0.0001. Newborns from ANC+/DEL- mothers showed elevated levels of Th-1 cytokines than those from DEL+ with significance in IFN-γ and IL-2 at heavy infection (0.792 pg/ml, 0.443 pg/ml, 0.379 pg/ml p<0.0001 and 0.223 pg/ml, 0.074 pg/ml, pg/ml 0.114, p= 0.047 respectively). Newborns from DEL+ mothers had significantly elevated IL-10 levels when compared to newborns from ANC-/DEL- (0.340 pg/ml, 0.456 pg/ml, 0.201 p=0.013). These preliminary results indicate that heavy infections have a significant influence on the immune response at birth. The high cellular response to malarial antigens in CBL from neonates of mothers with heavy infection in ANC may be due to early detection and treatment. Further analysis is needed to determine the implications of treatment of antenatal malaria on neonatal immunity

## 1676

### DEFORESTATION AND LAND COVER CHANGES: DRIVERS OF DISAPPEARING MALARIA IN NORTHERN LAO PDR?

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Between 2001 and 2011, Lao People's Democratic Republic (PDR) made substantial progress in reducing the burden of malaria, as the number of reported cases fell by 83%. In spite of a large-scale resurgence in southern Lao PDR in 2012-2015, the northern half of the country has successfully

maintained these gains. Exposure to *Anopheles* mosquitoes while working in the forest is considered a primary risk factor for malaria infection in much of Southeast Asia. This study aims to assess the environmental drivers of falling malaria transmission in northern Lao PDR, specifically considering land cover and forest changes. Landsat 7 Enhanced Thematic Mapper Plus (ETM+) data were obtained from the Google Earth Engine data library and 5 spectral bands were used as predictors of land cover. We collected training data (7210 points classified in 7 land cover classes) using manual digitization and photo interpretation techniques from high-resolution satellite imageries. A random forest algorithm was used to generate annual land cover maps from 2008 to 2016 (30m resolution). Malaria prevalence was determined using a cross-sectional survey of 1,500 households across 100 villages in 4 districts in northern Lao PDR in September-October 2016. Out of a total of 5,082 persons enrolled, 39 infections were detected by PCR, with substantial village-level clustering. The land cover layers will be used to assess forest change and included in a geostatistical model along with other climatic and environmental layers, intervention coverage, and epidemiologic risk factors to assess the relationship between recent forest change and observed malaria infection prevalence. Health facility confirmed case incidence collected from the surveillance system and serology results will also be incorporated into an historical assessment of land cover change and malaria infection. Finally, predictive risk maps will be produced to assist in targeting remaining *foci* of transmission as Lao PDR pursues subnational elimination.

## 1677

### HOW INCREASING ACCESS TO CASE MANAGEMENT COULD BE SUFFICIENT TO ACHIEVE AND MAINTAIN MALARIA ELIMINATION

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Malaria elimination efforts typically focus on intervention campaigns to reduce transmission through vector control interventions such as indoor residual spraying and clear the parasite reservoir through drug-based interventions such as mass drug administration. Yet routine, passive treatment of malaria infected individuals also plays an important role in reducing the reservoir of infections and thus diminishing onwards transmission. Evidence from several settings suggests that expanding timely, curative treatment for malaria may result in marked, sustained reductions in malaria transmission, but this effect is likely context-specific given heterogeneity in transmission risk, treatment accessibility, and human behavior. To evaluate the potential for strengthened case management to contribute to elimination goals, a mathematical model was constructed that takes into account level of malaria transmission, current access to treatment, the mobility of individuals, and the impact of vector control interventions. Using gridded data on population distributions and treatment-seeking behavior across Southern Africa, we evaluated the fraction of incident infections that could feasibly be cured by increasing case management access points (e.g., via community health workers) and strengthening testing and treatment practices at existing access points (including both public and private facilities and outlets). The impact on transmission of strengthening case management up to this feasible level across the region was then evaluated using the mathematical model. Results indicate that in Southern Africa, improving passive case management to effectively treat 60% of the malaria infections (currently around 45%) would result in interrupting transmission within 95% of the region's population currently residing within endemic areas. Given that strong case management can also result in the strong surveillance that will be critical for the sustainability of malaria elimination, achievement of the highest possible coverage of effective case management should be a priority for all malaria elimination programs.

1678

### MODELLING THE DYNAMICS OF *PLASMODIUM FALCIPARUM* HISTIDINE-RICH PROTEIN 2 IN HUMAN BLOOD STAGE CHALLENGE STUDIES

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Sensitive detection of malaria cases in a community is important for effective malaria control and elimination. Rapid diagnostic tests (RDTs) based on *Plasmodium falciparum* histidine-rich protein 2 (PfHRP2) are important public health tools. Although PfHRP2 persists in the circulation following successful treatment, the kinetics of PfHRP2 antigenaemia during infections are not well understood. This current study builds on an earlier model (Marquart et al 2012) using rich sampling of PfHRP2 and parasitemia data collected from 16 participants infected with *P. falciparum* in an induced blood stage challenge model. Participants were infected with approximately 1,800 *P. falciparum* (3D7 strain) asexual parasites, and received curative antimalarial treatment seven days after inoculation. Parasitemia and concentration of PfHRP2 were measured every 12 hours between days 4 and 11, using a 18s rDNA qPCR assay and a Quansys ELISA respectively. The updated kinetic model incorporated revised estimates of PfHRP2 half-life, the 40 hour asexual life-cycle of the 3D7 strain and individualised estimates of blood volume based on participant body weight. The half-life for PfHRP2 in the 3D7 strain was estimated assuming a first-order elimination model using individual PfHRP2 concentration-time curves from administration of treatment up to 4 days post-treatment. Calibration of the model with pre-treatment data predicted the amount of PfHRP2 produced per parasite lifecycle as a median of 31 fg (IQR: 20 - 39fg). Sensitivity analyses on key parameters were used to determine an optimal model of PfHRP2 kinetics in a human blood stage challenge model. This provides an opportunity to understand the performance characteristics of malaria RDTs detecting this antigen in low level infections.

1679

### NOVEL MODELING APPROACHES TO IMPROVE SPATIAL PREDICTIONS OF MALARIA PREVALENCE

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Accurate spatial prediction of malaria prevalence is important to help improve targeting of malaria prevention and control interventions, potentially leading to substantial cost-savings, as well as to assess the effectiveness of past interventions in reducing malaria risk. Current modeling approaches to create these spatial predictions have relied extensively on Bayesian geospatial models. Besides coherently representing uncertainty in predictions, these models leverage Gaussian Processes to generate better spatial predictions than simpler Generalized Linear Models. Here we explore novel classes of models including generalized Gaussian Processes and Bayesian Generalized Kernel Mixed Models (BGKMM). The advantage of these models is that they can represent nonlinear relationship and higher-order interaction terms, similar to Support Vector Machines, while still adequately representing the uncertainty in spatial predictions. We compare the out-of-sample predictive performance of these models to that of standard Bayesian geospatial models using the 2014 Demographic and Health Survey (DHS) from Ghana. Finally, we also explore how these modeling approaches can incorporate data from earlier surveys (i.e., 2011 Multiple Indicator Cluster Survey (MICS)) to generate improved spatial predictions. Our results are likely to be useful in generating better malaria prevalence maps, which can ultimately lead to more cost-effective spatially-targeted malaria prevention and control interventions.

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1680

### ASSESSING THE IMPACT OF IMPERFECT ADHERENCE TO ARTEMETHER-LUMEFANTRINE ON MALARIA TREATMENT OUTCOMES USING WITHIN-HOST MODELLING

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Artemether-lumefantrine (AL) is the most widely recommended and prescribed treatment for uncomplicated falciparum malaria worldwide. Clinical trials have clearly demonstrated its safety and efficacy. However, its performance in routine healthcare settings, where adherence to treatment may be suboptimal, is more difficult to assess. There are very few studies that record both detailed information on patients' adherence to AL and their treatment outcomes. Therefore, it is difficult to quantify the impact of imperfect adherence on the efficacy of treatment. In this work we develop a within-host modelling framework to explore the link between patient adherence to AL and the efficacy of treatment. We incorporate parasite dynamics, human immune responses, and pharmacokinetic-pharmacodynamic modelling. We use this model to estimate the role imperfect adherence plays in treatment failure. To obtain examples of real-life adherence behaviour we use individual-level data on pills taken and their timings in 482 malaria patients in Tanzania in 2012 as an input to our within-host model. The failure rate we estimate from our model using the Tanzanian adherence data for dose timings is higher (9%) than predicted for an equivalent cohort for which adherence was optimal (4%). The percentage of pills taken explains much of the variation in treatment outcomes found in the model simulations. Imperfect adherence was predicted to cause greatest increase in failure rates in young children, highlighting the importance of communications with caregivers. The addition of gametocyte production to the model enables us to assess the contribution of imperfect adherence to onward transmission of the parasite.

1681

### MODELING THE EFFECTS OF TRANSMISSION AND HOST POPULATION STRUCTURE ON MALARIA POPULATION GENETICS

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Renewed interest in malaria eradication has emphasized the need to accurately monitor changes in malaria transmission. Population studies have recently identified genomic signals associated with differences in transmission intensity. Genetic epidemiology models provide a framework for predicting how these signals react to changes in transmission, but the models typically lack host population structure and assume that infected individuals are equally likely to transmit to any other member of the population. Although epidemiological models indicate that host population structure and transmission heterogeneity can affect the spread of epidemics, little is known about how they can affect parasite population genomics or the magnitude of these effects. We simulated malaria epidemics on networks representing highly clustered populations or populations containing super-spreaders at different transmission intensities. We focused on how differences in host population structure affect the frequency and genetic composition of simulated polygenomic (multi-strain) infections. These results will improve our understanding of how both transmission intensity and host population structure can influence parasite population genomics and potentially impact the efficacy of eradication campaigns.

## 1682

### TO SCREEN OR NOT TO SCREEN: AN INTERACTIVE TOOL THAT INTEGRATES COSTS AND SPATIAL HETEROGENEITY TO DETERMINE WHEN MASS-SCREEN-AND-TREAT IS AN EFFECTIVE MALARIA CONTROL STRATEGY

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Resource management is critical to designing effective malaria interventions. The wide availability of rapid diagnostic testing has made pre-screening a viable method for reducing the waste and costs associated with mass drug administration. However, there has been mixed results regarding the effectiveness of mass-screen-and-treat (MSAT) interventions as a means of reducing morbidity and/or interrupting transmission. Two factors that influence intervention efficacy are (1) spatial heterogeneity of disease prevalence and diagnostic performance, and (2) cost of screenings and treatment. We propose a framework for interactively evaluating the cost effectiveness of presumptive treatment versus MSAT interventions. First, we modeled region-specific disease prevalence and diagnostic performances. With the model results, an interactive web application was created using R Shiny, which can incorporate relevant factors (e.g. urban/rural, age, fever history), allows the user to input cost of treatment, screening, and misdiagnoses, and compares the cost efficiency of these interventions in each region of a country. We present an example using DHS data from seven West African countries (Burkina Faso, Cote d'Ivoire, Ghana, Guinea, Mali, Nigeria, and Togo). We believe that this framework can be easily adapted for many contexts and is a useful tool for decision makers to design data-driven, cost-effective interventions.

## 1683

### MODELING THE GLOBAL BURDEN OF PREGNANCY-ASSOCIATED MALARIA DEATHS

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Approximately 125 million pregnancies occur annually in areas with malaria transmission. Malaria is an important cause of pregnancy-associated death in endemic areas. While Guyatt and Snow proposed in 2001 that 10,000 annual maternal deaths in sub-Saharan Africa could be explained by malarial anemia, no contemporary estimates exist. We conducted a systematic review to determine the proportion of pregnancy-associated deaths attributable to malaria and used the data to model the global burden. We conducted a systematic literature search on March 22, 2017 to identify observational studies on causes of maternal death, including the proportion of maternal deaths due to malaria, in malaria endemic areas. Two reviewers independently extracted data to create a primary dataset. A secondary dataset consisting of key predictors (antenatal attendance, delivery at a facility and with a skilled birth attendant, ITN, IRS, and IPTp3 coverage, Pf prevalence rate, and adult HIV prevalence) from malaria endemic countries was compiled. A predictive model was constructed using the primary dataset and applied to the secondary dataset to calculate the estimated number of pregnancy-associated malaria deaths worldwide. Monte Carlo simulation (10,000 simulations) was used to generate a 95% confidence interval (CI). Our search identified 11,870 records; after title review, 1665 abstracts were reviewed. Data from 71 articles from 25 countries with at least 1 year of data collection was included. On average, 7.9 (95% CI: 6.1-10.4) of maternal deaths were due to malaria. On preliminary estimate, this

translates to 30,404 (95% CI 1,615-230,458) annual maternal deaths associated with malaria infection, about 7% of malaria deaths in 2015. Despite improvements in coverage of malaria preventive interventions, maternal mortality due to malaria remains high. Furthermore, the total burden of malaria is likely underestimated, as our model does not account for maternal deaths related to severe anemia resulting from malaria infection. Continued interventions to minimize the effects of malaria in pregnancy are critical to achieve Sustainable Development Goal 3.1.

## 1684

### A NOVEL HUMANIZED PSEUDO-LIVER MOUSE MODEL FOR DISCOVERY OF ANTIMALARIAL DRUGS

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Of the *Plasmodium* species that infect humans, *Plasmodium vivax* has the largest geographical distribution and together with *Plasmodium falciparum*, contribute significantly to the burden of malaria associated morbidity and mortality worldwide. There are few preclinical animal models available to address the unique challenges of investigating the exoerythrocytic phase of *P. vivax* infection. This impedes our capability to develop effective antimalarial drugs that target the liver stage as well as the hypnozoite forms responsible for relapsing malaria. A biologically relevant, cost-effective, readily manipulable and adaptable animal model is needed to test new drugs for treatment and chemoprophylaxis, which is a key component in an era of malaria eradication as a global health agenda. To address this inadequacy, we have developed a humanized mouse model with a "pseudo-liver" that can host human *Plasmodium* infection. NOD/SCID IL2Rg<sup>-/-</sup> (NSG) mice at 6-8 weeks old were injected with 1x10<sup>7</sup> HC-04 cells subcutaneously (s.c.) and tumor growth was monitored by caliper measurement. Tumors approximately 1 cm in size were observed by 14 days post-injection. Mice received direct intratumoral (i.t.) or i.v. injections of 5-7 x 10<sup>5</sup> *P. falciparum* sporozoites. Packed human red blood cells were injected i.p. At 6-7 dpi, tumors were removed and evaluated for *P. falciparum* infection. Thick and thin blood smears were evaluated to confirm erythrocytic infection. Results show that hepatocyte cell lines engrafted s.c. in the flank of NSG immunodeficient mice developed into well vascularized tumors or "pseudo-livers" with liver-like characteristics. Following injection of *P. falciparum* sporozoites either i.t. or i.v., *P. falciparum* was detected in the pseudo-liver at 6 days post infection. In mice engrafted with human RBC, we also observed development of ring stage parasites in Giemsa stained blood smears. Validation of this model is currently ongoing. The NSG pseudo-liver model could provide a relatively low cost, reproducible, standardized liver stage malaria drug discovery tool to test antimalarial drugs in the context of human *Plasmodium* infection.

## 1685

### IMPACT OF HUMAN MIGRATION PATTERNS ON MALARIA ELIMINATION FEASIBILITY IN THE GREATER MEKONG SUBREGION

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Myanmar has the highest burden of malaria in the Greater Mekong Subregion, with 152,000 cases in 2014, and has pledged to eliminate falciparum malaria by 2025. Achieving this goal is particularly urgent given increasing levels of ACT resistance, especially along border regions with Thailand. Challenges to elimination in the area include high prevalence of

submicroscopic infection that makes detection of the infectious reservoir difficult, poorly understood patterns of human movement on both short and long time scales, and disparate access to health care for different ethnic and socioeconomic groups. We use a stochastic simulation-based model to test elimination feasibility under a number of scenarios for a high-burden region along the Thai-Myanmar border, accounting for submicroscopic infections, ACT resistance, and subpopulations systematically unable to access care. A village-level simulation of Karen state in Myanmar was calibrated to longitudinal data collected by the Malaria Elimination Task Force. Using this model framework, we tested the efficacy of different intervention combinations, including scaled-up case management, increased usage of insecticide-treated nets, and implementation of mass drug administration for high-prevalence villages. These intervention packages were considered under a combination of migration types, including short-term ambulatory migration, short-term road-based migration, medium-term migration to and from the forest, seasonal migration for farm or factory work, and long-term displacement due to internal conflicts, to quantify the impact of different migration patterns on success of elimination programs. We find that mass drug campaigns in particular lose impact in scenarios where index cases are primarily acquired in the forest or other areas far from where the campaign is implemented, and both case management and vector control also vary in effectiveness under different migration and accessibility patterns. Better data about true migration patterns in Karen and elsewhere is essential for planning effective elimination campaigns.

## 1686

### MAPPING MALARIA METRICS USING SURVEILLANCE DATA ACROSS HETEROGENEOUS LANDSCAPES

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As malaria endemic countries move towards elimination, the control strategies shift from mass intervention campaigns to targeted measures that aim at more focal and heterogeneous transmission landscapes. Routine or responsive surveillance are deployed to describe these settings, but the strength of surveillance systems vary both among and within countries. Understanding the accuracy of the surveillance and malaria situation between sentinel sites can be challenging. Newly developed spatial modelling techniques, along with remotely sensed high resolution environmental and socio-demographic variables, can be used to translate case data observed at varying surveillance resolutions to fine-scale malaria incidence maps. This includes down-scaling the most widely available surveillance data source, case counts reported at the administrative-level, to illustrate sub-unit heterogeneity. Case counts from health facilities are increasingly obtainable and can also inform pixel-level maps while also accounting for aspects that may contribute to under-reporting such as accessibility to health facilities and care-seeking behaviours. Finally, cases followed up from facility and geospatially to the household level increase the resolution of the predictions through point-process models. Bespoke modelling frameworks developed for countries based on the type of data available can produce pixel-level maps that support countries in developing informed and sustainable elimination strategies in a wide range of settings.

## 1686A

### PHARMACOKINETIC AND PHARMACODYNAMIC MODELING FOR THE PREDICTION OF THE MOSQUITOCIDAL EFFECT DURATION OF HIGH-DOSE IVERMECTIN (THE IVERMAL PK/PD MODEL)

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It was recently shown that the mosquitocidal effect on *Anopheles gambiae* s.s. populations feeding on malaria patients treated with high-dose ivermectin lasts for at least 28 days after the start of ivermectin administration when co-administered with 3 days of dihydroartemisinin-piperazine (DP). The current pharmacokinetic and pharmacodynamic analysis aimed to determine whether a drug interaction or an unidentified ivermectin metabolite could be contributing to the prolonged mosquitocidal effect of ivermectin. In the main trial, 141 adults with uncomplicated malaria were randomly assigned to receive ivermectin 0, 300, or 600 mcg/kg/day for 3 days. During 28 days of follow-up, 1,393 venous plasma samples were collected. Paired mosquito incidence rates for death (IDR) during 14-days post-feeding were available for 850 time points. Following liquid-liquid extraction, ivermectin concentrations were measured using liquid chromatography-mass spectrometry (LC-MS). Pmetrics@ 1.5.0 was used for the population modelling of both PK and PD data. Ivermectin concentrations were above the lower limit of quantification (LLOQ: 5 ng/mL) for 534 time points, of which 246 had paired IDR's. The population pharmacokinetics of ivermectin were best described by a two-compartment oral absorption model. Based on the PK/PD model there was a consistent association between (1) predicted and observed ivermectin concentrations and (2) predicted ivermectin concentrations and observed mosquitocidal effect throughout the entire duration of the study (28 days). The half maximal effective concentration (EC50) for incidence rate of death by day 14 was 17.1 ng/mL (IQR 15.1, 19.1). Predicted median concentrations remained mosquitocidal for at least 28 days. The PK/PD model accurately predicted mosquitocidal activity for the entire duration of the study (28 days) without the need to invoke unidentified variables such as an active metabolite. (ClinicalTrials.gov: NCT02511353)

## 1687

### RESULTS FROM A FORMATIVE EVALUATION OF THE MALARIA IN PREGNANCY CASE MANAGEMENT JOB AID IN NIGERIA

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Annually, nearly 7 million pregnant women in Nigeria are at risk of malaria in pregnancy (MIP). Although antenatal care is the platform for the prevention and treatment of MIP, malaria is also treated at outpatient departments. It is known that women of reproductive age (WRA) are often treated for malaria without assessing pregnancy status, although artemisinin combination therapies are contraindicated in the first trimester of pregnancy, and many pregnant women do not receive the recommended low cost interventions. In order to increase access to these MIP interventions, the President's Malaria Initiative supported the Maternal and Child Survival Program and partners to develop a two-page job aid for

case management of uncomplicated malaria among WRA. In collaboration with the Nigeria Malaria Elimination Program, the job aid was evaluated in Ebonyi State, a high malaria burden area, to determine providers' perceptions of its clarity, acceptability, and utility. A half-day workshop on use of the job aid was provided to 35 health workers (nurses - 20%; nurse-midwives - 20%; community health extension workers - 48%; and medical doctors - 12%) already trained on MIP case management, selected from 15 facilities where WRA seek care. After 3 months of use, a one-page questionnaire was administered to 34 health workers. One-hundred percent stated that the job aid helped them to do the following: identify pregnant women among the WCBA presenting with fever; use rapid diagnostic tests to diagnose malaria; and treat uncomplicated MIP. Sixty-eight percent used the job aid to provide correct treatment for severe malaria and 88% used it while providing services all or most of the time. The results indicated that after a half-day orientation on use of the job aid, health workers were able to use it to help them identify women who may be pregnant and provide appropriate treatment for uncomplicated MIP. They are also able to explain its use to colleagues. It is suggested that a poster-size version could be printed and disseminated to appropriate cadres of health workers in clinics where WRA seek care for fever, as it is anticipated that providers could benefit from its use.

## 1688

### CAREGIVER RESPONSES TO CHILDREN WITH UNCOMPLICATED AND SEVERE MALARIA: CHOICES AND DELAYED CARE SEEKING

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Delayed care seeking contributes to progression from uncomplicated to severe malaria. The impact of caregivers' choices on time to seek appropriate care has not been reported. We present results on choices of care and their determinants, and the impact of choices on time to seek care among caregivers of children with uncomplicated and severe malaria in rural Uganda. We used a case control study to enroll 325 children with severe malaria (cases) and 325 children with uncomplicated malaria (controls) in Jinja, Uganda. Caregivers' responses to their children's illnesses, including choice of action and time to seeking care were documented. Conditional logistic regression and Cox regression were used to determine risk factors for choices of care and delay in care seeking, respectively. Staying at home (72.9%) and seeking care at a drug shop (22.3%) were the most common initial responses to illness. Staying home was more common among controls compared to cases (82.7% vs. 63.0%; 95%CI 12.9, 26.2), in contrast with seeking care at drug shops, which was more common among cases than controls (32.3% vs. 12.3%; 95%CI 13.7, 26.2). Delayed care seeking (OR 4.21; 95%CI 1.52, 11.6) and longer distance to the nearest public health facility (OR 1.39; 95%CI 1.08, 1.79) were predictors of seeking care at drug shops. Over time, caregivers of cases were less likely to seek care compared to controls, with differences peaking in the first 48 hours (35.0% vs. 60.0%; HR 0.29, 95%CI 0.20, 0.43). Upon adjusting for known determinants of delayed care seeking, caregivers of cases whose initial response was staying at home (HR 0.29, 95%CI 0.17, 0.49) or who sought care at a drug shop (HR 0.37, 95%CI 0.19, 0.74) were more likely to delay care compared to caregivers of controls who stayed at home. Staying at home and seeking care at a drug shop were associated with delay in seeking care among caregivers of cases. Longer distance to a public health facility predicted seeking care at drug shops and delay. The impact of drug shops on time to seek appropriate treatment for children with malaria and influences of behavioral factors that impact caregiver response to illness need further study.

## 1689

### DEALING WITH G6PD DEFICIENCY ON THE WAY TO MALARIA ELIMINATION IN MYANMAR

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Glucose-6-phosphate dehydrogenase (G6PD) is the enzyme important in red blood cells required for pentose phosphate pathway. Its deficiency is the most common human enzyme condition which Myanmar people are no exception. Myanmar is striving for malaria elimination by 2030 although having long history of malaria. Being part of malaria treatment, the use of Primaquine (PQ) can cause haemolysis in G6PD deficient patients. It is prescribed to control transmission in falciparum malaria while it is used to eradicate liver stage in vivax malaria. This is encountered in malaria endemic country like Myanmar where there is no established system of recording individual G6PD status as well as national survey data. Such limited information makes healthcare providers overcautious to use PQ. To overcome setbacks in use of PQ by healthcare personnel, few studies were conducted to demonstrate the G6PD status in Myanmar. One of the studies in 2001 showed that 4.5% of 1079 samples were severely deficient whereas deficiency by races revealed 5.5% in Bamar, 3.2% in local Chinese, 3.4% in local Indians, 3.3% in Mon, 5.1% in Shan and 6.7% in Kayin. Another study in Chin race in 2009, showed G6PD deficiency of 2.9% and severe deficiency of 0.5%. In 2014, a study on 916 people in living in Yangon showed 6.6% of G6PD deficiency. Those studies were conducted in civilian population but malaria posed greater threats on the armed forces and police whose nature of work is at high stake to contract malaria. Filling the gap of G6PD status data in armed forces and police, two studies were carried out by DSMRC. In 2015, 567 military personnel were tested for G6PD enzyme activity. There were 12.4% of active servicemen found to have low enzyme activity reflecting enzyme deficiency and severe enzyme deficiency was 1.2%. In January of 2017, 129 samples of blood were collected from police force to determine the G6PD enzyme activity. There were only 14 police personnel found to have moderate enzyme deficiency but there was no severe enzyme deficiency. Further studies to establish national figures firmly supporting the use of PQ are anticipated.

## 1690

### AWARENESS AND USE OF MALARIA CONTROL STRATEGIES IN KANO AND ZAMFARA STATES, NIGERIA - 2016

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The Nigeria Malaria Frontline Project is a collaboration among the National Malaria Elimination Programme, State governments, United States Centers for Disease Control and President's Malaria Initiative, the National Stop Transmission of Polio Program, the Nigeria Field Epidemiology and Laboratory Training Program and other partners. Prior to its implementation in Kano and Zamfara states, we conducted a baseline household survey to assess awareness and use of malaria control intervention strategies. We surveyed women in reproductive age in Kano and Zamfara states, Nigeria from July to August 2016 using World Health Organization recommended cluster sampling method. We interviewed 551 women: 249 in 207 households (HHs) in Kano and 302 in 210HHs in Zamfara using standardized Malaria Indicator Survey questionnaire. We collected data on awareness and use of malaria control strategies. Of 551 women interviewed, 520 (94.4%) women were aware of malaria; 150 (63.6%) in Kano and 179 (63.0%) in Zamfara knew children were at risk. Of the 518 (99.6%) who responded to questions on ways to avoid malaria, 483 (93.2%) knew how to avoid malaria in pregnancy, sleeping inside net being the most mentioned (80.5% in Kano, 90.7% in Zamfara).

Awareness of use of artemisinin-based combination therapy (ACT) for malaria treatment was 14.8% in Zamfara and 28.8% in Kano. Radio was the main source of information on malaria prevention (77.6% in Kano, 76.6% in Zamfara). Ownership of long lasting insecticidal nets (LLINs) was 62.9% in Kano and 62.3% in Zamfara. Use of LLINs was 68.0% in Kano and 67.9% in Zamfara, with use being 70.8% in Kano and 81.8% in Zamfara among pregnant women. Heat / claustrophobia (Kano: 13.9%, Zamfara: 12.1%) and difficulty experienced during net hanging (Kano: 2.8%, Zamfara: 6.1%) were identified reasons for not using nets. The HH revealed high awareness of malaria prevention strategies, low awareness of use of ACT, high LLIN ownership and use among reproductive age women in Kano and Zamfara states Nigeria. Increased sensitization on ACT use and deployment of LLIN hanging initiatives are recommended.

## 1691

### FACTORS LIMITING THE EFFECTIVENESS OF STANDARDIZED MALARIA CONTROL STRATEGIES IN FORESTED HIGHLANDS OF VIETNAM: A QUALITATIVE STUDY

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Despite radical improvements in malaria control in Vietnam in the past decades, malaria persists among impoverished ethnic minorities in south-central Vietnam where the same strategies that lead to its reduction and elimination elsewhere in the country have been applied. An ethnographic study to assess socio-cultural and health systems factors limiting the effectiveness of malaria preventive measures among the Ra-glai ethnic minority was conducted in nine villages in Ninh Thuan province in south-central Vietnam. Qualitative data were collected through in-depth interviews, informal conversations and participation observation. Retroductive data analysis was concurrent to data collection and carried out using Nvivo 11. A combination of factors contributed to limiting effective malaria control in this minority setting. Firstly, the constant contact with the forest and slash-and-burn subsistence agriculture exposed the Ra-glai to the main early biting and outdoor-resting sylvatic vector *An. dirus*, limiting the effectiveness of bed nets and indoor residual spraying. Secondly, health messages on malaria prevention were not adapted to a context characterized by the Ra-glai culture, a different language, illiteracy and different living conditions than the majority society, making part of the messages irrelevant to the local situation. Thirdly, the doctor-patient hierarchy consisting of limited communications between health professionals and patients due to language barriers and the perspectives on minority patients further enhanced the difficulties during the health encounter. Waiting times and geographic distance in the context of high work burden during the malaria transmission season limited the use of the public health system. Private health practitioners and local shamans bridged this gap by providing services perceived as effective, friendly and accessible. This study provides the insights into barriers to the current malaria control strategies and the need to further assess how to improve interventions in minority settings, with different ecological and socio-cultural characteristics than the majority society.

## 1692

### MALARIA CASE MANAGEMENT AND PREVENTIVE PRACTICES IN KANO AND ZAMFARA STATES, NIGERIA: HEALTHCARE WORKERS PERSPECTIVE, 2016

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Preventive practices, prompt and effective case management of uncomplicated malaria is critical to malaria control. These involve accurate clinical assessment, laboratory confirmation by malaria rapid diagnostic test (RDT) or microscopy prior to treatment with an effective antimalarial. We conducted a study to assess the malaria case management and preventive practices of healthcare workers in Kano and Zamfara states, Nigeria. A health facility based cross sectional study was conducted in 23 primary health centers [PHCs] and 29 General Hospitals [GHs] in Kano State and Zamfara 23 PHCs and 16 GHs in Zamfara State states from July - August 2016. Two health care workers were purposively selected from each PHC and GH. We defined intermittent preventive treatment of malaria in pregnancy (IPTp-SP) as the administration of a dose of three white tablets of sulphadoxine-pyrimethamine (SP) to a pregnant woman. Data was collected electronically using open data kit to obtain knowledge on case management and prevention practices. Data was entered, cleaned and analyzed using Statistical Package for Social Sciences version 20 software and Microsoft Excel. Frequencies, means, and proportions was done. Overall, 145 and 75 HCWs were interviewed in Kano and Zamfara States respectively. One hundred and twenty-three (56%) from PHCs and 97 (44%) from GHs in both states. Majority (85.0%) of respondents in both states correctly defined IPTp-SP and 83.6% knew SP as the medicine of choice. One hundred and sixty-six (75.5%) respondents knew malaria is caused by *Plasmodium spp.* while 195 (89.5%) identified artemether-lumefantrine as a first line treatment. About 92.2% of health workers in both states adhered to the national guideline to confirm malaria diagnosis using RDT and nearly 38.6% used microscopy. Healthcare workers were knowledgeable on IPTp, cause of malaria and practice the test and treat strategy for the management of uncomplicated malaria. There is need to sustain adherence to the national guidelines on malaria diagnosis and treatment among healthcare workers in both states.

## 1693

### EVOLVING GOVERNANCE OPTIONS TO ENHANCE EQUITABLE DATA SHARING

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The WorldWide Antimalarial Resistance Network (WWARN) was established with the aim of generating new evidence to optimise antimalarial treatment through meta-analysis of individual patient data. Its success is driven by the collaboration with the research community in the analysis of research questions of relevance to their patients. Despite the perceived benefits and the success of such models, the recent obligation of mandatory sharing of clinical data from a number of funders and publishers has triggered concerns for researchers and research participants alike. Notably, the benefits of increasing access to data may be not be equally shared by patients or researchers in resource limited countries. To address these potential inequities, WWARN now offers two models for data access - the original model used by WWARN to date whereby data held within the WWARN Data Platform can only be accessed with the permission of the data contributor, and now a governed access model whereby decisions on data access can be delegated by the data contributor to the newly established independent Data Access Committee. Led by TDR, the Special Programme for Research and Training in Tropical Diseases, the Committee assesses requests for data access to ensure that they are ethically and scientifically sound, before releasing the data to

researchers who agree to a framework which protects the rights of both researchers and patients. These data access models enable researchers to maximise the use of existing data to further improve public health while ensuring that data generators receive due credit for their contribution to the research output and the identities of participants are protected. These models aim to increase cross collaboration between those who generate the data and those who request to use it for secondary analysis.

## 1694

### SCALING UP SEASONAL MALARIA CHEMOPREVENTION IN MALI: IMPLEMENTATION CHALLENGES AND LESSONS LEARNED

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Seasonal malaria chemoprevention (SMC), the administration of complete therapeutic courses of antimalarial to all children 3 - 59 months old during the malaria transmission season, is a strategy recommended by WHO in areas of highly seasonal transmission. Despite the challenges of organizing the delivery of SMC, which requires monthly contacts with health workers to receive antimalarial drugs, National Malaria Control Programs (NMCP) have been quick to adopt this new strategy, including Mali, which extended SMC to the entire country (65 districts) in 2016. In 2016, USAID/PMI supported the NMCP campaign in 12 districts in two regions of Mali reaching a total average of 573,040 children 3-59 months old over four rounds of therapeutic courses. We analyzed the SMC programmatic data together with malaria case reporting from the Malian health management information system to evaluate the impact of the SMC campaign on malaria morbidity. The average coverage rate in the Kayes and Sikasso regions respectively were 98% and 92%. In 2016, Mali also extended SMC to children 5 - 10 years old in 3 districts. The three districts reported a 27.7% seasonal reduction in confirmed malaria cases in 5-10 year olds: 2764 cases in 2015 to 1992 cases in 2016. The malaria cases reported decreased from 32.4% in 2015 to 24.9% in 2016, and the malaria mortality rate decreased from 0.42% in 2015 to 0 cases in 2016. These results were achieved through clear planning, strong coordination of partners, multi-channel social and behavior change communications, close supervision and rigorous monitoring. These strategies have been effective in addressing the challenges in the Malian context, which include difficult logistics during the rainy season, high opportunity costs to families who are busy with agricultural work, the adverse effects of the drug treatment in some children, and the limited capacity of some volunteer distributors. SMC has contributed to: (1) a reduction in reported malaria cases; and (2) a reduction of the workload in health centers and a reduction in anti-malarial required during the peak malaria season.

## 1695

### RESULTS OF AN EVALUATION OF THE TOOLKIT TO IMPROVE EARLY AND SUSTAINED INTERMITTENT PREVENTIVE TREATMENT IN PREGNANCY (IPTP) UPTAKE IN MOZAMBIQUE AND MADAGASCAR

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Malaria in pregnancy (MIP) is a leading cause of maternal and newborn morbidity and mortality; however uptake of intermittent preventive treatment in pregnancy with sulfadoxine-pyrimethamine (IPTp-SP), an effective prevention tool, remains alarmingly low across sub-Saharan Africa, including Mozambique and Madagascar. The WHO 2012 policy recommendations to prevent MIP include early enrollment into antenatal care (ANC), accurate estimation of gestational age (GA) and administration of IPTp-SP during ANC visits beginning early in the second trimester, spaced at least one month apart. Preventing MIP remains a challenge in settings with inconsistent application of these recommendations and inadequate provider training in estimating GA. To improve adherence to these recommendations, a toolkit was designed which includes a job aid with an algorithm to guide providers during ANC visits to determine IPTp-SP eligibility. Twenty-four providers from 24 facilities in Madagascar and 29 providers from seven facilities in Mozambique were trained on use of the job aid and interviewed three months later about their experience. Individual providers were interviewed using a questionnaire to assess the clarity and utility to the job aid, and their opinions of the practicality of the orientation. Interviewers also gathered information on years of experience and clinical certification. All providers reported that the job aid reminded them to estimate GA and measure fundal height, which is particularly helpful since few women remember the date of their last menstrual period (LMP). Health workers also reported that the job helped them encourage the use of long-lasting insecticide treated nets, and reminded them of the proper timing to start IPTp-SP. We conclude that the toolkit is useful to prompt providers to calculate GA and offer IPTp as early as possible in the second trimester. Future potential directions include revision of all Toolkit components to reflect input from this evaluation, including development of a wall poster version to enhance readability, and inclusion of a pregnancy wheel to facilitate calculation of GA and estimated date of delivery.

## 1696

### MONITORING SEASONAL MALARIA CHEMOPREVENTION CAMPAIGNS: LESSONS LEARNED FROM COVERAGE SURVEYS IN 7 COUNTRIES

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Household surveys are the primary method for assessing intervention coverage. They provide key information to support donor investment and to allow programme managers to improve the effectiveness of delivery. SMC (Seasonal Malaria Chemoprevention) programmes aim to ensure that children receive preventive treatments once a month during the malaria season, if the monthly campaigns are well timed four treatments can provide protection throughout the high risk period. Programme managers therefore need simple, reliable monitoring tools which allow assessment of coverage of SMC campaigns. We monitored coverage in 7 countries during the 2015 and 2016 transmission seasons, and based on this experience compiled key lessons learned for the assessment of SMC coverage as part of routine monitoring of national malaria control programmes. Receipt of SMC treatment was assessed from dates recorded on the child's SMC distribution card and from caregiver recall. Assessment

of validity, by comparing with administration registers which recorded the treatments administered to each child, showed excellent agreement with respect to the distribution of the number of treatments received among those reached by the programme. Overall, in 2015, 88% of children in targeted areas were reached by the programme, 74% of children received at least three monthly treatments and 56% received 4 treatments, and in 2016, 92% were reached, 71% received at least three treatments and 54% received four, in most countries the first years of full-scale implementation. SMC indicators should be included in national malaria indicator surveys but in countries with sub-national implementation of SMC, additional sampling in SMC areas may be required to yield sufficient precision for SMC coverage. This should be supplemented by assessment of the quality of SMC delivery, and adherence to the treatment regimen, through interviews with caregiver and community health workers during and immediately after SMC monthly campaigns.

## 1697

### IMPROVING INTERMITTENT PREVENTIVE TREATMENT FOR PREGNANT WOMEN (IPTP) COVERAGE IN 5 DISTRICTS IN CHAD (DOBA, BEBEDJIA, BODO AND BEBOTO) AND KRIBI DISTRICT IN CAMEROON

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Malaria is the leading cause of morbidity and mortality in Cameroon and Chad, where an estimated 500,000 and 1.5 million cases occur every year, respectively. In Cameroon, 55% of hospitalizations and 241 deaths among pregnant women reported in 2010 were due to malaria. In Chad, malaria accounted for 30% of hospital admissions and 41% of deaths among pregnant women in 2013. To improve uptake of intermittent preventive treatment for pregnant women (IPTp) for malaria in 5 districts in Chad and the Kribi district of Cameroon, Jhpiego adopted strategies targeting the 4 levels of the health system in each country: updating national policies and guidelines, building capacity of providers, building community health workers' (CHWs) capacity, and engaging in behavior change communication. Nationally, Jhpiego provided technical guidance to the Ministries of Health to develop tools including: training and malaria in pregnancy (MIP) reference manuals for providers and CHWs, guidelines on IPTp, and key supervision and data collection tools. At the regional/district levels, 38 supervisors were trained, and they conducted 248 supervisory visits in both countries, reaching 137 health facilities. At the facility level, 234 providers were trained in malaria prevention and management, MIP, data collection and commodity management. At the community level, 146 CHWs in both countries were trained to raise awareness on malaria prevention and control. In Chad, CHWs referred 6424 pregnant women for antenatal care/IPTp and 11679 pregnant women for malaria treatment in 2014 and 2015. Health facility and CHW data collection tools were revised and monthly validation of district data was implemented to improve data reliability, completeness, and readiness. As a result of Jhpiego's activities in Kribi, IPTp rates increased from the start of the project in 2012 to 2015: from 70% to 83% (IPTp1), 61% to 80% (IPTp2), and 12.7% to 28.1% (IPTp3). Similarly, from 2012 to 2015 in Chad, IPTp1 rates increased from 40% to 83% and from 30% to 50% for IPTp2. These gains are a result of training paired with coaching and supervision activities of trained providers and targeted facilities.

## 1698

### COSTS OF CONTINUOUS ITN DISTRIBUTION CHANNELS: A MULTI-COUNTRY CASE SERIES

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Sustained universal coverage with ITNs is essential to malaria control. While mass campaigns deliver nets in a single time-limited operation, continuous distribution systems (CD) use existing infrastructure such as clinics, schools, religious leaders and community-based workers to deliver nets continuously over time. WHO recommends the use of continuous distribution channels for maintaining universal coverage of insecticide treated nets (ITNs), but little is known about the cost or cost-effectiveness of this approach relative to others. The cost of ITN distribution was estimated in four settings including five distribution modalities: Zanzibar (community), Ghana (schools, ANC/EPI), Tanzania (schools, ANC/EPI), Mali (urban mass campaign, rural mass campaign, and ANC). The study utilized the provider perspective and estimated both economic and financial costs. Costs were collected retrospectively from financial and operational records and through stakeholder interviews at the national and regional level. A survey instrument was utilized to collect resource use and expenditure information at the subnational and distribution point levels. Preliminary results show a range of financial costs for LLIN distribution from 0.78 - 3.34 USD per LLIN incurred to international donors. Most of the ITN distributions studied (campaign and continuous) had similar donor costs ranging from 0.78 to 1.46 USD, with the Tanzania 2015 school distribution was an outlier, likely because donors covered costs that might have been contributed by the country in other settings. Continuous distribution systems involved higher contributions from countries compared to campaigns, suggesting greater buy-in or potential for health system strengthening with continuous distribution. Malaria transmission models will be used to estimate the epidemiological benefits of maintaining high coverage of ITNs between mass campaigns. These results will be combined with the estimated costs to produce estimates of the epidemiological effects, cost-effectiveness and net health benefit of continuous distribution.

## 1699

### IMPROVED METHOD FOR PURIFICATION OF *PLASMODIUM FALCIPARUM* LATE STAGE TROPHOZOITES AND SCHIZONTS FROM *IN VITRO* CULTURES USING MAGNETIC SELECTION

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*In vitro* culture of *Plasmodium falciparum* has provided important insights for parasite biology and pathogenesis as well as studies of drug resistance and targets of host immunity. Cultures are commonly maintained

asynchronized at low parasitemia. However, certain experiments require high parasitemia and/or highly synchronized parasites that can be difficult to achieve with current methods. Enrichment and synchronization of parasite-infected red blood cells (irbc) are currently based on density, osmotic fragility of irbcs or on magnetic properties of hemozoin, a disposal product from the digestion of hemoglobin by the parasite containing iron. The magnetic purification is less stressful to parasites compared to other methods because they are maintained in physiological buffer. The culture is loaded into a column containing small iron beads and placed in a magnet. Late stage irbcs that contain large amounts of hemozoin are preferentially retained on the column and subsequently eluted from the column by removing the magnet. Current protocols add physiological buffers to the column to elute irbcs by gravity, however many late stage irbcs that contain more hemozoin can be retained on the column. By eluting parasites using a syringe to increase negative pressure to the column, the quantity and purity of mature stage parasites, especially late stage schizonts, are routinely increased by 2 fold or more. Parasite growth and survival was not compromised by this method in culture. Other modifications such as changes in hematocrit, buffers and number of elutions per column were tested to further optimize the late stage irbcs purification and will be described. Obtaining larger quantities of late mature stages can be valuable in many different fields of malaria research.

## 1700

### IMPROVING MALARIA CASE MANAGEMENT AND SURVEILLANCE THROUGH A COMMUNITY-BASED PILOT IN PANAMA

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Panama aims to eliminate malaria by 2020. In 2016, 61% (496/811) of reported cases in Panama were detected in the Guna comarcas, indigenously populated areas requiring culturally appropriate case management and surveillance strategies. The comarca Guna Yala, located along the northeast coast of Panama is comprised of 49 communities, many of which are accessible only by boat. Due to the remote location of these communities, diagnosis, treatment, and reporting are often delayed. Time between symptom onset and diagnosis via microscopy may be up to 30 days. In an effort to strengthen case management and surveillance in Guna Yala, the Ministry of Health of Panama (MINSa), Guna authorities, and Clinton Health Access Initiative, launched a 12-month pilot program in October 2016. Community health workers (CHWs) were recruited and trained to perform malaria rapid diagnostic tests (RDTs), treat positive cases, report RDT results, and prepare blood smears for microscopy confirmation. CHWs were trained according to MINSa guidelines specific to malaria case management in remote areas. In October 2016, 44 CHWs from 42 communities in Guna Yala were trained and equipped. In the first three months of the pilot, CHWs tested 203 febrile patients using RDTs; 48 (24%) were both RDT and microscopy positive. An additional 10 cases were detected during microscopy confirmation. Cases found by CHWs represented 41% (58/143) of those reported in Guna Yala during this period. In January 2017, a supervision visit was made to CHWs. Of the 44 trained CHWs, 80% (35) were still active and 32 were evaluated on their malaria knowledge, diagnostic and treatment practices, and stock management. Almost all CHWs visited (29/32) performed RDTs appropriately, but only two-thirds (21/32) accurately interpreted RDT results. When presented with case scenarios, nearly two-thirds of CHWs (20/32) demonstrated that they would administer the correct treatment. In response, 30 CHWs were provided with additional training and a dosing wheel. Through community participation, this pilot program has helped to strengthen passive surveillance and to provide timely treatment to malaria cases.

## 1701

### ANTIMALARIAL PRESCRIPTION PRACTICES AT 21 PUBLIC OUTPATIENT FACILITIES LOCATED IN REGIONS OF VARYING MALARIA ENDEMICITY IN UGANDA

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In Uganda antimalarials are often prescribed when malaria is unlikely, a problem that is becoming critical following the adaptation of effective but expensive ACTs as the first and second line treatment for uncomplicated malaria. We present data on antimalarial prescription practices among outpatients seen at Malaria Reference Centers (MRCs) who had malaria test results. The Uganda Malaria Surveillance Program (UMSP) collects individual level data from 21 MRCs located in different regions of the country including the Northern region (Acholi sub-region (8); Lango sub-region (7)), South-west (2), West Nile (1), Eastern (1), East central (1) and Central (1). At each site data including medicines prescribed are captured in standard outpatient registers. The data are then entered into an electronic data base, and submitted to a core facility at weekly and monthly intervals for analysis and report generation. Between Jan 2015 and March 2017, a total of 1,651,950 patients were seen at all the MRCs; Acholi sub-region (669,050), Lango sub-region (549, 705) and other regions (433,195). Most (87.2%) patients had a malaria test performed, of which 70.3% were confirmed by Rapid Diagnostic Testing (RDT). Proportion of patients with a positive test was 71.3% in Acholi sub-region, 51.9% in Lango sub-region and 38.9% in the other regions. Of children with a negative blood smear, 11,888 (12.4%) in Acholi sub-region, 12113 (11.8%) in Lango sub-region and 6232 (5.9%) in other regions were prescribed an antimalarial. Artemether-lumefantrine was the most commonly prescribed antimalarial among children with positive (92.3%) and negative (91.2%) malaria test results. Among patients with a negative malaria test result, patients with a negative RDT (OR 1.2, 95%CI 1.17, 1.24, p<0.001) were more likely to be prescribed antimalarials compared to those with negative microscopy result. Prescription of antimalarials to patients with negative malaria test results is common at MRCs. With no clear benefit of this practice, there is an need to understand reasons why clinicians continue to prescribe antimalarials even when test results are negative.

## 1702

### CONTRIBUTION OF THE IMPROVING MALARIA CARE (IMC) PROJECT TO IMPROVING MALARIA CASE MANAGEMENT IN BURKINA FASO: STRENGTHENING THE CAPACITY OF HEALTH CARE PROVIDERS

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Malaria kills mostly children under five and pregnant women in Burkina Faso, and is the leading reason for medical consultation and hospitalization. Improving case management is a real challenge in reducing morbidity and mortality. The goal of the National Malaria Control Program (NMCP) was to reduce the morbidity by 75% by end of 2000 and malaria mortality to close to zero by the end of 2015. The United States Agency for International Development-supported Improving Malaria Care (IMC) project aims to reduce malaria morbidity and mortality. This includes strengthening the capacity of health providers to deliver high quality management- diagnosis and treatment, of malaria cases. Between 2014 and 2016 IMC and the NMCP revised malaria guidelines, oriented 163

national trainers, trained 1,819 providers at all levels and organized supportive supervision of these staff. As a result correct diagnostic testing of malaria cases increased from 62% to 82%. The proportion of people with uncomplicated malaria who received artemisinin combination therapy (ACT) increased from 85% to 94%. Strengthening of the data management system facilitated this information to be collected. Training these providers based on national guidelines and reinforcing their learning through supervision has enabled the NMCP to have a pool of health providers capable of treating the most vulnerable population and helping to reduce malaria mortality level in Burkina Faso. This training is accompanied by the implementation of formative supervision. Continued supervision and quality data management positions the NMCP to reach and document its goals.

## 1703

### PLASMODIUM RIBOSOMES DO NOT STALL ON POLYA TRACKS

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We have recently described a gene regulatory mechanism that is based on coding polyadenylate - poly(A) tracks that stall and frameshift translation apparatus in multiple model organisms. The levels of mRNAs of genes with A-rich motifs are regulated by the programmed mRNA decay through a combination of no-go (NGD) and nonsense-mediated (NMD) decay. Analysis of eukaryotic genomes coding sequence indicates a potential pool of polyA track regulated genes that range from 2% in most of the species, including human, to 60% in *Plasmodium falciparum*. The extreme AT richness (80%) of *P. falciparum* genome, an unusually high number (>2000 copies) and length of polyA tracks (111As in a row) indicates possible differences in the translational machinery and mRNA surveillance mechanisms compared to the other organisms. Here we show that *P. falciparum*, in contrary to the other tested organisms, can efficiently translate mRNAs with polyA tracks without notable effects on mRNA and protein stability. Biochemical and ribosome profiling analyses of endogenous genes and reporter sequences with engineered polyA motifs indicate that polyA tracks don't induce ribosomal stalling and/or frameshifting in *P. falciparum*. Biochemical analysis of the same reporter sequences in *T. thermophila*, a eukaryotic organism with 75% AT-rich genome, indicates the *Plasmodium* genus as a specific group of organisms with these capabilities. Further, we have dissected components of *P. falciparum* translational machinery and mRNA surveillance pathways. We find that in comparison to human cells, an absence of classical NGD pathway as well as specific features of *Plasmodium* ribosomes play a role in polyA track translation. Our analysis of RACK1 protein in human cells indicates a role for this protein in both NGD and non-stop decay (NSD) mechanisms. The lack of ribosome-associated *P. falciparum* RACK1 protein compared to the other eukaryotes suggests alternate mechanisms for targeting NSD and NGD substrates in malaria parasites.

## 1704

### MAPPING THE POLICY AND PROGRAMMATIC DECISION-MAKING LANDSCAPE FOR MALARIA CONTROL INTERVENTIONS: A CASE STUDY FOR MALI AND ZAMBIA

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Global efforts to fight malaria have proven successful in recent years, yet the financial resources required to sustain progress and further reduce the malaria burden remain limited. To best optimize available funding, countries and programs have to make evidence-based decisions when choosing if and how to implement malaria control interventions, including new vector control tools. However, the translation of evidence into policy and practice is a complex process unique to each country that must

be understood to better support evidence utilization. This study aims to describe the malaria control decision-making landscape in Mali and Zambia and provide an assessment of the facilitators and barriers that affect evidence generation and utilization. We employed a case-study methodology involving a comprehensive document review that was used to inform the development of a semi-structured interview guide for each stakeholder group and country program. Snowball and purposive sampling methods were used to identify relevant stakeholders, and semi-structured interviews were conducted with consenting participants. Questionnaires were distributed to informants not able to attend in-person interviews. Interview transcripts and questionnaires were then coded to conduct thematic analyses. The results presented provide details on the roles of stakeholders involved in the decision-making process, the platforms used for collaboration and strategy discussions, the variables and evidence considered when making decisions, the methods used to prioritize and allocate resources, the influence shifting entomological and epidemiological patterns have on decision making, and the challenges encountered in the decision-making process. This study provides a contextual understanding of how malaria policy and programmatic decisions are made in Mali and Zambia and the role of evidence use in each country. Understanding these complex landscapes is an important step in promoting collaboration between researchers and policy and program officials to maximize the utilization of evidence when making decisions about malaria control interventions.

## 1705

### CONTRIBUTION OF THE STANDARDS-BASED MANAGEMENT AND RECOGNITION (SBM-R) APPROACH TO FIGHTING MALARIA IN BURKINA FASO

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In 2015, Burkina Faso recorded 8,286,463 malaria cases, including 450,024 severe cases with 5379 deaths. The main reasons for these death are: Inadequate application of national malaria diagnosis and treatment guidelines, delays in seeking health care and poor quality of case management. The Standards-Based Management and Recognition (SBM-R) approach is used to improve quality of care using performance standards based on national guidelines. SBM-R includes the following steps: set performance standards; implement the standards; monitor progress and recognize as well as celebrate achievements. Areas covered by the approach are: services organization, case management at both health center and community, Intermittent Preventive Treatment in Pregnancy (IPTp), promotion of Long Lasting Insecticide treated Nets (LLIN) use and infection prevention and control. Since June 2016, 26 health facilities in three regions have been implementing SBMR. Therefore, 105 health workers have been trained. Performance progress was measured through 5 evaluations including baseline. Baseline has shown the highest score was 47% (Kounda) while the lowest was 9% (Niangoloko). The main issues observed were: lack of program activities, management tools, handwashing facilities, LLINs and misuse of Rapid Diagnosis Tests. Their cause was determined and an improvement plan was developed by each site. The second, third and final evaluations revealed a change in performance scores for all sites. The external evaluation showed 17 out of 26 health facilities with a score higher than 60%; among them 10 with a score above 80% (Bougoula, 94%). At the same time, IPTp 3 increased from 34.48% in 2014 to 78.38% in 2016 and no malaria death has been registered since October 2015. For the site under 80% the key reasons were: staff turnover, commodities stock-out and lack of infrastructure. The process continues with recognition of health facilities and supporting others (those at less than 80%) to reach the desired performance level. The SBM-R approach appears to be a great tool for improving quality and performance of health facilities.

## 1706

**THE KENYAN MALARIA MARKET AFTER AMFM**

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In Kenya, the Affordable Medicines Facility for malaria medicines (AMFm) pilot project was implemented in 2010-2013. The objective was to scale up quality assured ACTs (QAACT) by bringing down the price through factory gate subsidies, and flush out antimalarial monotherapies. The MOH recommends test before treatment of positive cases with ACTs across all malaria epidemiological zones and the administration of Sulphadoxine Pyrimethamine (SP) is restricted to endemic areas for malaria prophylaxis in pregnancy. ACTWatch survey is a multi-country research project implemented by PSI with the goal of monitoring key antimalarial market indicators at national level. Between 2010-2016, four rounds of descriptive cross-sectional surveys were conducted with representative sample of locations. In each sampled location, a census of all public and private outlets with potential to sell or distribute antimalarials or provide malaria blood testing was conducted. Data were analysed using Stata. Availability of quality assured ACTs (QAACTs) among public facilities increased from 88% in 2010 to 95% in 2016. In 2016, among public facilities, SP availability was 70% in endemic areas, and not available in other zones. In the anti-malarial stocking private sector, availability of QAACTs rose from 20% in 2010 to 46% in 2016. Availability of non-QAACTs in private facilities increased to 52% in 2016 and SP availability was common (76%). The private sector dominated the antimalarial market share at 70%, most commonly by pharmacies. SP was commonly distributed in the private sector. Availability of malaria testing was high in the public sector at 88%, but low in the private sector- 20%. There is a high readiness for the management of malaria in Kenya, particularly in the public sector. The high market share and low testing in the private sector indicates widespread presumptive treatment. The wide availability of SP in non-endemic areas in the private sector indicates that it is unlikely that it is being used for IPTp. The MOH and regulatory bodies should engage the private sector to decrease the use of non-QAACTs and ensure SP is not used for malaria treatment.

## 1707

**"IT'S BEEN USED FOR A LONG TIME": EXPLORING PRIVATE PROVIDERS' PREFERENCE FOR CONTINUED USE OF ORAL ARTEMISININ-BASED MONOTHERAPY IN MYANMAR**

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Continued use of oral artemisinin-based monotherapy (oAMT) is widely considered to be one of the main factors in the development and spread of resistance to artemisinin and its derivatives. Importation and registration of oAMT was banned in Myanmar in 2011. Since 2012 a program has been underway to crowd-out oAMT from the private sector with subsidized, quality assured ACT (QAACT). Outlet survey data show oAMT availability decreased from 67% in 2012 to 20% in 2016 and market share from 44.3% to 10.6%. Despite these impressive gains, oAMT use persists. We conducted a qualitative study at 24 private sector outlets in May 2016 to better understand provider motivation to stock and sell oAMT. Providers were purposively sampled for inclusion from townships with the highest oAMT availability reported in the 2015 outlet survey. In-depth interviews were conducted at the provider's outlet or a location of their choosing in local languages, and were recorded. Discussions covered providers' historical and current malaria treatment practices, reasons for stocking/selling oAMT and business decision-making, among other topics. Transcripts were coded, and codes were compiled to generate emerging themes. Providers perceived oAMT as being an effective medicine with few side effects and a quick action, requiring few tablets (compared to

QAACTs). Providers were either motivated by continued demand from clients or reluctance to change a familiar approach to malaria treatment. Providers reported a higher profit margin with oAMT, compared with price-controlled ACTs. Most respondents described oAMT as a replacement drug for older antimalarials but none were motivated to switch from oAMT to ACT. Few providers were aware of the ban on monotherapy. Findings from this study can inform the revision of provider communication messages to better address the reasons why providers continue to stock oAMT. Future efforts should also focus on strengthening consumer behaviour change communication to address patient demand for oAMT. In addition, increased government enforcement of the oAMT regulations could help counter the profit incentive driving the sale of some oAMTs.

## 1708

**RESULTS FROM A BASELINE SURVEY TO EVALUATE DIFFERENT INDOOR RESIDUAL SPRAY IMPLEMENTATION STRATEGIES FOR MALARIA CONTROL**

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Indoor residual spray is an important intervention for the control of malaria vectors. Most national malaria control programs do not have large IRS operations due to the costs involved, and those that do are rarely able to protect more than 30% of the population at any risk of malaria transmission. To implement IRS therefore begs the question, if we cannot spray all the houses at risk how should we prioritize which houses we spray? IRS works through a type of herd protection, wherein a blood-seeking mosquito may feed on an individual in a house protected by IRS but dies while resting on the sprayed wall following the bloodmeal. Therefore at the local spatial scale, we know that IRS needs to be applied in a high concentration. Unfortunately, information is scarce on the threshold needed for herd protection, but the WHO sets an 85% coverage target as the standard for successful IRS implementation at local spatial scales. Yet, at larger spatial scales it remains unknown whether concentrating limited resources in one area (at the expense of others) is better than spreading limited resources across the entire area (while still maintaining high concentration at local spatial scales). We are currently conducting a trial of different IRS implementation strategies at the district level. Three strategies will be compared: 1) concentrating all available IRS resources in a single district, 2) spraying highest risk communities in 2 districts with risk defined as proximity of a community to health centers with the highest incidence of malaria, and 3) spraying highest risk communities in 2 districts with risk defined as communities with the highest likelihood of having *Anopheles funestus* populations. The three outcomes of *Plasmodium falciparum* infection, counts and densities of *Anopheles* mosquitos, and health facility incidence will be analyzed using a difference-in-differences approach to assess the effectiveness of each strategy. Herein we will present the study design and results from the baseline cross-sectional survey.

## 1709

**USING SMS TO REPORT STOCK-OUTS OF ANTI-MALARIA MEDICINE AND BED NETS IN GUINEA**

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The millennium development target 6c1 calls for the halt and reverse of malaria incidence by 2015. The global malaria mortality rate reduced by 58% between 2000 and 2015. It is estimated that 6.2 million deaths from malaria were averted, the majority of which are children under five years old in Sub-Saharan Africa. While great strides have been made in malaria control, in 2015 over 212 million new cases were reported worldwide, 90% of which were from the WHO Africa Region<sup>3</sup>. In 2015 Guinea

reported an estimated 4.6 million cases and 9,900 deaths. The global technical strategy for malaria 2016-2030, which targets a 90% reduction of malaria incidence and mortality globally by 2030 was approved by the world health assembly in May 2015. The first step to better provide services to those at risk of malaria in Guinea is an efficient and effective reporting system. RTI International is conducting a formative evaluation that aims to determine if weekly SMS reporting is more beneficial than monthly paper based reporting through evaluating report quality, reporting cost and frequency of antimalaria medication and bed net stock-out and the secondary aims of the evaluation are to determine the perception of a SMS reporting system. As a result of analyzing the StopPalu's intervention outcomes, RTI International proposed to integrate technology to accelerate the development outcomes of the project. The integration of technology into a project intervention is a new area that is being explored across USAID and other donor-funded projects as the rapid spread of mobile technologies to developing countries continues. The findings of this evaluation can serve as a base for similar project interventions.

## 1710

### SEASONAL MALARIA CHEMOPREVENTION SCALING UP AND ITS IMPACT ASSESSMENT IN MALI

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Seasonal Malaria Chemoprevention (SMC) was recommended in March 2012 by World Health Organization for sahelian countries as control strategy. Mali was one of the first countries to introduce SMC and reaching nationwide coverage in 2016. From 2014, the ACCESS-SMC project funded by UNITAID has supported the scale up and monitoring and evaluation (ME) of SMC in seven sahelian countries including Mali. As part of the ME the coverage of the intervention using cross-sectional surveys and its impact on malaria burden were assessed. Children aged from 3 to 59 months years old received rounds of SMC with Sulfadoxine-pyrimethamine plus Amodiaquine at monthly interval during rainy season up to four months. SMC drugs were given at fix point in village or area. Rapid diagnostic test for malaria was performed in case of fever during the drug distribution campaign and malaria cases was treated on site during the campaigns. Cross-sectional survey was undertaken in 2015 and 2016 in December to assess coverage in 50 clusters (villages or quarters of urban areas) randomly selected with probability proportional to size in 5 districts. In each cluster about 20 children aged between 4 months and 7 years were surveyed. Impact of SMC on malaria burden was assessed using data routine malaria data from 2013 to 2016 from health centers. In addition, routine individual malaria data from health center registers are being collected using tablets PC. Coverage of SMC defined as proportion of children who received the four rounds of SMC was 43% in 2015 and 53% in 2016. The coverage rate was relatively lower (42%) in children from parents considered themselves poor than in children of non poor parents (58%). Preliminary analysis indicated that SMC was associated with a reduction of 49% of malaria cases. Additional results on the impact of SMC using data from sentinel sites being collected from health center registers will be available and presented.

## 1711

### SCALING-UP OF SEASONAL MALARIA CHEMOPREVENTION IN SOKOTO AND ZAMFARA STATES, NIGERIA: MONITORING DELIVERY AND IMPACT

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Following pilot schemes in Katsina and Jigawa, SMC was introduced in Zamfara and Sokoto states in 2015 and 2016 through the ACCESS-SMC project, an area, which includes 37 Local Government Areas (LGAs) and 1.8 million children (2016 estimate). SMC was introduced in 17 LGAs in 2015 and all 37 LGAs in 2016. A sentinel surveillance system was established to monitor impact of SMC on malaria by collecting routine data on malaria cases before and after SMC scale-up from 24 health facilities, including details of cases of all ages, tested or treated for malaria. At first visit to each facility, retrospective data from 2013-2015 was collected. Subsequently, selected facilities were visited every 3 months to assemble data from the registers. Community-based surveys were conducted at the end of 2015 and 2016 transmission seasons to assess coverage and quality of SMC delivery. Each year, 60 settlements were selected with probability proportional to size, and compact segment sampling used to select survey households. Children up to 7 years of age were included in the survey in order to be able to assess SMC coverage in the target age group (at least 3 months old at the time of SMC administration, and who were less than 5 years at the first cycle), and to determine whether older children were being treated. Receipt of SMC was determined from SMC record cards held by caregivers for each child, and from caregiver recall. In 2015, 84% of eligible children received SMC at least once, 77% received at least three SMC treatments and 54% were protected for four months. In 2016, delivery was expanded to cover all 37 LGAs, but delays in registration of dispersible tablets led to a shortage of drugs at the start of the campaign so that not all areas could be reached at cycle 1. 81% of children received SMC at least once, 46% at least three times, and 21% received four treatments. SMC is part of Nigeria's strategic plan for malaria control for 2014-2020 with nine states eligible to receive SMC, representing 12 million children under 5 years of age. Careful supervision of delivery will be required to maximize the impact of this intervention.

## 1712

### A FATAL CASE OF IMPORTED MALARIA IN MONGOLIA

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On day nine from onset of symptoms, Mongolia reported its first ever malaria case fatality. A 31-year-old Mongolian engineer who was infected with *Plasmodium falciparum* while working in the Democratic Republic of the Congo died of severe malaria shortly after his return to Mongolia. He initially presented at Darkhan-Uul provincial hospital on day five from onset of first symptoms, with malaria like symptoms. He was later hospitalized on day six, when his condition failed to improve. On day 6, the patient tested positive for malaria by microscopy (32% parasitemia). The results were confirmed using a Pf/Pan specific malaria rapid diagnostic test (RDT) and PCR on day seven. Treatment for malaria was initiated

on the evening of day seven. After entering into a coma on the 8th day, the patient died of complications due to malaria the following day, day 9th. Increased international travel and expanded efforts for global development in Africa have led to a rise in imported cases of malaria into non-endemic developing countries like Mongolia. This report focuses on the tenth imported case of malaria in Mongolia since 2005, with more cases expected in the future. This fatal case illustrates the need for readily available RDTs and treatment for suspected cases of imported malaria in Mongolia.

## 1713

### AUTOMATION TRIPLES THROUGHPUT OF PFSMZ MALARIA VACCINE EXTRACTION FROM MOSQUITOES WITH 20-FOLD REDUCTION IN TRAINING TIME

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The global death toll from Malaria is estimated to be upwards of 600,000 people each year, with more than 1,000 children succumbing every day. *Plasmodium falciparum* (Pf) Sporozoite (SPZ)-based vaccines are the only intervention in humans, proven to induce robust, high-level (>90%) and long-lasting (at least 14 months) protective efficacy against malaria, forming the basis of Sanaria's unique technology platform of aseptic, purified, cryopreserved live *Plasmodium falciparum* Sporozoites (PFSMZ). PFSMZ Vaccine contains live PFSMZ attenuated by radiation, PFSMZ-GA1 is attenuated genetically, and infectious parasites in PFSMZ-CVac are arrested at the blood stage by drugs like chloroquine. Driven by stellar clinical safety and efficacy results, and backed by an uncommon consortium of international investigators from >35 research groups in 18 countries, Sanaria plans to receive approval for a Biologics License Application (BLA) by the beginning of 2019 for travelers and by 2020 for all age groups and for mass vaccination programs, and has received Fast Track Designation in 2016 from the FDA for PFSMZ Vaccine, in recognition of its progress. In keeping with scaling-up manufacturing operations, our goal was to automate a key step of isolating SPZ from the salivary glands of mosquitoes based on amalgamating and integrating various biological, physical, chemical and engineering principles. Significant technological advancements included the first-ever development of prototypes for batch-processing mosquitoes in two steps of decapitation and gland extrusion and collection, resulting in at least a 3-fold higher throughput with reduced operator fatigue, a 15-20 fold reduction in operator training time, and significantly improved product purity. Further increase in efficiency is expected through leveraging alternative task-distribution methods that have already been implemented for manual dissection. Data from rigorous workflow analysis and the steps involved in transitioning the semi-automated mosquito microdissection system from laboratory to cGMP-compliant prototypes will be presented.

## 1714

### USING ANTIBODIES TO SIMULATE THE CO-ADMINISTRATION OF TRANSMISSION-BLOCKING AND PRE-ERYTHROCYTIC VACCINES ACCELERATES MALARIA ELIMINATION IN MICE

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Vaccines that reduce human-to-mosquito and mosquito-to-human transmission have the potential to accelerate malaria elimination and can help to achieve eventual eradication. Currently, the lead candidates for transmission-blocking vaccines (TBVs, which restrict parasite development in the mosquito) are in the early stages of development, while pre-erythrocytic vaccines (PEVs, which inhibit hepatocyte invasion) have been shown to be partially effective against clinical malaria in a Phase 3 trial. We explored the benefit that co-administering TBVs and PEVs could have by administering antibodies to a murine population. These antibodies would be upregulated in hosts as a consequence of using vaccines. Multigenerational assays, passaging *Plasmodium berghei* between murine and *Anopheles stephensi* mosquito populations, were conducted simulating different doses of TBV, (these doses were titrated to reduce the percentage of oocyst-infected mosquitoes by 50%, 65% or 85%), or PEV (titrated to reduce the percentage of infected mice by 47.2%). These vaccines target different life-stages of the parasite life cycle and were found to enhance the efficacy of the PEV by 70% when used in combination compared to the expected efficacy were the two vaccines acting independently. This synergism is produced by reductions in parasite density caused by the partially-effective interventions. Using a rodent multigenerational system, we demonstrate that simulating the co-administration of a TBV with a PEV has the potential to accelerate elimination.

## 1715

### CONTROLLED *PLASMODIUM VIVAX* BLOOD STAGE REPEATED INFECTION INDUCES PROTECTION AGAINST HETEROLOGOUS CHALLENGE IN THE NON-HUMAN PRIMATE MODEL

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Malaria is caused by parasites of the genus *Plasmodium* that are transmitted to humans by the bites of *Anopheles* mosquitoes. After the elimination of *Plasmodium falciparum* it is predicted that *Plasmodium vivax* will remain a major cause of morbidity and mortality outside of Africa, stressing the importance of developing a vaccine against *P. vivax* malaria. The blood stage antigens AMA1 and MSP142 have individually been shown to induce partially protective immune responses in *Aotus* monkeys. Current studies to develop a *P. vivax* vaccine are focused on inducing stronger antibody responses in combination with already robust T cell responses. At present there is no benchmark to assess the level of protection (cellular or humoral) needed to achieve sterile protection against a heterologous *P. vivax* blood stage challenge in both *Aotus* and humans. We have previously demonstrated successful induction of sterile protection in *Aotus* monkeys by repeated blood stage infection with homologous and heterologous *P. falciparum* strains. Here we present a model to induce sterile protection against *P. vivax* by repeated blood stage

infections and challenge with homologous (Sal-1) or heterologous (AMRU-1) strains. Blood samples were collected across the experiment to measure parasite and host parameters, including correlates of protection against blood stage antigens such as AMA1, MSP142 and DBP. This study will provide a benchmark for testing the efficacy of candidate blood stage *P. vivax* malaria vaccines in the non-human primate model.

## 1716

### HUMAN TO MOSQUITO TRANSMISSION OF SUB-MICROSCOPIC *PLASMODIUM FALCIPARUM* GAMETOCYTE DENSITIES DURING CONTROLLED HUMAN MALARIA INFECTION AND QUANTIFICATION OF MALE AND FEMALE GAMETOCYTES

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The controlled human malaria infection (CHMI) model has been used successfully to assess efficacy of drugs and vaccines targeting the pre-erythrocytic and blood stages of malaria infection. However, existing models have yet to be used to assess the ability of interventions to interrupt transmission of malaria from humans to mosquitoes. Such a model would be an invaluable tool for selecting the most promising transmission blocking interventions (TBIs) for further evaluation. Here we report the reproducible induction of gametocytes in CHMI participants and the transmission of these gametocytes to *Anopheles* mosquitoes. We used the induced blood stage malaria (IBSM) model with *P. falciparum*-infected red blood cells to initiate blood stage infection in malaria-naïve volunteers (n=17). Volunteers were then treated with piperazine (480mg) to clear asexual parasitemia and allow development of gametocytes. Utilizing RT-qPCR with sex specific mRNA markers we were able to detect and quantify sub-microscopic levels of male and female gametocytes circulating in all 17 volunteers. Following gametocyte detection, transmission studies were performed on a subset of volunteers using direct skin feeds and membrane feeding assays. Successful transmission of gametocytes to laboratory reared *An. stephensi* mosquitoes was achieved from 73% of participants (8/11), and transmission success was related to gametocyte density. Prevalence of infection was up to 16.7%, and quantitative analysis of specific biomarkers was undertaken to further evaluate factors that may contribute to transmission efficiency, including male:female gametocyte sex ratios and gametocyte commitment. This is the first step in the development of a valuable new tool that may accelerate the discovery and prioritization of the most promising TBIs. These studies also provide a novel insight into the dynamics of sexual stage malaria parasite development and transmissibility in a controlled infection setting. This work may therefore contribute to the current understanding of natural malaria transmission, a critical consideration of malaria elimination and eradication agendas.

## 1717

### IMMUNOGENICITY OF THE RTS,S/AS01E VACCINE IN AFRICAN CHILDREN: EFFECT OF AGE, MALARIA TRANSMISSION INTENSITY AND ASSOCIATION WITH PROTECTIVE EFFICACY

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RTS,S/AS01E is the most advanced malaria vaccine candidate in the clinical pipeline. The vaccine efficacy is moderate, lower in infants (6-12 weeks) than children (5-17 months), and of limited duration. Furthermore, little is known about its mode of action, epitope specificity, the quality of antibodies elicited, including Ig subclass patterns, and how they are affected by age and malaria endemicity. We have applied an in-house multiplex bead-based antibody assay to assess the immunogenicity of RTS,S malaria vaccine in children and infants participating in the Phase 3 African trial. We performed a case control study analyzing IgG subclasses 1-4 and IgM elicited by RTS,S in participants from Kintampo (high-medium malaria endemicity, n=80) and Manhica (low malaria endemicity, N=125). Additionally, the effect of age and malaria intensity transmission were determined, and associations with malaria protection were explored. A total of 393 samples at baseline and month 3 (peak response) in RTS,S vaccinees and comparators were analyzed against 3 CSP antigen constructs: the CSP full length, the NANP central repeat, and the C-terminal, and HBsAg by Luminex. The RTS,S vaccine induced high antibody responses to CSP full length, NANP and C-terminal one month after 3 vaccinations for all Ig isotypes and subclasses (p-value < 0.001). We found higher IgG and subclass antibody levels in children than in infants (p-value < 0.01), except for IgM and IgG2. Baseline levels of anti-CSP IgG and IgG subclasses were higher in Kintampo (p-value < 0.001) but no differences between sites were found for IgM levels. Furthermore, the vaccine induced higher levels of anti-CSPs IgM at month 3 in Manhica than in Kintampo, whereas no differences at peak response on antibody levels were found for IgG. Finally, we will report data on the association between anti-CSP antibody levels at month 3 and occurrence of clinical malaria cases in an exploratory single-marker analysis in this set of volunteers. A better characterization of the mode of action of RTS,S will greatly facilitate the development of more efficacious and long-lasting vaccines for malaria eradication.

## 1718

### IMMUNE CORRELATES OF PROTECTION AFTER VACCINATION WITH RTS,S-AS01E: ROLE OF ANTI-CSP ANTIBODY AVIDITY

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The completion of the RTS,S phase 3 trial and its planned pilot implementation in endemic countries constitute landmark events in the history of malaria vaccine development. The results generated during this unprecedented trial have also shown the compelling need to better understand the mechanisms underlying the protection induced by RTS,S/AS01E but also to describe the factors influencing the immune response induced by the vaccine during the early stages following the vaccination. To this perspective we have analyzed the IgG antibody avidity to the circumsporozoite protein repeat (NANP) and C-terminal regions of CSP in plasma/serum of participants in the phase 3 trial at baseline (M0) and at 1

month (M3) after the third dose of RTS,S. The samples were obtained from volunteers enrolled in the African multicenter RTS,S phase 3 trial. In total, 500 children aged between 5 to 17 months and 309 infants aged between 6 to 12 weeks at the time of first dose of RTS,S/AS01E inhabiting Nanoro (Burkina Faso), Kintampo (Ghana) and Bagamoyo (Tanzania) participated our study. We found that RTS,S vaccination resulted in a highly significant increase in IgG responses not only to NANP but also to the C-terminal CSP antigens at M3. We will present the comparison of the magnitude and avidity of response between the three sites of different malaria transmission intensity and between the two age cohorts. Furthermore, we will show data on the importance of the differences in CSP IgG avidity at M3 between protected and non-protected RTS,S vaccinated children after one year follow up. Data from this study provides valuable information on the fine specificity of the vaccine and the quality of the antibodies induced and their importance towards protection against malaria.

## 1719

### A CONTROLLED HUMAN MALARIA INFECTION MODEL COMPARING LOW-DOSE PIPERAQUINE AND SULFADOXINE-PYRIMETHAMINE TO INDUCE INFECTIOUS MALE AND FEMALE *P. FALCIPARUM* GAMETOCYTES

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The renewed focus on malaria elimination has increased the priority of research towards development of transmission blocking interventions (TBI) such as vaccines targeting the sexual, sporogonic, or mosquito stages of the parasite, and gametocytocidal drugs. Whilst there is broad consensus that TBI can play a significant role in malaria elimination initiatives, an effective model for the early clinical evaluation of TBI is currently unavailable. Here, we present a model to induce gametocyte carriage in participants by four different drug regimens to attenuate asexual parasitaemia without affecting developing or circulating gametocytes: low-dose piperazine (LD-PIP) followed by curative piperazine (PIP); LD-PIP followed by curative sulfadoxine-pyrimethamine (SP); low-dose sulfadoxine-pyrimethamine (LD-SP) followed by PIP; and LD-SP followed by SP. Four participants were enrolled per arm. All participants (16/16) developed mature gametocytes. Male (by novel PF3D7\_14699000 qRT-PCR) and female gametocytes (Pfs25 qRT-PCR) were observed 10-12 days after the first appearance of asexual parasites, supporting the hypothesis of gametocyte commitment during the first wave of asexual parasites. Gametocyte densities and the area under the curve of gametocytaemia versus time were highest in the LD-PIP/SP arm ( $p=0.04$ ). We demonstrated that the peak and total gametocyte burden are associated with the preceding asexual parasite biomass. Upon release in the circulation, male gametocytes had a mean circulation time of 2.68 days (95% CI 1.46 – 3.91) compared to 5.12 days (95% CI 4.10 – 6.14) for female gametocytes. The estimated female/male sex ratio of gametocytes in our study participants was 2.1 (SD=1.3). Exploratory mosquito feeding assays confirmed the viability of these gametocytes by demonstrating successful mosquito infections in a small subset of participants. In this study we present promising data for a critical unmet need by making available a more biologically relevant assay to address gametocyte biology and dynamics, and potential to evaluate TBI against *falciparum* malaria during early clinical development.

## 1720

### ANTIBODY CORRELATES OF NATURALLY ACQUIRED IMMUNITY AGAINST MALARIA IN CHILDREN PARTICIPATING IN THE RTS,S/AS01E PHASE 3 AFRICAN MULTI-CENTER TRIAL

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RTS,S/AS01E is the most advanced malaria vaccine candidate in clinical development, having undergone a phase 3 trial for licensure. Many gaps in knowledge remain as to how and why vaccine efficacy is affected by age and malaria endemicity, and what is the impact of RTS,S vaccination on naturally acquired immunity. In this study, we assessed antibody responses to a large panel of *Plasmodium falciparum* antigens and their association with RTS,S/AS01E vaccination and protection from clinical malaria, as well as the effect of age and malaria endemicity. We performed an exploratory case control study including 80 children (5-17 months) and infants (6-12 weeks) from Kintampo (high-medium malaria endemicity) and 117 from Manhica (low malaria endemicity). Samples were analyzed at baseline and month 3 (peak vaccine response) in RTS,S vaccines and comparators. We applied an in-house multiplex assay to measure IgG, IgG subclasses 1-4 and IgM levels to 34 blood-stage, 3 pre-erythrocytic and 4 RTS,S-specific antigens. We will report the effect of RTS,S vaccination in naturally acquired antibody responses to diverse antigens in both sites and age cohorts, taking into account endemicity and maternal antibodies. We will also report preliminary data of the association between levels and breadth of antibody isotypes/subclasses and future clinical malaria over a one year follow up. Finally, this study will allow to down-select and prioritize immunological variables, before conducting a larger longitudinal immune correlates study in multiple trial sites. A systems biology approach will be adopted to integrate the data and identify signatures associated with vaccination and protective immunity. Identifying the immune correlates of protection against malaria in the context of the large phase 3 trial gives unprecedented potential to obtain valuable information useful for the development of more efficacious and long-lasting vaccines for malaria eradication.

## 1721

**PARASITE MULTIPLICATION RATES DURING CONTROLLED HUMAN MALARIA INFECTIONS IN TANZANIAN ADULTS**

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Controlled human malaria infection (CHMI) using aseptic, purified, cryopreserved, infectious *Plasmodium falciparum* strain NF54 sporozoites (Sanaria® PfSPZ Challenge) is being used to investigate novel malaria drugs, vaccines, and innate and naturally acquired immunity. Standardized use of 3,200 PfSPZ administered by direct venous inoculation (DVI) enables comparison of outcomes across sites and trials. Conduct of CHMI in African populations ensures that results are generated in the individuals most in need of novel interventions against malaria. During the past 4 years we have conducted CHMI in 94 Tanzanian adults with self-reported minimal recent malaria. Monitoring of asexual blood stage parasitemia during CHMI was done by thick blood smear (TBS) microscopy and DNA based qPCR assays. In 3 of 36 non-vaccinated control volunteers, qPCR but not microscopy positivity thresholds were reached within the pre-defined 28 days of follow-up. Parasite genotyping based on MSP1, MSP2 and microsatellite size polymorphisms confirmed that all parasites detected during CHMI were NF54. On average, volunteers were treated for malaria 5.5 days after parasites were detected in blood by qPCR. Thus, there were ~2 cycles of asexual parasite growth between detection and treatment. Post CHMI blood samples were collected daily enabling calculation of parasite multiplication rates (PMR). We will present data on the PMR in Tanzanians and compare these with the PMR from malaria naïve volunteers. A group of five placebo control volunteers in the BSPZV2 trial underwent two consecutive homologous CHMIs ~6 months apart. During the first CHMI, 5 of 5 volunteers has positive TBSs by microscopy during the 28 day follow up. However, during the second CHMI, 4 out of 5 volunteers remained negative by TBSs, but were identified as malaria positive by qPCR. We conclude that CHMI in malaria pre-exposed volunteers leads to a significant boost of acquired asexual erythrocytic stage immunity as indicated by a highly reduced PMR. CHMI studies in Tanzanian volunteers provide a unique tool to identify immune effector mechanisms under highly controlled conditions.

## 1722

**BUILDING MALARIA VACCINES USING IN SILICO ANALYSIS AND REVERSE ENGINEERING TECHNIQUES TO TARGET CRITICAL T AND B CELL EPITOPES**

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Effective malaria vaccines capable of preventing disease in a wide range of populations living in regions with vastly different transmission rates and with a multitude of genetically diverse *Plasmodium* species/strains are needed. Multi-antigen vaccines provide a unique opportunity to address this need in a manner that permits inclusion of critical cross stage and cross-species/strain epitopes into a single vaccine candidate. Therefore, we took advantage of the recent advances regarding in silico epitope prediction and analysis tools to identify class I and class II epitopes across a range of malaria antigens including both well-known and lesser known targets. Predictive binding analysis was used to identify sets of epitopes that provide broad HLA coverage. Sequence conservation analyses were performed to identify epitopes conserved across malaria parasite strains or to include additional sequences to fill gaps in cross-strain coverage. In addition, sequence similarity to the human proteome regarding the potential for cross-reactive epitopes, which may induce regulatory T cell responses, was also analyzed. Of interest is that several well-studied malaria vaccine candidates, such as TRAP, CSP, and EBA-175 score low regarding overall levels of class I and class II T cell epitope content, whereas, more novel proteins, such as Rh5 and SPECT-2, score higher in these areas. Further several of the proteins analyzed show regulatory T cell flags that overlap regions containing T cell epitope clusters. To identify critical B cell epitopes, we employed bacteriophage display to bio-pan for mimotopes of antibodies with strong neutralizing activity against conserved protein regions. The resulting VLP-based mimotopes are evaluated for their ability to elicit a parasite neutralizing humoral response. Further, different strategies for displaying these critical epitopes on a VLP platform capable of incorporating multiple insertions are employed to reverse engineer a vaccine. These data along with strategies for epitope selection and testing will be presented.

## 1723

**DIVERSITY-REFLECTING PEPTIDE MICROARRAY AND MUTATION SCAN ANALYSES DEMONSTRATE HIGH STRAIN SPECIFICITY OF VACCINE-INDUCED ANTIBODIES TO A CRITICAL RESIDUE IN THE PLASMODIUM FALCIPARUM AMA1 CLUSTER 1 LOOP**

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A Phase 2 vaccine trial of *Plasmodium falciparum* apical membrane antigen 1 (AMA1), FMP2.1/AS02A failed to demonstrate clinical efficacy against the primary study endpoint, but did show significant strain specific efficacy with parasites expressing AMA1 homologous to the vaccine strain with respect to key polymorphic amino acid residues. Sieve analyses of this strain-specific efficacy pinpointed polymorphisms in a hypervariable region of the AMA1 cluster 1 loop (c1L). Earlier molecular epidemiological and *in vitro* antibody-binding studies also pointed to c1L as a key determinant of immunity to AMA1. To determine the underlying strain- and epitope-specific antibody responses in vaccinated children that these molecular and *in vitro* analyses predicted, we used a high-density peptide microarray to measure the difference in seroreactivity to AMA1 peptides in sera from

Malian children who had received the AMA1 vaccine or a control rabies vaccine. Post-immunization sera from AMA1-vaccinated children had 6-fold greater mean seroreactivity to peptides spanning c1L matching the vaccine strain, compared to the seroreactivity to heterologous c1L peptides. To identify the precise amino acids responsible for this allele-specific efficacy, we tested sera against a collection of peptides containing substitutions of every possible amino acid or a deletion at each c1L position. None of the substitutions were associated with differences in seroreactivity in pre-vaccinated sera. However, in post-vaccination sera, perturbation of the vaccine-homologous glutamic acid residue at position 197, whether by substitution or deletion, significantly reduced seroreactivity. These results establish an immunologic basis for the earlier molecular epidemiological evidence that a single amino acid at position 197 in the AMA1 c1L is the primary determinant of strain-specific efficacy of the AMA1 vaccine. This result suggests that strain-transcending efficacy can potentially be achieved using multiple AMA1 antigens that vary at this single amino acid position despite polymorphisms at other positions in a multivalent next-generation malaria vaccine.

## 1724

### IMPACT OF PROTEIN TARGETING ON IMMUNOGENICITY OF Pfs25 ENCODED BY DNA VACCINE PLASMIDS

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Pfs25 is a leading candidate for the development of *Plasmodium falciparum* transmission blocking vaccines (TBV). Many different vaccine delivery platforms are currently being evaluated to develop an effective vaccine and considerable progress has been made with Pfs25 based DNA vaccines, evaluated in mice and nonhuman primates. Subsequent studies have also shown improved immunogenicity of codon optimized Pfs25 DNA plasmid delivered by intramuscular electroporation. In an effort to further optimize immunogenicity of DNA vaccines we evaluated the impact of protein targeting. We designed plasmids that would allow immunogenicity differences among encoded proteins destined for (1) secretion, (2) endosomal targeting, and (3) retention in the cytoplasm. Female Balb/c mice (5-6 weeks old) were immunized (intramuscular) with three doses, at one month apart, by *in vivo* electroporation with a final Pfs25/alum protein boost. Immune response parameters analyzed included Pfs25-specific antibodies and T cell responses. Studies revealed significant differences between targeted and non-targeted encoded Pfs25 and further emphasize evaluating similar approaches for any future DNA vaccine.

## 1725

### IMMUNIZATION WITH MULTIPLE ALLELES OF *PLASMODIUM FALCIPARUM* FULL LENGTH VAR2CSA DNA CONSTRUCTS TO GENERATE A PLACENTAL MALARIA VACCINE SHOWING BROAD HETEROLOGOUS PROTECTION

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Placental malaria (PM) is caused by adhesion of *Plasmodium falciparum* (Pf) infected erythrocytes (IE) to the placental receptor chondroitin sulfate A (CSA), mediated by the *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) family member called VAR2CSA. Over successive pregnancies, women acquire antibodies against VAR2CSA as they acquire clinical

resistance to malaria. VAR2CSA is a 350kDa, multi-domain protein: many different domain combinations of the protein used as immunogens have failed to generate broadly neutralizing activity against diverse CSA-binding isolates. Recent studies have shown that the full length extracellular domain of the protein has a 100,000-fold higher affinity for CSA compared to any individual domain. Moreover, the extracellular domain has also been shown to be structurally more compact compared to individual domains and antibodies raised against the full length (FL) extracellular protein could completely block homologous IE binding to CSA indicating that the full-length protein could serve as a better vaccine. In preliminary studies with FL VAR2CSA from PfNF54 and PfFCR3 we found that FL VAR2CSA DNA vaccine constructs delivered by electroporation induced neutralizing activity against diverse field isolates. To extend these findings, we have prepared codon-optimized glycosylated versions of six different Pf VAR2CSA alleles: NF54, M1010, Brazil 7G8, HB3, FCR3 and Malayan Camp, and cloned them into the mammalian expression vector VRC8400 such that the proteins will be expressed on the cell surface. A rat immunization study using these constructs either individually or as a blend of six alleles has been initiated. We will report the ability of these individual constructs to show anti adhesion activity against multiple Pf isolates as well as the ability of antibodies generated against the blend of six different Pf VAR2CSA alleles to show broad anti adhesion activity.

## 1726

### BLOOD TRANSCRIPTOME RESPONSES TO *P. FALCIPARUM* INFECTION AND IMMUNIZATION

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Immunization with radiation attenuated *P. falciparum* sporozoites provides protection from infection by non-attenuated parasites. We performed RNAseq transcriptional analysis on blood samples from subjects who were immunized with purified, cryopreserved radiation attenuated sporozoites and had protection was determined by controlled human malaria infection (CHMI) challenge. We determined responses in two vaccine trials that differed by prior malaria exposure and modes of immunization and CHMI and responses of non-immunized subjects to infection. We employed statistical methods that take into account uncontrolled variations within individual volunteers and differences between trials and performed multiple comparisons to determine host gene expression responses at various time points after vaccination and/or parasite challenge. We also compared responses between protected and non-protected people after vaccination and parasite challenge. Numerous changes in mRNA expression levels occurred following immunization and CHMI. Similar kinetics of gene expression responses to vaccination and CHMI were observed including between trials. For example: genes associated with neutrophils and erythrocyte development were significantly up-regulated 7 days after the 2nd vaccination. In addition, genes associated with myeloid lineage, coagulation cascade and metabolism had similar responses to CHMI. Furthermore, genes associated with neutrophils and erythrocyte development had similar kinetics in response to vaccination and to CHMI of non-immunized subjects. Analysis of genes for interferon responses, erythrocyte development, and hematopoietic precursors showed a trend for higher expression in protected compared to non-protected subjects after CHMI. In contrast, B-cell-related genes were more highly expressed in non-protected subjects. These changes in the blood likely reflect a combination of changes in immune cell gene expression, proliferation, apoptosis and cell trafficking and provide insights into immunological processes that are associated with infection and protection.

1727

### A NOVEL BLOOD-STAGE VACCINE CANDIDATE MEDIATES PROTECTION AGAINST FALCIPARUM MALARIA IN MICE AND CHILDREN

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There is an urgent need to develop new, highly effective malaria vaccines. We previously screened a *P. falciparum* blood-stage cDNA lambda phage library and identified a novel protein (PF3D7\_1134300) that is uniquely recognized by antibodies from a group of malaria-resistant Tanzanian children who controlled parasite density during infection but not by those from a group of susceptible children whose non-immunological risk factors were carefully balanced. The objectives of this study were to 1) confirm our findings in a large human cohort, 2) immunolocalize the protein, 3) test the hypothesis that this protein induces growth-limiting antibodies and 4) assess protection against parasite challenge in a murine model. After adjusting for repeated measures and potential confounders using generalized estimating equations, we confirmed that high levels of monospecific human antibodies (upper 5th percentile) from an actively monitored longitudinal cohort of 450 Tanzanian children 2 to 3.5 years of age (1020 observations), were associated with an almost 50% reduction in parasite densities on blood smears (124 vs 68 parasites/200 WBC;  $p=0.04$ ). We showed by means of immunolocalization studies that the native protein is expressed in trophozoite- and schizont-stage parasites. It is trafficked via Maurer's clefts to the RBC membrane inner leaflet despite lacking a recognizable export signal, thereby constituting a novel PEXEL-negative exported protein (PNEP). In antibody-mediated growth inhibition and schizont arrest assays, we showed that murine purified anti-PF3D71134300 IgG decreased parasite replication and egress from RBCs in a dose-dependent fashion by up to 75% compared to pre-immune controls. We then immunized mice with an adjuvanted *P. berghei* ANKA ortholog of PF3D7\_1134300 or adjuvant alone and found that actively immunized mice challenged with 1x10<sup>4</sup> *P. berghei* ANKA had significantly longer median survival ( $p=0.014$ ). These data support development of PF3D7\_1134300 as a viable vaccine candidate, and present a unique opportunity to elucidate poorly understood mechanisms of protein export and immune protection.

1728

### CORRELATION BETWEEN PLASMODIUM FALCIPARUM NF54 STRAIN OOCYSTS AND SPOROZOITES COUNTS IN ANOPHELES STEPHENSI MOSQUITOES

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Sanaria® PfSPZ Vaccine and PfSPZ-CVac (chemoattenuated PfSPZ vaccine) are highly protective vaccines composed of aseptic, purified, cryopreserved *Plasmodium falciparum* (Pf) sporozoites (SPZ) that are currently being evaluated in clinical trials in the U.S., Europe, and Africa. The immunogens, PfSPZ, are manufactured using *Anopheles stephensi* mosquitoes as bioreactors. We have systematically collected data on numbers of oocysts and sporozoites in mosquitoes fed the same blood meal of Pf strain NF54 gametocytes to characterize parasite developmental kinetics, and determine how accurately we can predict salivary gland sporozoite numbers from oocyst counts. For this analysis we are using mosquitoes that had a range of a mean of 43 to 165 oocysts/mosquito and a mean of 32,000 to 185,037 PfSPZ/mosquito. Conversion rates from

oocysts to salivary gland PfSPZ varied from 990-2905 PfSPZ/oocyst. We will present the results of analyses to establish how accurately oocyst counts can be used to predict numbers of PfSPZ per mosquito.

1729

### IMPROVED DISPLAY OF THE MALARIA TRANSMISSION BLOCKING PFS25 ANTIGEN ON A SECOND-GENERATION PLANT-PRODUCED VLP

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The Pfs25-VLP produced in plants using transient expression technology is a fusion of the *Plasmodium falciparum* Pfs25 protein to the alfalfa mosaic virus coat protein antigen display molecule. This Pfs25-VLP has previously been shown in animal studies to be highly effective at preventing the transmission of malaria, and was tested through a Phase 1 clinical trial, where it showed no adverse reactivity. The effectiveness of this VLP molecule was hampered, however, by in *planta* cleavage events within the coat protein display molecule, resulting in considerable loss of the Pfs25 antigen from the VLP. Through N-terminal sequence analysis and molecular engineering, a second generation Pfs25-VLP molecule has been developed, reducing the loss of the Pfs25 antigen. This second-generation molecule has more Pfs25 antigens displayed per VLP than the original Pfs25-VLP and has equivalent or better malaria transmission blocking activity in animal models. At the same time, the Pfs25-VLP purification method was simplified to a two step all chromatography purification approach, resulting in improved Pfs25-VLP purity and yield.

1730

### EFFECTIVENESS OF COMMUNITY-BASED LARVICIDING PROGRAM ON MALARIA VECTOR ABUNDANCE ON BIKO ISLAND, EQUATORIAL GUINEA

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Recommended as a supplement to the core vector control interventions of IRS and ITNs, several African countries are introducing larval source management (LSM) into their malaria control programs. Vectors resistance to insecticides and the change in the biting behavior of the vectors to more of outdoor biting have renewed the interest in LSM. However, the operational delivery strategy that will realize the full potential of LSM in reducing malaria transmission in sub Saharan Africa is still not clear. The Bioko Island Malaria Control Project (BIMCP) in Equatorial Guinea introduced a pilot larviciding program using community volunteers to assess the sustainability and the impact on malaria vector densities on Bioko Island. After community engagement in each community, community members were identified and recruited as volunteers with the help of the community leaders. The community-based larviciding program was introduced in 2015 in 13 communities where community volunteers were willing to participate. In phase I of the program in 2015, the volunteers were fully supported by the BIMCP. The community volunteers were trained and assisted in identifying, monitoring, and treating all potential breeding sites, including those that they had missed to treat. However, during phase II of the program in 2016, the community volunteers took ownership of conducting all larviciding activities while the BIMCP monitored and evaluated the program. Though there was no significant difference in the average number of breeding sites monitored in the two phases (N=1,033 in phase I and N=970 in phase II), the treatment coverage was lower in phase II than in phase I by about 25%. This was also reflected in the total amount of larvicide consumed in phase I (5,585.8kg) as compared to phase II (2,936.9kg). The average human

biting rate of the vector also doubled from an average of three bites per person per night in phase I to six bites per person per night in phase II. A community-based larviciding program in Bioko Island can significantly reduce the vectors abundance on the Island only through strong operational and managerial expert support.

## 1731

### FACTORS ASSOCIATED WITH THE UPTAKE OF AT LEAST TWO DOSES OF SULFADOXINE/PYRIMETHAMINE FOR THE PREVENTION OF MALARIA IN PREGNANT WOMEN, BENIN, 2015

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In Benin, an estimated 29% of pregnant women receive at least two doses of sulfadoxine/pyrimethamine (SP) as a prophylaxis against malaria in pregnancy (2015 Benin Malaria Indicator Survey). This is well below the national target of 80%. To determine factors associated with SP uptake during pregnancy, we sampled responses from 3,555 women aged 15-49 in the 2015 Benin MIS database. We used a logistic regression model ( $\alpha = 0.05$ ) and 10 variables related to women's demographics and access to health care to describe the factors associated with successful and unsuccessful uptake of two or more doses of SP. A correlation matrix was created to identify associations between variables. Variables with a positive effect on the uptake of at least two doses of SP were: High socioeconomic status ( $p = 0.010$ ); early use of antenatal care (ANC) services during the first trimester of pregnancy ( $p = 0.000$ ); a live birth preceding the most recent delivery ( $p = 0.038$ ), and; southern (higher coverage) vs. northern (lower coverage) geographic region of origin ( $p = 0.000$ ). Factors that did not influence the use of at least two doses of SP were: Education level, ANC visit during the third trimester of pregnancy, and; urban vs. rural residence. To increase SP uptake among pregnant women, behavior change communication interventions should be reinforced, especially among women with low socioeconomic status and primigravidae in geographic areas with low coverage. Emphasis should be placed on early ANC visits during the first trimester of pregnancy.

## 1732

### TRACKING LONG LASTING INSECTICIDAL NET (LLIN) PHYSICAL INTEGRITY AND BIOEFFICACY 2 YEARS AFTER A MASS CAMPAIGN IN BENIN

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Benin's National Malaria Control Program (NMCP) distributed three types of long-lasting insecticide-treated nets (LLINs) during a mass campaign in 2014: PermaNet 2.0®, DuraNet®, and DawaPlus 2.0®. Based on the manufacturers' claims and WHO recommendations, the NMCP assumed that each population of nets would meet accepted norms of fabric integrity and bio-efficacy during a three-year distribution cycle. To validate this assumption, we monitored standard LLIN durability indicators for bio-efficacy and physical integrity during a 2-year period following distribution.

We selected one LLIN in each of the 300 enrolled households in each of the 3 health districts. In each district, a total of 300 LLINs from rural ( $n=150$ ) and urban ( $n=150$ ) settings were randomly selected and tracked. Physical integrity was assessed using methods recommended by the World Health Organization (WHO). Bio-efficacy was monitored using the WHO cone test method. After 2 years, only 46% (416/900) of HHs were opened to the surveyors who were able to assess only 31% (283/900) of the LLINs on track. This high rate of nets lost-during the follow up could be explained by the lost or the replacement of the LLINs in the enrolled households. Out of the 283 LLINs found, we observed that 87% (248/283) had some physical damage after two years and cannot be used. Both rural and urban areas showed similar rates of net loss associated with fabric integrity: rural 91% (131/144) and urban 84% (117/139) ( $p>0.05$ ). The range of proportionate hole index (PHI) values in rural versus urban areas, (754-870) and (663-929) respectively, were similar. None of the 3 types of LLINs distributed by the NMCP in 2014 were in 'good shape', defined as a PHI < 64, after 24 months. The bio-efficacy of PermaNet 2.0® and DuraNet® after 2 years was 80% mosquito mortality for each net type, was somewhat better than DawaPlus 2.0®, which showed 69% mosquito mortality. In summary, after two years, each population of LLINs showed loss of fabric integrity at greater than expected levels. Such results argue for the strengthening of behavior change communication efforts aimed at improving net care behaviors among LLINs recipients.

## 1733

### CURRENT PRACTICES IN URBAN INSECTICIDE-TREATED NET DISTRIBUTION ACROSS SUB-SAHARAN AFRICA

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Universal coverage campaigns are the primary distribution channel for insecticide-treated nets (ITN) across malaria endemic countries. Campaigns distribute ITNs to diverse populations across socioeconomic and urban/rural gradients. Urban settings present distribution challenges as a byproduct of unique social patterns and population diversity that is rarely found in rural zones. Gated communities, urban slums, population density, security issues, and busy social movement patterns represent some of these challenges. Using the 2013 report *Experiences in Long Lasting Insecticidal Net (LLIN) Urban Distribution Campaigns* as a baseline to measure progress, the VectorWorks Project conducted interviews with consultants, implementing partners, and national malaria control program staff from eleven countries where urban distribution or its planning occurred in 2016. The results indicate that the vast majority of countries are registering urban households during evenings and weekends to accommodate urban social movement patterns and have increased the number of distribution points to manage urban density. Despite this progress, many aspects of urban distribution require attention. The lack of standardization in determining which municipalities are operationally "urban" needs to be addressed. Key recommendations include the need to employ technology to aid in supervision and harmonization; potentially limiting or ceasing ITN distribution in high socioeconomic areas that are difficult to register; and creating an urban distribution task force during the planning phase in more urbanized countries. The ramifications of not developing comprehensive urban-specific strategies can include reduced ITN coverage and the need for expensive duplicate activities to make up for poor initial registration or distribution. Best practices and opportunities for improvement will be discussed, with a look toward creating a set of recommendations for future ITN distribution in urban settings.

## 1734

**LESSONS LEARNED AROUND IMPROVED IRS PLANNING AND MONITORING IN SOUTHERN AFRICA**

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Indoor Residual Spraying (IRS) is a critical but resource-intensive intervention of malaria elimination programs. Using Geographical Information System (GIS) technologies, electronic data collection and dashboard visualizations, malaria programs in Botswana, Namibia, and Zimbabwe have improved IRS operational planning, implementation, and during- and post-season data analysis. In the planning phase, household enumeration, surveillance, and malaria risk data were used to determine operationally feasible and priority target clusters to spray. This information was shared with IRS teams using simple tables and easy-to-read maps. Pre-implementation household enumeration data and geo-locations collected during IRS campaigns provided the programs with timely data for estimating process and impact indicators. Post-implementation, government programs across these countries documented lessons learned and stakeholders provided feedback on the new process, technical challenges, and on timeliness, completeness, and utility of resulting data. Several thematic lessons emerged across the countries: 1) timely initiation of planning is essential to uptake and comfort with new data collection and dashboard tools; 2) selection of target clusters is an iterative process, so quick-to-update mapping and analysis tools are required; 3) considering map literacy, maps should include context-specific information such as key landmarks and have easy-to-read legends and scales; 4) readable screens, long battery life, and extra battery packs are important attributes of hardware; 5) data collection tools should mirror existing paper-based forms; and 6) technical guidance and assistance via a qualified consultancy on enumeration, data management, and overall process flow are necessary for at least one season to improve familiarity with new tools. Lessons learned in these countries including technical and operational issues show the utility of incorporating spatial and other analytical software into IRS planning and monitoring, while highlighting areas for improvement in future spray seasons and in other countries implementing IRS.

## 1735

**NET MIGRATION OR NON-USE? BED NET OWNERSHIP FOLLOWING MASS DISTRIBUTION CAMPAIGNS ON BIKO ISLAND, EQUATORIAL GUINEA**

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The objective of this study was to describe the patterns of use and explore factors related to attrition following two long-lasting insecticide treated net (LLIN) distribution campaigns. A large discrepancy was measured between the numbers of LLINs that households received during mass distribution versus the numbers reported and observed in the same households at three intervals. In 2015, the National Malaria Control Program (NMCP) led a mass distribution campaign on Bioko Island. At least one new LLIN for every two people was delivered to 88.2% of households, and the numbers of LLINs physically hung were recorded. One

year later, before a campaign to hang LLINs in 3,510 high-risk households, the NMCP recorded the number of LLINs households reported owning. During an indoor residual spraying (IRS) campaign, 78% of the high-risk households were revisited, and the number of LLINs observed was compared to the number hung during previous distributions. Before the top-up campaign, the number of LLINs households reported owning fell 55.6% from the number that had been hung during mass distribution. During the targeted IRS campaign that occurred on average 30 days after top-up, the number of LLINs observed by sprayers decreased 40.5% from the number hung during the top-up campaign. Approximately 58 days after top-up, the annual malaria indicator survey visited 267 of the high-risk households. Only 58.6% of respondents reported sleeping under a bed net the night before. Among respondents who reported no longer owning a bed net, 21.3% reported transferring a net to others. The average number of LLINs observed decreased from the number previously distributed by -1.63 among households that reported not travelling off the Island in the previous 8 weeks and by -2.33 among households that did. Despite achieving target LLIN coverage on Bioko Island, attrition rates pose a threat to one of the principal vector control strategies established by the NMCP. Work should be undertaken to address the lack of vector control interventions on the mainland of Equatorial Guinea, where it is believed many LLINs are being transferred to populations at high risk of malaria infection.

## 1736

**SUSTAINING HIGH NET OWNERSHIP THROUGH CONTINUOUS COMMUNITY DISTRIBUTION**

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The main malaria vector control strategies in Kenya are Long Lasting Insecticidal Nets, Indoor Residual Spraying and Integrated Vector Management. LLINs are distributed through mass campaigns every three years, routinely through ANC/EPI clinics, social marketing and commercial. Sustaining this high net coverage attained after mass net is a challenge and hence, the continuous community net distribution was piloted. The pilot strategy included a mechanism where trained community health volunteers visit households, verify net need and issue a coupon to HH head to redeem the net at the nearest distribution point for free. In addition, the CHVs integrated interpersonal communication to address barriers to net use. The pilot was implemented between August 2014 and May 2015 within Samia Sub-county Busia county in Kenya. Using a community-based cluster randomized control trial; the study site was divided into 29 clusters (sub-locations) and randomized to Intervention (received intervention), and a Control group. Using a design effect of 2, a 95% confidence interval with an 80% power, and the estimated prevalence of 59.6% of universal coverage the sample size was calculated at 875 per arm. A pretested household questionnaire was administered at baseline and end line. Data were analyzed using Stata. A significant proportion of respondents in the intervention group had more than one LLIN (Baseline; 76.9 % versus Endline; 93.9%) as compared to the control (Baseline; 75.8 % versus Endline; 78.1%). The majority of respondents in the intervention area attained universal coverage (Baseline; 50.4% versus Endline; 78.7%) as compared to the control (Baseline; 49.7% versus Endline; 44.6%). More household members significantly slept under an LLIN the previous night in the intervention group (Baseline; 91.4% at versus Endline; 96.4%) compared to the control (Baseline; 87.9% versus Endline; 85.8). In conclusion, continuous community net distribution is a feasible approach to sustaining high net ownership, universal coverage and use at household level. However, there is need to invest in community health systems and volunteers.

### USING THE ANTENATAL CARE QUALITY IMPROVEMENT TOOL AND TARGETED TRAINING TO STRENGTHEN ANC SERVICES INCLUDING MIP IN KAGERA AND MARA REGIONS, TANZANIA

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Malaria in Pregnancy (MiP) is a major, preventable cause of maternal morbidity and poor birth outcomes. In collaborations with partners, Tanzania's National Malaria Control Program (NMCP) and the Reproductive and Child Health Unit has been working to promote the World Health Organization's three-pronged approach to address the burden of MiP.

A malaria training for 180 supervisors and 360 ANC providers from 221 health facilities was conducted in the Kagera and Mara regions. Updates included an orientation on MiP as well as malaria case management, screening, data management and ITN promotion.

Prior to the training, facility baseline assessments were conducted using the Ministry of Health, Community Development, Gender, Elderly and Children (MOHCDGEC) antenatal care quality improvement (ANC QI) tool to identify gaps in knowledge and skills of health providers to better target trainings to improving the quality of ANC services. A second assessment took place six months post training. Both assessments included hospital, health facility and dispensary levels and included observation, interviews, record reviews and skills assessments. Results demonstrated that over 90% of the facilities scored below 30% across all categories in the overall baseline assessment with a high score of 35 %, while the 2nd assessment showed a large improvement with 40% of the facilities scoring below 30% and a high score of 70%.

The ANC QI tool is effective in determining the impact of ANC health provider's knowledge and skills to target training to improve ANC service quality.

### NET USE AND PREFERENCE AMONG INDIVIDUALS SLEEPING IN FORESTS OR FARMS IN MALARIA MULTI-DRUG RESISTANT AREAS

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Strengthening vector control measures among mobile and migrant populations (MMPs) is crucial to malaria elimination, particularly in multi-drug resistant areas. We aimed to assess mosquito net use and preference among those who slept in a forest or farm in a priority area of Vietnam. Census data from 2015 revealed a total of 4668 households in 18 villages in northwest Phu Yen Province, Vietnam. These data were used to select 20% of the households and survey identified residents who sleep in forests or farms on their mosquito net preferences. Data were collected using a smartphone application (KLL Collect®) and uploaded into an online database (ONA®). Logistic regression were used to calculate prevalence odds ratios (PORs) and 95% confidence interval

(CI) to assess differences among those who slept in forests and farms. Of 301 respondents, 258 slept in forests and 43 on farms. Most respondents preferred to bring hammock nets (277, 92%) rather than bed nets (74, 25%) to their sleeping sites. They preferred to bring thick hammock nets with zippers (52%) as opposed to 'hard' long lasting insecticidal nets (LLINs). Most respondents regularly used hammock nets with a separate flip over (RAI hammock net) in forests (18 [67%]) compared to those on farms (4 [36%]). Respondents sleeping in forests were more likely to want hammock nets only rather than carry both bed nets and hammock nets (adjusted POR=4.63; 95% CI 2.33-9.21) compared to the 43 respondents slept on farms. Respondents sleeping in the forest preferred the RAI hammock net (POR=20.0; 95% CI 2.20-181.55), but were less likely to receive a RAI hammock net (adjusted POR=0.34; 95% CI 0.15-0.75). We recommend scaling up the distribution of LLINs or hammock-nets to 100% coverage in high-risk population areas. Interventions could include distributing forest-packages to forest goers including insecticide treated hammock nets, repellent and information on the benefits of protective clothing, nets and repellents; as well as engaging the private sector to establish malaria focal points within workplaces associated with malaria risk. Additional implementation research is needed to the uptake of net use by MMPs.

### OBSERVATIONAL EVIDENCE OF A COMPLIMENTARY EFFECT OF COMBINING NEXT GENERATION INDOOR RESIDUAL SPRAYING AND SEASONAL MALARIA CHEMOPREVENTION IN THE SÉGOU REGION OF MALI, 2014

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By 2014, concerns about pyrethroid resistance in the Ségou Region of Mali had prompted a shift in indoor residual spraying (IRS) products to a micro-encapsulated formulation of the organophosphate insecticide pirimiphos-methyl. Also in 2014, Ségou was in the midst of expanding a pilot program to provide seasonal malaria chemoprevention (SMC) to children aged 3 to 59 months in select districts. The timing of these decisions presented an opportunity to analyze the impact of both interventions, deployed individually and in combination, using quality-assured passive surveillance data. District-level analysis of 2014 monthly malaria incidence (total rapid diagnostic test-confirmed cases/total district population) showed that compared to contiguous non-intervention districts in Ségou: SMC by itself in San corresponded to 306 fewer cases/10,000 person-months; IRS by itself in Barouéli corresponded to 492 fewer cases/10,000 person-months; and combined implementation of both IRS and SMC in Bla corresponded to 661 fewer cases/10,000 person-months. Further analysis of epidemiologic curves, by plotting the reduced incidence observed in intervention districts as a percentage of the background incidence observed in non-intervention districts, is useful for describing the magnitude and timing of an intervention's impact. This analysis suggests that the SMC only intervention had a more moderate (24% fewer cases in the first month) but longer duration (6 months) effect while the IRS only intervention had a rapid, comparatively large impact (50% fewer cases in the first month) of shorter duration (4 months); the impact of the combined intervention was both rapid (68% fewer cases in the first month) and of a longer duration (6 months). Though observational studies should always be interpreted with caution, these results suggest a possible combinatorial effect of implementing both IRS and SMC in central Mali. This work also supports the utility of quality-assured and validated

routine surveillance data in rapidly assessing the impact of malaria control interventions in operational settings to empower evidence-based decision making.

## 1740

### MALARIA VECTOR DENSITY AND PROXIMITY OF HUMAN RESIDENCE TO AN IRRIGATED AGRO-ECOSYSTEM IN MALAWI

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Although malaria control interventions have led to a decline in transmission intensity, the disease continues to be a major public health problem in Malawi. Proximity of vector breeding sites to human residences affects malaria risk. Irrigation plays an increasingly important role in Malawi to improve food security and the rural economy. However, land transformation for irrigated agriculture could increase malaria vulnerability for those residing in proximity to irrigation schemes by creating more *Anopheles* breeding habitat, thereby increasing local transmission. Therefore, we aimed to determine the effect of irrigated agriculture on variation in *Anopheles* mosquito density in 14 villages around the Bwanje Valley rice irrigation scheme in eastern Dedza District. Two sampling efforts were conducted at the end of the rainy seasons of April 2016 and 2017 involving 338 households per year. A total of 6700 mosquitoes were collected from 344 households sampled by indoor CDC light trap without dry ice and played in sleeping rooms. Mosquitoes were identified morphologically and by PCR methods to species. Of the malaria vectors, *Anopheles arabiensis* (68%) was the dominant mosquito in collections and *Anopheles funestus* was second most common (10%), whilst *Culex* sp represented 18% and *Mansonia* sp represented 2% of total collections, respectively. An average of 19 mosquitoes per household were collected (5-56 range) per trap-night and six villages had counts above the average. All were close to the scheme. The malaria transmitting species were most abundant in six of the fourteen villages and in these villages. *An. funestus* was relatively more abundant in four villages only. Villages most proximal to the scheme had the highest *Anopheles* abundance compared to those those further away. Vector density declined as an exponential function of distance of house from the irrigation scheme.

## 1741

### MONITORING THE PROTECTIVE EFFICACY OF SEASONAL MALARIA CHEMOPREVENTION USING CASE-CONTROL STUDIES: METHODOLOGY AND RESULTS FROM 5 COUNTRIES

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During the 2015 and 2016 rainy season, we conducted case-control studies in countries implementing SMC to estimate the protective efficacy of SMC against malaria, and to explore the duration of protection, and any changes in efficacy over time. Malaria cases in children below five years of age were recruited at health facilities, with caregivers then interviewed at home to document prior receipt of SMC and potential confounders, including age, socio-economic status and use of insecticide-treated nets. Recruitment of cases was stratified over the entire transmission season to

allow estimation of efficacy in different time periods. Two controls were selected from households in the same community at the same time that each case was recruited, and the same information collected. The sample size of approximately 250 cases and 500 controls per country. All data collection was carried out using tablet-PCs, allowing geo-location of cases and controls. These studies illustrate several important practical issues in the use of case-control studies to estimate SMC efficacy, and intervention efficacy more generally. These include measurement of exposure (the date of receipt of SMC and adherence to the regimen) and potentially confounding factors, which require a home visit to assess carefully; the definition of cases (in terms of parasitology and clinical symptoms) and the selection of controls; the recruitment strategy over time; and choice of reference group. Case control studies are vulnerable to bias, but a useful feature of SMC is that efficacy since efficacy is known to be very low more than 6 weeks after treatment, this permits a check of validity. Results from 2015 indicate protective efficacy of SMC over 80% in the first month after treatment, and preliminary analysis of data from 2016 indicate a similar picture of high SMC efficacy. By linking estimates of efficacy from these studies to results from large-scale surveys of molecular markers of drug resistance conducted nearby under the ACCESS-SMC project, we will suggest how these two approaches can be combined for ongoing monitoring of SMC efficacy in the future.

## 1742

### SEASONAL BIONOMICS OF MALARIA VECTORS IN KILWA AND KASHOBWE, HAUT-KATANGA PROVINCE, DEMOCRATIC REPUBLIC OF CONGO

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The goal of the Southern and Central Africa International Centers of Excellence for Malaria Research is to address critical research questions on barriers to malaria control and elimination in Zambia, Zimbabwe and the Democratic Republic of Congo (DRC). Of importance for regional malaria control is movement of parasites and vectors across international borders. In this study, baseline data are presented on malaria vectors in the southern Haut-Katanga Province, DRC, which shares a border with Luapula Province, northern Zambia along Lake Mweru and the Luapula River. Two cross-sectional surveys were conducted in Kilwa and Kashobwe in Haut-Katanga Province in February (rainy season) and July 2016 (dry season). Kilwa is located near Lake Mweru and Kashobwe is located near the Luapula River, providing abundant vector breeding sites throughout the year. Six villages were selected at each site. Mosquitoes were collected using CDC light traps and pyrethroid spray catches. Species identifications of female *Anopheles* mosquitoes were determined morphologically and confirmed by PCR. Sources of blood meals were identified by PCR. In total, 479 female anophelines were caught and analyzed (189 in February and 290 in July 2016). The majority of mosquitoes caught in the rainy season were *An. gambiae* s.s., whereas the majority of mosquitoes caught in the dry season were *An. funestus* s.s. Blood meal PCR revealed that the captured mosquitoes were highly anthropophilic. In Kilwa and Kashobwe, malaria vectors are abundant throughout the year; however, *An. gambiae* dominates during the rainy season whereas *An. funestus* dominates during the dry season. This bionomic pattern for the anopheline malaria vectors is very similar to that observed in a few sites along Lake Mweru in Luapula Province, in Zambia. Further cross sectional studies are necessary to expand the findings and better delineate the foraging and resting behaviors of these mosquitoes such that they may be better exploited for malaria control.

### OUTDOOR MALARIA TRANSMISSION IN DANGASSA, A COMMUNITY WHERE MALARIA CONTROL IS FAILING DESPITE THE USE OF LONG-LASTING INSECTICIDAL NETS (LLINS)

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Long-lasting insecticide-treated bed nets (LLINs) and indoor residual spraying (IRS) are key elements of malaria vector control in Mali. Both these strategies target endophilic and endophagic mosquitoes, and therefore may select for changes in the trophic behavior of *Anopheles gambiae s.l.*, such as outdoor feeding. The purpose of these studies was to assess the level of transmission occurring outdoors in a rural Malian setting where conventional malaria control strategies have failed to reduce the either intensity of transmission or the incidence of human disease. Human landing catches were performed indoors and outdoors from 2012 to 2016 in the village of Dangassa. A total of 2463 female *Anopheles gambiae s.l.* were collected: 1225 indoors and 1238 outdoors. The human biting rates were similar indoors and outdoors (132.5 and 134.0 bites per person per night indoors and outdoors) from 2012 to 2013 before universal coverage with LLINs. Likewise, before universal coverage with LLINs, Entomologic Inoculation Rates (EIRs) were similar indoors and outdoors (69.0 and 67.6 infective bites per person per month, respectively). Similar results were again observed for biting rates three years after the introduction of universal coverage with LLINs. At that time, human biting rates were 71.7 and 72.3 bites per person per night indoors and outdoors, respectively. However, three years after the introduction of universal coverage with LLINs, the outdoor EIR rose while the indoor EIR remained stable (indoor and outdoor EIRs of 56.0 and 92.3 infective bites per person per month, respectively). Based on changes in the EIR (an increase in the outdoor EIR), these results may explain in part the failure of conventional malaria control strategies in communities such as Dangassa. If confirmed, these results suggest that the exclusively indoor focus of most malaria control strategies such as LLINs and IRS may be a serious limiting factor in the current global effort to improve malaria control. They suggest that additional strategies which reduce outdoor biting and transmission will be needed to achieve better malaria control in communities with intense transmission.

### ANALYSES OF THE IMPACT OF SHIFTING IRS OPERATIONS ON MALARIA TRANSMISSION RATES IN THE NORTHERN, UPPER EAST AND UPPER WEST REGIONS OF GHANA: 2014-2015

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The indoor residual spraying (IRS) of insecticides has contributed substantially to the recent successes enjoyed by many malaria control programs, and IRS is positioned to remain one of the central components of the global malaria control strategy moving forward. Nonetheless, insecticide resistance concerns in Ghana prompted a switch from pyrethroids to a microencapsulated formulation of the organophosphate insecticide pirimiphos-methyl beginning in 2012. These changes in active ingredient, as well as other logistical considerations, contributed to some shifts in IRS operations and changes in the IRS status of several districts in the north of Ghana between 2013 and 2015. These changes allow

for observational study to better describe the public health impact of IRS with a next generation product, with a particular focus on the impact of (1) shifting active ingredients and (2) introducing IRS into previously unsprayed districts in the Northern, Upper East, and Upper West Regions of Ghana. Routine epidemiological data from the national District Health Information Management System II allow for the calculation of monthly malaria Rapid Diagnostic Test (RDT)-positive incidence rates at the health facility level across the three districts of northernmost Ghana. Changes in incidence rates observed at health facilities experiencing a change in IRS status from one year to the next are compared to changes in incidence observed at health facilities where IRS implementation status does not change, providing an estimate of intervention impact standardized for seasonal changes in baseline transmission. Particular attention is given to the impact of: suspension of IRS in Upper East Region in 2015 and Upper West Region in 2014; introduction and reintroduction of IRS into five districts of Upper West Region in 2015; in Northern region, suspension of IRS in Savelugu-Nanton district in 2015 and reintroduction of IRS in Kumbungu district in 2015. Analyses will also include results from routine entomological surveillance conducted by the President's Malaria Initiative (PMI)/Africa Indoor Residual Spraying (AIRS) project.

### COVERING HOUSE EAVE GAPS AND CEILINGS WITH INSECTICIDE TREATED NETS MAY REDUCE THE RISK OF PLASMODIUM INFECTION AMONG CHILDREN IN SIAYA SUB-COUNTY, KENYA

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Long-lasting insecticidal nets have been widely used for reducing malaria cases. A persisting challenge is how to protect children who do not adhere to net use. This study examined whether covering house eave gaps and ceilings with a fabric made of insecticide treated nets (ceiling nets) reduces the risk of *Plasmodium* infection among children in a village in Siaya Sub-County, Kenya. Ceiling nets were installed in half of the houses that were randomly selected. Two to three months after the installation, indoor resting anophelines were sampled from the houses with ceiling nets and those without the nets. Then, nearly all children aged between 0 and 17 in the village were tested for *Plasmodium* infection and hemoglobin concentration. Within one month after the test, ceiling nets were installed in the remaining houses. Monthly hospital records of malaria cases were compared before and after the intervention for the village and an adjacent village. The number of indoor resting anophelines was significantly fewer in the houses with ceiling nets compared with those without the nets ( $P < 0.001$ ,  $n = 40$ ). The Giemsa stained slide-positive prevalence for children with and without ceiling nets was 42.5% and 50.0%, respectively. The difference was not statistically significant (adjusted odd ratio: 0.61, CI: 0.22-1.60,  $P = 0.319$ ,  $n = 102$ ). On the other hand, the mean hemoglobin concentration of children with ceiling nets was 12.55 (sd=1.27) g/dL, and that of children without the nets was 11.50 (sd=2.04) g/dL. The difference was statistically significant ( $P = 0.029$ ,  $n = 102$ ). The proportion of the number of malaria cases to non-malaria cases for the village was significantly lower after the intervention ( $P < 0.001$ ,  $n = 36$ ) while the difference was not significant for the adjacent village ( $P = 0.328$ ,  $n = 36$ ). As the children were tested for infection two to three months after the intervention, the duration might not have been long enough to evaluate the effects with Giemsa staining. These results suggest that covering house eave gaps and ceilings with insecticide treated nets may reduce the risk of *Plasmodium* infection among children.

## 1746

**HPLC-FLUORESCENCE METHOD FOR DETECTION OF IVERMECTIN IN MOSQUITO BLOOD MEALS**

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Ivermectin (IVM) is an endectocidal drug that can be lethal to many biting arthropod vectors, including mosquitoes. We have been developing IVM mass drug administrations (MDA) as a tool for integrated malaria control due to its effect on *Anopheles* mosquitoes when they bite IVM-treated people, and as a control method for West Nile virus transmission due to its effect on *Culex tarsalis* when they bite birds given IVM-treated feed. While IVM has been shown to be toxic to these species, IVM detection and quantification in mosquito blood meals has not been published. This is important as IVM pharmacokinetics is studied using venous-drawn serum or plasma samples, however, given the highly lipophilic nature of IVM, mosquitoes may be ingesting a different amount of IVM from subdermal capillaries as they are often in close proximity to subdermal fat deposits. *An. gambiae* were fed serial dilutions of IVM in artificial blood meals at concentrations of 50, 25, 12.5, 6.25, 3.125, and 1.56 ng/ml and frozen at 0 hr and 12 hr post blood meal. IVM was detected at the lowest blood meal concentration of 1.56 ng/ml at both time points in individual blood fed *An. gambiae* using HPLC-fluorescence methods. This represents a potential ability to detect IVM in *Anopheles* caught resting in houses after blood feeding on treated humans the previous night, and IVM serum concentrations that can be found in humans at least a week following MDA on onchocerciasis control. Our method was also able to detect IVM in individual *Cx. tarsalis* given an artificial blood meal of 25 ng/ml and held for either 0 hr or 20 hr post-blood feed. Future experiments will include determining the limits of quantification and analyzing *in vivo* IVM blood meals of *Cx. tarsalis* from birds and *An. gambiae* from humans. This method could be used to determine IVM coverage within mosquito populations following IVM application to hosts, and could provide a critical link in understanding mosquito mortality dynamics in relation to IVM pharmacokinetics in humans and birds. This could contribute to a better understanding of the use of IVM for integrated vector control.

## 1747

**A MURINE MODEL OF DIARRHEA AND GROWTH IMPAIRMENT WITH *SHIGELLA FLEXNERI* INFECTION AND THE ROLE OF ZINC DEFICIENCY**Pedro Henrique Q. Medeiros<sup>1</sup>, Solanka E. Ledwaba<sup>1</sup>, David T. Bolick<sup>1</sup>, Reinaldo B. Oriá<sup>1</sup>, Aldo A. Lima<sup>2</sup>, Richard L. Guerrant<sup>1</sup><sup>1</sup>University of Virginia, Charlottesville, VA, United States, <sup>2</sup>Federal University of Ceara, Fortaleza, Brazil

*Shigella* is a highly infectious enteric pathogen often associated with bloody diarrhea worldwide. However, the lack of a robust murine model impairs study of *Shigella* pathobiology and the pursuit of vaccines. This study aimed to establish a murine model of *S. flexneri* infection. C57BL/6 mice received either standard chow (nourished controls, n=8) or zinc-deficient (n=8) diets for two weeks, as zinc deficiency has been implicated in worse diarrheal illness in children. Prior to *S. flexneri* infection, a broad-spectrum antibiotic cocktail was given in the drinking water for three days. Then mice were infected with *S. flexneri* 2457T strain (10<sup>9</sup> CFU/mouse) by gavage and followed daily for measures of weights and stool collection. Stools were used to quantify intestinal inflammation biomarkers (myeloperoxidase-MPO and lipocalin-LCN) using ELISA assays. In addition, bacterial DNA was extracted from stools for *S. flexneri* detection by qPCR (*ipaH* gene). *S. flexneri*-infected mice showed diarrhea and significant weight loss on days 2 and 3 post-infection, recovering subsequently. Nourished mice showed significantly higher *S. flexneri* stool shedding on day 1, whereas zinc-deficient mice showed higher stool shedding on day 5 and thereafter. Both infected groups had robust stool shedding until day 15, when nourished mice stopped shedding. Zinc-deficient mice continued to shed at least until day 33 post-challenge. Increased levels

of fecal MPO and LCN were found in the nourished infected mice on day 2. Increased levels of fecal LCN were found in the zinc-deficient infected mice on days 23, 25 and 27. IL-10 and TNF- $\alpha$  levels from cecal contents were significantly increased in the zinc-deficient infected mice on day 15. This study describes a novel murine model of shigellosis leading to growth impairment, diarrhea, heavy stool shedding and intestinal inflammation. Our findings also show that zinc deficient mice challenged by *S. flexneri* infection showed more prolonged colonization and inflammation.

## 1748

**PLASMA IGA RESPONSES AGAINST THREE *SALMONELLA* TYPHI ANTIGENS IDENTIFY PATIENTS WITH TYPHOID FEVER WITH EXCELLENT SENSITIVITY AND SPECIFICITY IN DHAKA, BANGLADESH**Richelle C. Charles<sup>1</sup>, Farhana Khanam<sup>2</sup>, Jason R. Andrews<sup>3</sup>, Motaher Hossain<sup>4</sup>, Meagan Kelly<sup>1</sup>, Stephen B. Calderwood<sup>1</sup>, Taufiqur Rahman Bhuiyan<sup>2</sup>, Firdausi Qadri<sup>2</sup>, Edward T. Ryan<sup>1</sup><sup>1</sup>Massachusetts General Hospital, Boston, MA, United States,<sup>2</sup>International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, <sup>3</sup>Stanford University School of Medicine, Stanford, CA, United States, <sup>4</sup>Stanford University School of Medicine, Dhaka, Bangladesh

There is a need for a reliable and simple diagnostic assay for typhoid fever. Currently, available commercial serologic assays using plasma and serum for typhoid fever have sensitivities and specificities of 50-75% and 70-90%, respectively. We have used a number of high throughput immuno-screening technologies and have identified immuno-reactive *S. Typhi* antigens that appear promising for inclusion in a new assay. Our data also suggest that assessing IgA responses in plasma can accurately identify patients with acute typhoid fever, even in highly typhoid-endemic areas. We therefore assessed IgA responses in plasma drawn on patients presenting with a febrile illness in Dhaka Bangladesh (day 0; n=28) who subsequently were determined to have *S. Typhi* bacteremia. We compared these responses to those detected in febrile (confirmed non-typhoid; n=15) and healthy endemic zone controls in Dhaka (n=20). We assessed IgA responses against HlyE, LPS, and MP (membrane preparation) via standard ELISA. We compared results to those obtained by using two commercially available tests: Tubex and Typhidot, as well as to a recently developed Typhoid/Paratyphoid test (TPTest). Of the 28 bacteremic individuals, 28 (100%) were positive by HlyE/MP IgA, HlyE/LPS/MP IgA, and TPTest; whereas 27 (96%), 21 (75%) and 18 (64%) patients were positive by HlyE/LPS IgA, Tubex and Typhidot, respectively. In healthy endemic zone and other febrile controls, the HlyE/MP IgA, HlyE/LPS IgA, HlyE/LPS/MP IgA, TPTest, Tubex and Typhidot were negative in 97%, 94%, 94%, 97%, 89% and 80%, respectively. Conclusion: Assessment of acute phase plasma IgA responses by ELISA for HlyE/MP (+/-LPS) gave a sensitivity of 100% and specificity of >95% in this small study and warrants further evaluation.

## 1749

**PREVALENCE OF CHILDHOOD DIARRHEAL ILLNESSES IN A PERUVIAN AMAZON RIVER BASIN COMMUNITY**Sanika Gadkari<sup>1</sup>, Marian Poley<sup>1</sup>, Ricardo Abedie<sup>2</sup>, Rosa Burga<sup>2</sup>, Melita Pizango<sup>2</sup>, Claudio Rocha<sup>2</sup>, David Craft<sup>1</sup><sup>1</sup>Pennsylvania State College of Medicine, Hershey, PA, United States, <sup>2</sup>Naval Medical Research Unit-6, Lima, Peru

Diarrheal disease continues to be a leading cause of childhood morbidity and mortality in resource-poor settings lacking water sanitation infrastructure. This longitudinal study focuses on the prevalence of enteric bacterial pathogens and parasites in stool samples from children under the age of 5 in the underserved community of Belén, Perú. Under an internationally approved IRB, consent was obtained from the mother or primary caregiver of each child under the age of 5. Households were excluded from the study if there was no adult over 18 present or child under 5 in the home. One hundred surveys were conducted and 87 stool samples were collected from children under the age of 5 from Jul - Aug

2016. Each survey was conducted in Spanish by translators. Stool samples were processed and cultured for bacterial pathogens, and processed and examined by microscopy for the presence of parasites, using standardized clinical microbiology procedures. Stool samples were further analyzed using QUIK CHEK (TechLab, Blacksburg, VA) test kits for the rapid detection of shiga toxin producing *E. coli* (STEC), Giardia, Cryptosporidia and *Entamoeba histolytica*. 16.9% of samples yielded at least one enteric bacterial pathogen and 60.9% of samples yielded at least one enteric parasitic pathogen. The most prevalent parasites were *Giardia lamblia* (37.9%), *Entamoeba coli* (14.9%), and *Ascaris lumbricoides* (10.3%). The most prevalent bacterial pathogens were *Aeromonas* spp. and *Salmonella* spp. (4.6% each), and *Shigella* spp. (3.4%). There is no evidence of STEC or *E. histolytica* in this population. Although clinical data was not collected to determine disease incidence, the presence of enteric parasites (60.9%) and enteric bacteria (16.9%) in the microbial flora of the stool is concerning for the potential for diarrheal disease. High parasite burden is consistent with previously presented data in which parasite burden was inversely proportional to chlorinated drinking water and enteric bacterial burden. Future studies are planned to include interventions such as a public health infection prevention seminar (charla) in the surveyed community.

## 1750

### MONITORING AND EVALUATION IN A MULTI-COUNTRY SURVEILLANCE SYSTEM: SEVERE TYPHOID IN AFRICA PROGRAM

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The Severe Typhoid in Africa (SETA) is a standardized, multi-country, surveillance network with the purpose of estimating the burden and severity of invasive salmonellosis. Monitoring and evaluation (M&E) of the surveillance network/system is important for data quality and comparability across sites; however, there is limited evidence on the best approaches to implement adequate M&E for multi-country, standardized surveillance studies. We present the process to develop the M&E plan for SETA and the lessons learnt during the pilot testing of the plan. Different steps were undertaken to develop the SETA M&E plan. First, the key elements and data flow through the surveillance system were ascertained and described. Second, the core activities and minimum standards required for the project to meet its main deliverables were identified. Third, using the two pieces of information mentioned above, a compilation of monthly monitoring data, indicators, targets associated with indicators and thresholds for actions were developed. Fourth, systematic field monitoring assessment visits were scheduled, and tools to report information on a monthly basis and during the monitoring visits were drafted. Lastly, pilot testing of the M&E strategies and documents took place at two of the 6 SETA countries. During the field visits, the core activities and minimum standards listed were observed and documented. Two main lessons were learnt. First, each site organized the logistics to implement the study standard procedures differently. This resulted in a variety of approaches that needed to be registered and documented. Second, not all study procedures could be observed. This was due to absence of patients at the recruitment healthcare facilities at the time of the visit, and follow-up visits scheduled outside of the time period of the visit. The logistics and organization to implement the study standard procedures may vary across SETA sites. A M&E plan than can leverage on the unique strategies or approaches of each site to implement the surveillance system will help to ensure data quality, comparability, and good performance across sites.

## 1751

### LONGITUDINAL ASSESSMENT OF ANTIBIOTIC RESISTANCE IN *E. COLI* ISOLATED FROM THE MAL-ED BIRTH COHORT STUDY IN RURAL TANZANIA

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Antimicrobial resistance is a serious global public health threat. Drug-resistant gram negative bacteria (GNB), such as *Escherichia coli*, are of particular concern. Despite the increasing prevalence of drug-resistant GNB in low resource countries, there is a lack of data concerning risk factors for acquisition. We aimed to characterize the prevalence, phenotypes, and risk factors for acquisition of drug-resistant *E. coli* from stool collected in the Etiology, Risk Factors, and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health and Development (MAL-ED) birth cohort study in rural Tanzania. 262 children were enrolled in the MAL-ED Tanzania site. We randomly selected 100 children who had *E. coli* isolates or stools archived every 6 months until 60 months, totaling 928 specimens. Susceptibility testing of 18 antibiotics was performed by disk diffusion. CLSI interpretive criteria were used for determination of resistance. Azithromycin was tested by E-test (MIC, µg/mL). From 270 *E. coli* specimens tested, resistance was identified in some samples for all drugs except ertapenem (100% susceptible). The highest rate of resistance was for ceftazolin (89%) followed by ampicillin (84%) and cotrimoxazole (81%). 18% of specimens had an azithromycin MIC  $\geq 32$  µg/mL. Four (1.5%) met criteria for ESBL based on combination disk testing with cefotaxime/cefotaxime-clavulanate and ceftazidime/ceftazidime-clavulanate. In conclusion, ESBL *E. coli* carriage appears to be rare in this community. Use of, and resistance to, ampicillin is common. After finishing all testing, we will analyze the longitudinal surveillance data of MAL-ED for potential risk factors of drug-resistant *E. coli* acquisition, including antibiotic use, hospitalizations, sociodemographic determinants, and diarrheal illnesses.

## 1752

### ETIOLOGY-SPECIFIC DIARRHEA BY QPCR AND LINEAR GROWTH DEFICITS IN BANGLADESHI INFANTS

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Childhood diarrhea has been identified as a risk factor for linear growth shortfalls. This association has been inconsistent, and the relationship between diarrhea attributable to specific pathogens and growth has not been comprehensively evaluated. In this study, we sought to characterize the etiology of diarrhea, as well as determine the relationship between pathogen-attributable diarrhea with linear growth and systemic inflammation by analyzing data collected during the Performance of Rotavirus and Oral Polio Vaccines in Developing Countries (PROVIDE) study. In this study, children were enrolled from birth to age 7 days and were followed via in-home surveillance for diarrhea. Diarrheal stools were collected, and subsequently tested with Taqman Array Cards (TAC) for causal pathogens. To account for asymptomatic carriage, we attributed pathogens as the etiology of diarrhea by calculating attributable fractions using odds ratios derived from the Global Enteric Multicenter Study. To assess growth impact, we fit a multivariable linear regression model with an outcome of length-for-age Z score (LAZ) at 12 months and used generalized estimating equations to assess potential delays in noticeable growth outcomes as well as resultant systemic inflammation. Of 700 enrolled children, 603 were included in the analysis. *Campylobacter jejuni*/*coli* was the primary bacterial etiology of diarrhea. Diarrheal episodes

independent of etiology did not have an impact on growth at 52 weeks. When analyzed by pathogen, both *Cryptosporidium* and *C. jejuni/coli* decreased LAZ (-0.26,  $p=0.05$  and -0.17,  $p=0.03$ , respectively), although *C. jejuni/coli* had a larger effect in the first 6 months of life (-0.52 decrease in LAZ per additional AE,  $p = 0.009$ ). Both *C. jejuni/coli* and *Shigella/EIEC* were associated with heightened inflammation (0.169 increase in log CRP,  $p=0.06$  and 0.183 increase in log CRP,  $p=0.04$ , respectively). *C. jejuni/coli* was the most common bacterial etiology of diarrhea in this cohort. Pathogen-specific diarrhea is associated with decreased linear growth at 52 weeks, as well as heightened systemic inflammation.

## 1753

### FOAM DRY STABILIZATION OF TYORASS, THE RECOMBINANT TY21A-SHIGELLOSIS VACCINE

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The usual method for thermostabilizing live attenuated viral and bacterial vaccines is lyophilization. However, this involves initial freezing, which is damaging, and product stability is frequently less than optimal with bacterial products. Here we investigated the use of foam drying to thermostabilize an orally-administered *Salmonella* Typhi Ty21a-vectored vaccine expressing both the *Shigella sonnei* protective antigen and its acid resistance genes. TyOraSs was grown in supplemented CY medium to early stationary phase, harvested, resuspended in CY medium and mixed 1:1 with a 2x foam dry protective additive (FPA) cocktail. FPA ingredients evaluated included trehalose, sucrose, gelatin (porcine and piscine), agar, methionine, serine, glycerol, DMSO and potassium phosphate, at a range of pH values. Samples were aliquoted into 50 mL Schott vials, foam dried using a VirTis lyophilizer; varying the vacuum, number of vacuum steps and shelf temperature(s). Stoppering was performed after argon purging. Optimization of FPA, pH and lyophilizer program will be reported. Water content was determined using the Karl Fischer method. Foam dried vaccine was stored at 4 oC, ambient and 37 oC for accelerated stability testing. Samples were removed and assayed for viable counts by reconstituting to the original pre-foam dried volume, serially diluted and quantitated by colony enumeration on TSA plates. Residual water contents in the samples ranged from 2-6% at t=0. Our FPA formulation demonstrated the lowest post-process loss of 53% when trehalose, fish gelatin and glycerol were included; in comparison, experimental foam dried vaccines produced by others gave an average process loss of 70%. Work is continuing with agar-based FPA cocktails which have initially shown post-process viability of 36%. Long term stability for a similar strain, Ty21a-PA, at ambient and 4 oC at 20 months was 47% and 100%, respectively, compared to t=0 viability, which we hope to replicate in TyOraSs. Immunogenicity studies of foam dried TyOraSs in mice are ongoing. These results provide a basis for future development of large scale physically stable formulations of TyOraSs.

## 1754

### SALMONELLA ENTERICA SEROVARS ISOLATED FROM STOOLS OF CHILDREN ENROLLED IN THE GLOBAL ENTERIC MULTICENTER STUDY

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The Global Enteric Multicenter Study (GEMS) determined the etiological agents of moderate-to-severe diarrhea (MSD) in children under the age of five years in four countries in Africa and three countries in Asia. *Salmonella* was not strongly associated with MSD. However, non-typhoidal *Salmonella* (NTS) infections are an important cause of septicemia in infants in Africa. Here, we present the NTS serovars isolated during GEMS from Africa. *Salmonella* spp. identified in Kenya, Mali, the Gambia and Mozambique were shipped to the Center for Vaccine Development in Baltimore for complete identification of serovars using antisera and biochemical tests. We tested 181 African *Salmonella* isolates, of which 101 were from cases and 80 were from controls. Multiplex PCR was employed to confirm agglutination results and to determine *Salmonella* Typhimurium sequence types. We also tested sensitivity to selected antibiotics by the Kirby Bauer disk diffusion method. Of the 181 *Salmonella* enterica strains, 138, 35, 2 and 6 were from Kenya, the Gambia, Mali and Mozambique, respectively. 38.7% were *S. Typhimurium*, 9.4% were *S. Virchow*, 6.6% were *S. Newport*, 6.6% were *S. Enteritidis*, 5.5% were *S. Heidelberg*, 3.9% were *S. Paratyphi B Java*, 1.7 % were *S. Hissar*, 1.1% were *S. Wingrove*, *S. Aberdeen*, or *S. Bovismorbificans*, 0.6% were *S. Muenster*, *Ssilla*, *Larochelle*, *Eastbourne* or *S. Muenchen*, 8.3% were other *Salmonella* serovars and 13.3% were non-typable. Antibiotic resistance of *S. Typhimurium* was 96% for ampicillin and trimethoprim-sulfamethoxazole (SXT) and 79% and < 1 percentage for chloramphenicol and gentamicin, respectively. 88.2% of tested *S. Typhimurium* strains were ST313. The most common *Salmonella* serovar that was isolated in GEMS was *S. Typhimurium*, followed by *S. Virchow* and *S. Newport*. Although *S. Typhimurium* ST313 has been associated with invasive disease in sub-Saharan Africa, we determined that it was isolated from the stools of children in this region too.

## 1755

### PREVALENCE AND ANTIBIOTIC RESISTANCE OF ENTEROPATHOGENS BACTERIAL ISOLATED FROM FECAL SAMPLES AT A HOSPITAL OF THE PERUVIAN AMAZON

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Diarrheal disease is the major cause of death in children under 5 in developing countries, and is a significant threat to public health. Acute gastroenteritis also remains a very important cause of morbidity in adults, and morbidity and mortality in children in Latin America. Antimicrobial resistance among enteric pathogens has also been increasing over the last decade in Latin America due to the indiscriminate use of antibiotics without prescription. Poor infection control standards and few antibiotic stewardship programs have resulted in many hospitals continuing to use ineffective antibiotics for the treatment of resistant enteric infections. In the present study, 174 samples of diarrhea were collected from August 2015 to March 2017. These samples were processed by standard laboratory culture techniques for bacterial pathogen identification and antibiotic susceptibility was determined by disk diffusion on all isolates. Of these samples, 16% (29/174) were positive for enteropathogenic bacteria. A total of 31 bacteria were isolated (27 samples with only one pathogen and 2 samples with two pathogens each). *Shigella* sp. and *Salmonella* sp. were the pathogens most common with 11 and 10 isolates, respectively, comprising 67% (21/31) of the enteric bacterial isolates, followed by 5 *Plesiomonas shigelloides* (16%), 2 isolates of *Aeromonas* spp. (6%), and one isolate of *Campylobacter* spp., *Vibrio cholera* Non-O1, and *Yersinia enterocolitica* each. Of the *Shigella* isolates, 90% were resistant to trimethoprim/sulfamethoxazole and tetracycline, followed by 81% resistant to chloramphenicol and ampicillin, and finally 9% were resistant to azithromycin. Of all the tested *Salmonella* spp, 70% were resistant to

nalidixic acid, 50% to ampicillin and tetracycline, 40% to chloramphenicol, 30% to trimethoprim/sulfamethoxazole, and 90% of the *Salmonella* spp showed intermediate resistance to ciprofloxacin. Because of growing resistance in the two most common enteric bacterial pathogens, *Shigella* and *Salmonella*, new strategies for the treatment of diarrheal diseases in Peru should be adjusted to control the emerging antimicrobial resistance.

## 1756

### COST-EFFECTIVENESS OF INTERVENTIONS TO PREVENT DIARRHEA: INTRODUCING A MICROSIMULATION MODEL FOR CHILDREN UNDER FIVE IN EVERY COUNTRY FROM 2005 TO 2015

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Diarrhea is one of the leading causes of morbidity and mortality among children under five. The global under five diarrhea mortality rate declined by 39% between 2005 and 2015, but the burden remains immense. Policymakers need to be informed as to which interventions better reduce the burden of diarrhea. We developed a cost-effectiveness analysis microsimulation (CEAM) model using an individual-based discrete-time Markov model. CEAM has three sections: a general, modular framework for running agent-oriented microsimulations, a repository of applications specific to public health, and a repository of data ingestion code that pulls, transforms, and preprocesses data from the Global Burden of Disease (GBD). We leveraged data from GBD to estimate the health burden due to specific diarrhea-causing pathogens (e.g. rotavirus) as well as the effect of treatments that target specific pathogens (e.g. rotavirus vaccines). We have decided to compare two vaccines - RotaTeq and BRV-PV - that reduce the burden of diarrhea due to rotavirus as an example. We will generate estimates of deaths, YLLs, DALYs, and costs of supplying the rotavirus vaccines using a population of 20,000 simulants in Kenya. We will compare RotaTeq and BRV-PV in terms of cost-effectiveness. Our simulation will provide a tool to facilitate comparisons between interventions and guide policymakers, governments, and funding organizations to make informed, evidence-based decisions for prioritizing investments in diarrheal disease interventions. Our work will allow for a better allocation of limited resources to reduce the burden of diarrhea.

## 1757

### GENOMIC AND PHENOTYPIC CHARACTERIZATION OF TYPHOID VACCINE STRAIN TY21A REVEALS INSIGHTS IMPACTING FUTURE VACCINE DEVELOPMENT AND OPTIMIZATION

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The live, oral, attenuated *Salmonella* Typhi vaccine Ty21a is one of only two licensed typhoid vaccines. Ty21a was developed in the 1970s by chemical mutagenesis of the virulent strain Ty2. Ty21a triggers long-lasting immunity and has been safely administered to > 200 million recipients, a safety record that is unrivaled by any live attenuated bacterial vaccines or vaccine candidates produced by modern, gene-specific attenuation. Nevertheless, bystander mutations that reduce overall fitness of the bacterium without contributing to safety can reduce vaccine efficacy. We use Ty21a as a platform technology for multivalent vaccines and such information are of utmost interest. The total genome sequence of both Ty2 and Ty21a are available in NCBI GenBank. However, these assemblies harbor ambiguous nucleotides and errors, particularly in the assembly of Ty21a. Therefore, we carried out *de novo* assembly of Ty2 and Ty21a genomes using next- and third-generation sequencing and compared the assembled sequences

to the reference genomes by variant analyses. Our preliminary results showed that a long-range translocation due to rDNA recombination occurred in Ty21a but was not accounted for previously. There were at least 41 and 119 previously misannotated nucleotides in Ty2 and Ty21a reference genomes, respectively. Of the 689 previously identified SNPs between Ty2 and Ty21a, only 522 were identified in our analysis and 1 more SNP was identified. Additionally, we detected the signature mutation of Ty21a, C444Δ in the *galE* gene that was inadvertently missed in the Ty21a reference genome. We are currently verifying our findings by PCR-sequencing of independent sources of Ty2 and Ty21a. Furthermore, SNP mutations that might contribute to overall fitness and survival of the bacterium are being characterized by genetic complementation tests. Such a detailed characterization will provide insights in future optimization of the Ty21a vaccine platform as a vector for development of multivalent vaccines. Furthermore, our findings will also benefit the development of novel live attenuated bacterial vaccine platforms.

## 1758

### ETIOLOGY AND SEVERITY OF DIARRHEAL DISEASE IN INFANTS IN BRAZIL SEMIARID REGION: A CROSS-SECTIONAL CASE-CONTROL STUDY

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We designed this study to identify the etiology and severity of diarrheal disease in children in the low-income Brazil semiarid region. The study is a cross-sectional, age-matched case-control of diarrhea in children aged 2-36 months residing in six cities from Brazil semiarid region. We recruited children with diarrhea seeking care at health centers along with selected age-matched community control children. We obtained informed consent from their parents, clinical and epidemiological data, and a fecal sample to identify enteropathogens using Luminex System. We enrolled 1200 children, 600 children with diarrhea and 600 children without diarrhea. By bivariate analysis the enteric pathogens associated significantly with diarrhea were: *Norovirus* GII (OR 5.14, 95% CI 2.13, 12.46), *Adenovirus* (OR 3.84, 95% CI 1.42, 10.36), typical enteropathogen *Escherichia coli* (tEPEC) (OR 3.16, 95% CI 1.33, 7.49), enterotoxigenic *E. coli* (ETEC LT-ST producing toxins) (OR 2.61, 95% CI 1.01, 6.77) *Rotavirus* (OR 1.93, 95% CI 1.22, 3.06), enterohemorrhagic *E. coli* (EHEC) (OR 1.76, 95% CI 1.15, 2.68), enteroaggregative *E. coli* (EAEC) (OR 1.42, 95% CI 1.13, 1.79) and *Giardia* (OR 1.41, 95% CI 1.07, 1.87). By logistic regression, the best predictors of diarrhea were in descending order: *Norovirus*, *Adenovirus*, *Rotavirus*, *Giardia* and EAEC. Severity diarrhea scores were significantly associated with *Norovirus* and EAEC ( $p < 0.034$ ). Five enteric pathogens (*Norovirus*, *Adenovirus*, *Rotavirus*, *Giardia* and EAEC) are associated with diarrhea in children in the low-income Brazil semiarid region.

## 1759

### HUMAN CHALLENGE MODEL REFINEMENT FOR B7A, AN ENTEROTOXIGENIC *ESCHERICHIA COLI* (ETEC) CHALLENGE STRAIN THAT EXPRESSES CS6

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Enterotoxigenic *Escherichia coli* (ETEC) is a major cause of diarrhea in children under the age of 2. The primary objective of this randomized, open label phase 1 trial was to refine the ETEC strain B7A (CS6, LT, ST positive strain) challenge model: to determine the safety of B7A and to identify a dose and fasting regimen that reproducibly induced moderate-severe diarrhea in at least 70% of naïve subjects. Twenty-eight subjects were randomized to one of 4 study groups for Cohort 1 with either an overnight (108 or 109 cfu) or ninety minute (109 or 1010 cfu) fast. Of the subjects receiving B7A doses of 108 cfu with an overnight fast or 109 cfu following a 90-minute fast, 3/7 met the primary endpoint of moderate to severe diarrhea. The groups receiving B7A at a 1010 cfu dose with a 90-minute fast and 109 cfu dose following overnight fast had a higher attack rate with 5/7 experiencing moderate to severe diarrhea. To assess the reproducibility of the attack rate of the target regimen and to determine homologous protection against rechallenge, 11 subjects who met the moderate-severe diarrhea endpoint in cohort 1 were re-admitted along with 19 naïve subjects; all subjects received 109 cfu of B7A following an overnight fast. The proportion of naïve subjects with moderate-severe diarrhea was lower than observed in the initial cohort with only 11/19 (58%) meeting the primary endpoint. Additionally, the incidence of moderate-severe diarrhea in previously challenged subjects was not significantly different than in naïve subjects 5/11 (46%). Antibody responses to CS6 were modest as measured by ELISA or ALS across all dosing groups. LPS was the most immunogenic antigen measured. In this study, we attempted to identify an optimal dosing and fasting regimen that maximizes attack rate while minimizing the inoculum necessary to achieve a high and reproducible attack rate. We found that increasing the fasting time was not sufficient to allow for a decrease in the inoculum dose while maintaining a consistent attack rate, but it caused more symptoms in the volunteers. In addition, there was a lack of protection upon rechallenge with the 109 cfu with an overnight fast dosing regimen.

## 1760

### CORRELATING THE PREVALENCE OF *VIBRIO CHOLERAE* AND *SHIGELLA* SPP. IN HIV-SERO POSITIVE PEDIATRIC PATIENTS WITH THEIR CD4 T-CELLS COUNT ATTENDING THE ART REFERENCE LABORATORY IN CENTRAL HOSPITAL OF NEPAL: FIRST CASE STUDY FROM THE COUNTRY

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The diarrheal disease in children living with HIV/AIDS (CLWHA) being an indicator of lack of social development continues to be a major public health problem in Nepal. Opportunistic infection (OI) diarrhoea affects

nearly 90% of CLWHA in underdeveloped countries like Nepal. The cardinal aim of this research was to correlate the level of CD4 T-cells with the prevalence of *Vibrio cholerae* and *Shigella* spp. among the CLWHA. Right antibiotics treatment can reduce the volume of diarrhoea and reduce the disease burden. Diarrhoeal stool samples of HIV sero-positive paediatric (below 16 years) patient's visiting the antiretroviral therapy (ART) reference laboratory of Sukraraj Tropical and Infectious Disease Hospital (STIDH) in Kathmandu were collected and analysed following microbiological procedures recommended by the Clinical Laboratory Standards Institute. Serological confirmation and serotyping of *Shigella* and *V. cholerae* isolates were done using specific antisera from Denka Seiken Co., Tokyo, Japan. CD4 T-cell counting was done using computerized Partec flow-cytometer. Among 337 stool samples analysed, 59 samples were found to be positive for enteric bacterial pathogens, of which 33 were *V. cholerae* (9.8%) and 26 were *Shigella* spp. (7.7%), the total prevalence being 17.6%. The identified enteropathogens showed that *V. cholerae* was found to be the highest with 9.8%, followed by *Shigella flexneri*, *Shigella sonnei*, *S. dysenteriae*, and *S. boydii*. All isolated *V. cholerae* strains belonged to the serogroup O1 and serovar Ogawa and of biotype El Tor. There was high rate of culture positivity (63.7%) among the children patients having low level of CD4 count. All the *Vibrio* isolates were resistant to nalidixic acid and cotrimoxazole while 91.7% were sensitive to ciprofloxacin. *Shigella* isolates were mostly susceptible to cefotaxime (80.9%) while ciprofloxacin (19%) was the least effective drug. Among the isolated *Shigella* spp. 61% were multiple drug resistant (MDR) while 56% of *V. cholerae* were MDR. HIV positive paediatric patients are in high risk of OIs like diarrhoea hence treatment of diarrhoea on time is becoming crucial.

## 1761

### VILLAGE-LEVEL MEDICATION LOGBOOK AUDIT FOLLOWING A TRACHOMA MASS DRUG ADMINISTRATION CAMPAIGN IN AMHARA REGION, ETHIOPIA IN 2016

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Trachoma programs throughout the Amhara National Regional State of Ethiopia use village-level medication logbooks to track distribution of azithromycin during mass drug administration (MDA) for trachoma. However, audits of the quality of this approach have not been commonly reported. This survey assessed MDA coverage as recorded in logbooks for a random selection of households in a representative sample of villages in the Amhara region. Following a post-MDA cluster-randomized coverage survey in Amhara, data collectors visited health posts for each cluster (village) to assess presence of logbooks and household pages for participating households from each cluster. When located, drug dosage information for each household member was recorded. Overall, logbooks were located for 296 clusters (93%). From these clusters, family pages were located for 3,495 households (72.7%) and contained data for 18,547 individuals. Treatment coverage as recorded in family pages for all ages was 76.3% for Amhara region, and ranged from 64.9% in South Gondar zone to 89.7% in East Gojjam zone. Recorded treatment coverage among children ages 1-9 years was 81% regionally, and was higher than the all-ages coverage in all zones, ranging from 74.3% in North Wollo to 93.5% in East Gojjam zone. Recorded treatment coverage differed widely across surveyed districts within each zone, with coverage as high as 97.7% and as low as 13%. Recorded treatment coverage for Amhara was close to the World Health Organization's recommended threshold of 80%, and suggests that the MDA campaign in this region reached a high percentage of eligible residents. Reasons for missing household pages, and reasons for low recorded coverage in certain areas should be investigated. Further analyses to compare self-reported coverage from the post-MDA coverage survey to recorded coverage from medication logbooks at the individual level is ongoing. The results of this audit demonstrate that the quality of

medication record keeping as well as the recorded MDA coverage varies within a mature MDA program. Lessons learned from this audit should help regions scaling up MDA programs throughout Ethiopia.

## 1762

### ONE ROUND OF TARGETED SUB-DISTRICT TREATMENT IN THE DISTRICT OF OUSSOUBIDIAGNA, MALI ACHIEVED THE CRITERIA OF STOPPING TRACHOMA MASS DRUG ADMINISTRATION

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Oussoubidiagna health district (HD) in Mali was originally part of Bafoulabé HD that was found to be endemic for trachoma in 1997, with a baseline active trachoma prevalence of 42.5% (range: 37.5-47.2%) in children aged under 10 years. Three rounds of annual mass drug administration (MDA) with azithromycin and 1% tetracycline eye ointment was conducted in 2003-2005, and MDA was stopped in 2006 following an impact assessment showing prevalence of trachomatous inflammation follicular (TF) had dropped to 1.2%. However, a surveillance survey in 2009 showed a recrudescence of trachoma in Bafoulabé (TF: 15%), and three further rounds of MDA were conducted in 2010-2012. A sub-district level impact assessment was conducted in the HD in 2013 per WHO recommendations. The results indicated that one out of five sub-districts in Bafoulabé had a TF prevalence of 5.58% (3.68-8.37%) in children aged 1-9 years and should receive one more round of MDA per the WHO guidelines. In 2014, Bafoulabé was split into two HDs: Bafoulabé and Oussoubidiagna. The Oussoubidiagna HD inherited the sub-district requiring MDA. In February 2016, the national program conducted MDA targeting the sub-district only (9 of the 20 health areas), achieving a programmatic coverage of 88.1%. A district-level assessment using a cross-sectional, randomized, two-degree cluster survey of 20 clusters was conducted in Oussoubidiagna in January 2017, one year after the MDA to assess the TF prevalence in children aged 1-9 years. The clusters in the treated sub-district accounted for 40% (8/20) of the total clusters surveyed. The WHO simplified grading system was used to identify TF cases. A total of 1,116 children aged 1-9 years were examined with a male-to-female ratio of 1.27. No cases of TF were detected (0/1116), resulting in a TF prevalence of 0%. These data indicate that implementing MDA at the sub-district level, rather than the district level enabled the country programme to reach the TF elimination threshold (<5%) in Oussoubidiagna while saving resources. Surveillance is needed to avoid further recrudescence.

## 1763

### PROGRESS TOWARDS ACHIEVING TRACHOMA ELIMINATION: RESULTS FROM 6 LOCALITY-LEVEL IMPACT SURVEYS IN SUDAN

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In 2016 and 2017 The Carter Center supported the Sudan Federal Ministry of Health to conduct trachoma impact surveys to evaluate the success of program interventions and determine if additional rounds of mass drug administration (MDA) with azithromycin were needed. For each locality-level survey, a multi-stage cluster-random sampling method was used whereby clusters (villages) were selected using probability proportional

to population size in the first stage, and households selected randomly from a village household register and segmentation method in the second stage. Households within a cluster were selected with equal probability and all present household members examined using the WHO simplified trachoma grading scheme. A total of 14,741 individuals in 3,692 households were surveyed across six localities spanning five states (Red Sea, Gedarif, Sinnar, Blue Nile and South Kordofan). The prevalence of trachomatous inflammation follicular (TF) in children age 1-9 years ranged from 0.4%, (95% Confidence Interval (CI): 0.0, 0.8) in El Fashaga locality, Gedarif to 8.3%, (95% CI: 0.6, 15.9) in El Quraisha locality, Gedarif. In those 15 years and older, trachomatous trichiasis (TT) ranged between 0.1% (95% CI: 0.1, 0.3) in Abu Jebaiha locality, South Kordofan to 2.5% (95% CI: 0.8, 4.2) in Gaissan locality, Blue Nile. The prevalence of water and sanitation indicators were moderate with an average of 78% of children age 1-9 years having a clean face and household latrine coverage ranging from 13.2% to 86.5%. The prevalence of TF among children ages 1-9 years was less than 5% in five out of six localities which allows the government to stop MDA in these 5 localities. The sixth locality, Al Quraisha, will require one more round of MDA before another impact survey. The prevalence of TT in those 15 years and older was above the WHO thresholds of 0.2% in five out of six localities, demonstrating the need for continued surgical interventions to avoid blindness. Results from these impact surveys show that the program is on-track to reaching its target of elimination of trachoma as a public health problem by 2020.

## 1764

### RELIABILITY OF THE ABBOTT REALTIME ASSAY FOR THE QUANTIFICATION OF CHLAMYDIA TRACHOMATIS IN CONJUNCTIVAL SAMPLES FROM A TRACHOMA-ENDEMIC AREA OF ETHIOPIA

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The World Health Organization recommends monitoring trachoma programs by assessing trachomatous inflammation-follicular in children aged 1-9 years (TF1-9). Unfortunately, TF1-9 correlates poorly with infection, particularly after mass antibiotics. Commercial Nucleic Acid Amplification Tests can be used to estimate the prevalence of ocular infection with *Chlamydia trachomatis* in a community, and have become standard in research. Home-brew tests using real-time PCR are even used to quantify load of infection in individuals and burden in communities. The Abbott RealTime assay allows for quantification of load of infection, as opposed to earlier qualitative tests. To assess the reliability of Abbott RealTime for detection of *C. trachomatis* on the m2000 platform, conjunctival samples were taken from 69 children aged 0-9 years from 6 communities in the Amhara region in Ethiopia. Three swabs were taken from each child: 2 per right eye and 1 per left eye. Calibration curves were calculated using known concentrations of *C. trachomatis*. Four aliquots were run for each child: 2 from 1 right eye swab, 1 from the other right eye swab, and 1 from the left eye swab. A linear mixed effects model with child, eye, and swab included as random effects was used to evaluate intra-class correlation coefficients (ICC) for the following comparisons: 2 aliquots from the same swab, 2 swabs from the right eye, and 1 right and 1 left eye swab from the same child. Twenty-one children (30.4%) had at least 1 positive swab. The range of elementary bodies detected was 0-28,390 (median=0, IQR=0, 0). The ICC for 2 runs of the same swab was 0.97, indicating that results from duplicate aliquots are essentially the same. The ICC for the 2 right eye swabs was 0.88, suggesting some variation in sample collection. Specifically, 3 children (0.04%) had 1 positive and 1 negative swab from the right eye. The ICC for right and left eye swabs was 0.71, consistent with the belief that some children have

unilateral infection. Overall, the majority of variance was due to between-child variability in all comparisons, indicating that the Abbott RealTime assay provides highly concordant chlamydial loads.

## 1765

### PROVIDING TRACHOMATOUS TRICHIASIS SURGICAL SERVICES IN RURAL TANZANIA

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The World Health Organization (WHO) targets global elimination of trachoma as a public health problem by the 2020 through the SAFE (surgery, antibiotics, facial cleanliness, environmental change) strategy. Since inception of SAFE in 1999, Tanzania implements trichiasis surgical services in rural areas based on WHO guidelines with the aim of achieving GET 2020 goals. According to WHO elimination of trachomatous trichiasis (TT) is considered when the prevalence of trichiasis cases un-known to the health system is <1/1000 in the whole population (i.e. <0.2/1000 in people aged 15 years and above). In Tanzania TT surgical services are provided through camps and static units. Trained case finders search for TT cases around their community and report back the number of cases identified to the nearest health facility. Case finding is followed by social mobilization around the endemic community and visit by a TT surgeon to review the cases identified and plan for TT surgery camp. During social mobilization information on the date and location of TT camp are given to each community so that all identified TT cases can visit for TT surgical services. Based on the latest survey data, there are an estimates 53,426 TT un-operated TT cases. Account for elimination target of 1/1000 population, there were an estimated 28,478 TT cases requiring surgery for TT before intervention. Over the 2012 to 2016 implementation period, a total of 10,739 TT cases received surgery. The number of people that still need trichiasis surgery to reach elimination of TT are estimated to be 17,739. Implementing TT surgical services in rural areas has been a challenge especially in pastoralist areas which are nomadic in nature. This challenge has been addressed by using cultural leaders to advocate for the services provided and in other areas to use them as advocates and champions of TT surgical services. With concerted effort, Tanzania is on track to reach the TT elimination goal by 2020.

## 1766

### CONFIRMING TRACHOMATOUS TRICHIASIS PREVALENCE: PILOT TT-ONLY SURVEY IN TOUBORO HEALTH DISTRICT IN NORTH CAMEROON

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Through baseline mapping of trachoma following World Health Organization (WHO) recommended methodology, 47 health districts (HDs) in North, Far North and Adamaoua regions of Cameroon were found endemic for trachoma, with rates of active trachoma >10% for 16HDs, between 5-9.9% for 4HDs, and <5% for 27 HDs. Following the WHO SAFE strategy - Surgery, Antibiotics, Face washing and Environment improvement -, the ministry of public health implemented mass drug administration in the 20 HDs with active trachoma of at least 5%. 31 of the 47 HDs (66%), including 4HDs in Adamaoua, 8HDs in North and 19HDs in Far North regions, were identified as having trachomatous

trichiasis (TT) as a public health problem, with TT >0.1%. The Ministry started implementing TT surgery in the North and Far North Regions with these baseline data. Recently, trachoma experts have suggested that TT-only surveys may be needed in some regions where it is unclear whether TT is a public health problem, given that baseline mapping surveys might not have been statistically powered to provide a precise estimate of TT. WHO suggested pilot-testing TT-only surveys (TT-only), and Cameroon was selected for the pilot testing. Data from the baseline mapping showed Touboro HD in North region had active trachoma of <5% and TT of 0.23%. The teams used the WHO trachoma grading system to examine 5,378 people and entered the data into the Tropical Data collection system using smartphones. TT prevalence was 1.73% (95% CI: 1.37-2.19%) in 15 years olds and above and 1.26% (95% CI: 0.10-0.16%) in all ages. The TT prevalence from the TT-only survey is over 5 times higher than the rate found with the baseline mapping carried out in 2011. Moreover, the TT-only survey suggests a backlog of 2,497 TT cases while the Ultimate Intervention Goal is 198. Further investigations are needed to understand why the rates of TT from the two methods do not align.

## 1767

### TRACHOMA ENDGAME IN TANZANIA: TEMPORAL-SPATIAL ANALYSIS OF CLUSTERING OF TRACHOMATOUS INFLAMMATION FOLLICULAR (TF) IN DISTRICTS WITH PERSISTENT TF FOLLOWING MASS DRUG ADMINISTRATION WITH AZITHROMYCIN

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Tanzania began implementation of SAFE strategy in 1999 and has made tremendous progress towards trachoma elimination with 40 of 55 endemic districts having stopped mass drug administration (MDA). Impact surveys have shown that some districts continue to have TF prevalence of 5-9.9% thus warranting further MDA. We defined TF as persistent when a district continued to have TF ≥5% at two consecutive impact surveys. We investigated temporal-spatial distribution of TF prevalence in districts with persistent TF. Data were reviewed for 6 districts with TF=5.0-9.9% after the first impact surveys. Data from impact surveys were summarized to provide cluster level TF prevalence in children 1-9 years. Distribution of cluster TF prevalence were assessed for each impact survey using box plots. Household coordinates were averaged to provide a single set of coordinates for each cluster. Cluster level data were plotted on a map using QGIS to investigate temporal spatial clustering. Of 6 districts that had a second impact survey, TF prevalence was <5% in 2; 5-9.9% in 3; and ≥10 in 1. In one district TF has remained between 5-9.9% for three impact surveys. Distributions of cluster level prevalence showed that in districts where TF was <5% at the second impact survey, TF prevalence declined across all clusters. However, where TF was persistent the distribution of cluster prevalence remained the same or had a wider distribution at subsequent survey. In districts with persistent TF, the findings suggest that clusters with highest prevalence of children with TF were located in the same areas across the different survey periods. In 3 districts, trachoma was widespread throughout the district while in the fourth trachoma was clustered in specific areas. Based on these findings, it appears that districts with TF>5% at the first impact survey have high possibility of continued TF>5% and potentially an increase in TF prevalence at the subsequent impact surveys, even after a single round of MDA. Further studies are needed to investigate ocular chlamydial infection and associations of persistent TF with chlamydia infection, antibiotic coverage and other potential risk factors.

## THE CHANGING FACE OF TRACHOMA CONTROL IN TANZANIA: RESULTS FROM THE 2016 TRACHOMA IMPACT SURVEYS

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The World Health Organization (WHO) recommends evaluation of the surgery, antibiotic therapy, facial cleanliness and environmental change (SAFE) strategy after three to five years of implementation. In Tanzania, a total of 95 districts were surveyed for baseline trachoma prevalence between 2004 and 2014, and total of 59 districts were identified as endemic for trachoma. In 2016, 16 districts were eligible for trachoma impact surveys (TIS). Between July and September 2016, the Tanzania NTD Control Program (TZNTDCP) investigated: 1) the prevalence of trachomatous inflammation-follicular (TF) in children aged 1-9 years; 2) prevalence of trachomatous trichiasis (TT) in people aged 15 years and above; and 3) estimated TT backlog. A two stage cluster random survey design was used to select sample, with clusters (villages) selected in the first stage, and households selected using systematic random sampling in the second stage. In sampled households, children aged 1-9 years were examined for TF and persons aged 15 years and above were examined for TT using the WHO standard simplified grading system. Of the districts surveyed, 9 had reached the WHO threshold for stopping mass drug administration (MDA) of TF prevalence of <5%. A total of 5 districts had TF prevalence of 5-9.9% thus MDA was needed for one more annual round before another impact survey could be undertaken. Two districts had TF of > 10% thus another 5 round of annual MDA were warranted. Prevalence of TT by district ranged from 0.00% to 0.94%. TT backlog was estimated to be 67,000 across the 16 districts. Survey findings over the years suggest that, the ultimate intervention goal for TF of <5% has been attained in 48 surveyed districts therefore MDA with Zithromax should be stopped and surveillance surveys undertaken after 2 years. However, in several districts TT still remains a public health problem and therefore trichiasis surgery is required to clear the TT backlog. Concurrently, F&E (WASH) implementation need to be accelerated in all districts in order to sustain the gains so far achieved in trachoma elimination efforts in the country.

## IMPROVING TRACHOMA MASS DRUG ADMINISTRATION UPTAKE AMONG NOMADIC AND PASTORALIST COMMUNITIES OF RURAL TANZANIA

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According to baseline surveys conducted between 2004 and 2014, 59 districts in Tanzania are endemic for trachoma. Following baseline surveys, implementation of the SAFE (surgery, antibiotics, facial cleanliness and environmental change) strategy was initiated in a phased approach. The Tanzania NTD Control Program (TZNTDCP) has carried out mass drug administration (MDA) with azithromycin in endemic districts since 2011; reaching all endemic districts by 2015. MDA epidemiological coverage across trachoma endemic districts nationwide has ranged from 43% to 95%. Uptake in nomadic and pastoralist communities has been particularly challenging because community members do not fully participate in MDAs, leading to poor coverage rates. In January 2017, the program developed a behavior change communication (BCC) strategy for nomadic and pastoralist communities. The BCC strategy aims to improve

the understanding of the MDA approach and to encourage participation in NTD control activities. The strategy will be piloted in 3 districts of Arusha region (Ngorongoro, Monduli and Longido). A two-phased approach will be implemented before and during MDA. Phase 1 will focus on primary school children and women attending health facilities, in addition to key opinion leaders such as cultural leaders, local government authorities and community health workers. Phase 2 will target areas with severe cases of trachoma, while a more general campaign will cover the rest of the campaign area. A number of BCC approaches will be employed including football leagues in specific districts, creation of primary school hygiene and sanitation clubs, health facility campaigns for women during antenatal visits, open-market day events, an SMS campaign using geo-location of mobile phones, and mainstream media campaigns (radio, TV and newspapers). Output and outcome indicators will be used to monitor and evaluate the process. The campaign is expected to raise awareness of NTD interventions, improve MDA coverage, and increased uptake of TT surgery. Results of the BCC strategy's effect on MDA coverage will be determined after the next MDA (scheduled for July/August 2017).

## FOLLOW-UP OF TRICHIASIS PATIENTS OPERATED DURING AN EVALUATION OF THE SURGICAL SIMULATION DEVICE HEAD START

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From September 2015-January 2016, a study was carried out in Niger's Maradi region to determine whether the HEAD START surgical simulation device helps improve experienced trichiasis (TT) surgeons' operating skills. As part of this research, study participants operated on 2-4 TT patients, for a total of 61 patients. Patients should be followed up post-surgery to determine whether further care is required and for National Trachoma Programs to monitor program quality. In addition, as HEAD START has been introduced to improve surgical quality, patient follow-up is necessary to determine whether this tool is successful. A follow-up of patients operated on during the HEAD START study took place in January 2017. Only villages where  $\geq 2$  patients had been operated were visited, yielding a list of 50 patients. An examination of the patients' eyelids was conducted by the HEAD START master trainers and a questionnaire administered to each patient. Thirty-five patients were followed up; 15 were absent at the time of the study team's passage. Post-surgical guidelines had been followed: dressings were removed 1 day post-surgery and sutures removed 7 days following the surgery in all patients. Three patients complained of watery eyes and 8 patients had post-operative TT; of these, 7 had both eyelids operated during the study. Since the follow-up took place between 12-16 months after their surgery, we cannot conclude that the post-operative trichiasis is due to the surgery; it may be due to further disease progression. This may be evidenced by the fact that 7/8 of the post-operative TT cases had TT in both eyes, indicative of more severe TT. However, this follow-up provided an opportunity to refer these patients for further services. We also cannot conclude whether the HEAD START training improved patient outcomes, as we had no data on patients operated by these surgeons prior to the training. To determine whether HEAD START can improve patient outcomes, we recommend collecting data on patients operated by surgeons who are to undergo HEAD START training prior to the training and ensure patient follow-up 3-6 months following their surgeries.

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## FACTORS ASSOCIATED WITH MORTALITY AMONG PATIENTS WHO ABSCONDED FROM JINJA CHILDREN'S HOSPITAL

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Patients admitted to hospital may abscond before discharge or completing treatment, predisposing them to poor health outcomes and increased likelihood of mortality. We determined factors associated with mortality among patients who absconded from Jinja Children hospital in eastern Uganda. We reviewed case records of children who absconded from Jinja Children's hospital from 2012 to 2013 and managed to trace 313 patients in the surrounding community. Caretakers of children who absconded from hospital were interviewed. About 14 % (44/313) of the children that absconded from hospital had died by the time of the survey. Mortality was significantly lower among patients who consulted a pediatrician before abscondment compared to those who did not (OR 0.1, 95% CI 0.17-0.59). Mortality was not associated with social demographic characteristics of the patient and caretaker. Mortality was high among patients who abscond from hospital and lack of review previous review of the patients by the specialist's increases mortality among those who absconded. Further studies are needed to examine factors associated with abscondment and review of patients by specialised health workers should be strengthened at facilities.

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## COMMUNITY SENSITIZATION AND DECISION-MAKING FOR TRIAL PARTICIPATION: A MIXED-METHODS STUDY FROM THE GAMBIA

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Ensuring individual free and informed decision-making for research participation is challenging. It is thought that preliminarily informing communities through 'community sensitization' procedures may improve individual decision-making. This study set out to assess the relevance of community sensitization for individual decision-making in research participation in rural Gambia. This anthropological mixed-methods study triangulated qualitative methods and quantitative survey methods in the context of an observational study and a clinical trial on malaria carried out by the Medical Research Council Unit in rural Gambia. Although 38.7% of adult community members were present during sensitization sessions, 91.1% of the respondents were inclined to participate in the trial when surveyed after the sensitization and prior to the informed consent process. This difference can be explained by the informal transmission of information within the community after the community sensitization, the expectations such as the benefits of participation based on previous research experiences, and the positive reputation of the research institute. Commonly mentioned barriers to participation were blood sampling and the potential disapproval of the household head. Community sensitization is effective in providing first-hand, reliable information to communities as the information is cascaded to those who could not attend the sessions. However, further research is needed to assess how the informal spread of information further shapes people's expectations, how the process engages with existing social relations and hierarchies (e.g. local political power structures; permissions of heads of households) and how this influences or changes individual consent.

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## IMPACT OF SEASONAL MALARIA CHEMOPREVENTION ON HOSPITAL ADMISSIONS AND MORTALITY IN CHILDREN UNDER 5 YEARS IN OUELESSEBOUGOU, MALI

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Over the past 15 years, overall mortality rates in African children under five have been reduced by 57% due to expanded health interventions including control of malaria, a major killer in sub-Saharan Africa. Seasonal malaria chemoprevention (SMC) has been recommended for malaria prevention in areas of high seasonal malaria transmission since 2012, and is now in large-scale implementation in most Sahelian countries including Mali. To assess the impact of SMC on hospitalizations, we conducted surveys in December 2016 to assess and compare the rate of hospital admissions and deaths in children under 5 years of age from two health sub-districts of Ouelessebouougou receiving SMC and two health sub-districts where SMC had not yet been implemented during the malaria transmission season. Information on hospital admissions and deaths in children under 5 years of age in 2015 were collected by interview. A total of 7867 children (4637 from the intervention areas and 3230 from the nonintervention area) were surveyed. Preliminary analysis indicated a mortality rate per 1,000 children of 3.0 in the SMC intervention area compared to 8.01 in the non SMC area (age and gender adjusted mortality rate ratio (MRR) = 0.39, 95% CI: 0.19- 0.78, p = 0.008). Incidence rate of hospital admission per year per 1,000 children was 23 in the SMC intervention area compared to 34 in the non SMC area (age and gender adjusted incidence rate ratio (IRR) = 0.69, 95% CI: 0.52- 0.91, p = 0.009). SMC implementation was associated with a substantial reduction in all-cause mortality and hospital admission in children under 5 years of age in Ouelessebouougou, Mali.

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## DEVELOPMENT OF AN ANTISENSE RNA (ASRNA) STRATEGY FOR GENE SILENCING IN *LEPTOSPIRA* SPP.

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The genus *Leptospira* encompasses both pathogenic and saprophytic species. Pathogenic *Leptospira* are the etiological agents of leptospirosis, while saprophytic bacteria are environment free-living organisms. The mechanisms responsible for leptospirosis pathogenesis are yet poorly understood, mainly due the lack of genetic tools available for the pathogen. Antisense control in bacteria is now recognized as an efficient and specific mechanism for gene regulation. This control occurs at many levels, including premature transcription termination and direct or indirect blocking of translation. In order to expand the possibilities of genetic tools for *Leptospira* gene silencing, we evaluated the effect of expressed asRNA upon gene expression; we selected the  $\beta$ -galactosidase gene in order to assess the enzyme activity in the saprophyte strain. Four different constructs were employed, encompassing two different promoters (leptospiiral *lipL32* and borrelial *flgB*) controlling the transcription of the asRNA containing or not a paired-termini (PT) secondary structure. The antisense sequence was designed to be complementary to the 5' UTR region of the  $\beta$ -galactosidase mRNA. The constructs were ligated into the pMaOri vector, replicative in *Leptospira* and recombinant plasmids were used to transform *L. biflexa*. The expression of the asRNA in the leptospiral

strains was validated by Northern Blotting and  $\beta$ -galactosidase activity was assessed by chromogenic substrate assay. Northern blotting analysis showed that all constructs were able to successfully express the asRNA and the inclusion of the PT rendered higher stability of the transcripts. *L. biflexa* cells containing asRNA1 and asRNA2 (*lipL32* promoter with and without secondary structure, respectively) presented less enzymatic activity. The results demonstrate the feasibility and applicability of the antisense RNA molecules as a tool for gene silencing in *Leptospira*. This strategy will help elucidating the function of different genes and consequently shed light to the basic aspects of biology and pathogenesis of *Leptospira*.

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### DIFFERENCES IN SYMPTOMATOLOGY OF CHILDHOOD DENGUE, CHIKUNGUNYA AND MALARIA INFECTION IN KENYA

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Diagnosis of dengue (DENV) and chikungunya (CHIKV) occurs infrequently during the evaluation of childhood febrile illness in Kenya, leaving the burdens of arboviral infections in Kenyan children largely unknown. To investigate, we enrolled children (age 1- to 17-years) in an ongoing study (2014-2018) who presented with fever to one of four study sites in western and coastal Kenya. Malaria was diagnosed by peripheral blood smear examination, and DENV and CHIKV infections were diagnosed by RT-PCR of acute blood samples, or IgG seroconversion between the acute and convalescent samples. To date, we have enrolled 3835 subjects. 33.4% had malaria parasitemia. 6.2% (83/1346) of samples tested thus far by PCR were positive for DENV RNA. An additional 7 DENV infections were identified based on IgG seroconversion. None of the samples tested by CHIKV PCR were positive, however 0.8% (14/1738) individuals seroconverted for CHIKV IgG. Vomiting and aches and pains were reported by half of the subjects, regardless of infectious etiology. Rashes, edema, and eye complaints occurred rarely and did not differ by pathogen. However a higher proportion of CHIKV-infected subjects reported abdominal pain (43%) than did subjects with either DENV infection (15%) or malaria (26%,  $p=0.025$ ,  $\chi^2$ ). Unexpectedly, bleeding symptoms were reported by 7% of CHIKV-infected subjects, compared with 0.01% and 0.0056% of DENV- or malaria-infected subjects, respectively ( $p=0.011$ ,  $\chi^2$ ). Our findings suggest some differences in patterns of clinical symptomatology of DENV or CHIKV infection in children, which may aid clinicians in establishing alternative diagnoses to malaria when evaluating febrile illness. In our cohort, 86% of CHIKV- and 58% of DENV-infected subjects were treated with antimalarial medications despite available malaria test results. Given the overtreatment of malaria in sub-Saharan Africa, prioritizing development of arbovirus clinical diagnostic aids, in the form of accessible rapid diagnostic tests or clinical disease pattern recognition algorithms, is an important strategy for combating the growing problem of antimalarial and antibiotic overuse.

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### IMPROVING EFFICIENCY AND PATIENT EDUCATION IN THE DARTMOUTH TRAVEL CLINIC

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The number of US citizens traveling internationally increased from 59 million to 74 million from 2011-2015. Many of these travelers stay healthy by visiting specialized travel clinics before their trips. The Dartmouth

Travel Clinic sees ~1400 patients annually. Surveys regarding areas for improvement were sent to 90 patients over a 3-month period. Based on 28 responses as well as provider input, we identified better efficiency and patient education as target areas for improvement. Toward this aim, we developed a questionnaire requesting information about travel plans and medical and vaccination history that was mailed or emailed to patients when their appointment was scheduled. Providers received the completed forms prior to the appointment in order to improve encounter efficiency and personalization. We also developed four internet-based interactive decision tools for frequently-discussed vaccinations so patients could understand key elements of vaccination recommendations prior to their appointment. We re-surveyed 49 patients after implementation of these interventions to assess their impact. After implementation of the pre-appointment questionnaire, the number of patients who provided their vaccination history prior to their appointment increased (~half to 72%), and 74% of patients reported that the pre-appointment questionnaire helped their provider address their specific needs. Comparing before and after questionnaire implementation, providers were able to decrease the amount of time spent acquiring history from 10 to 8 minutes. Patient satisfaction scores increased from 78% prior to quality improvement efforts to 100% after. Data analysis of online vaccine decision tools is underway. By collecting patient data before appointments, providers were able to make appointments more efficient and personalized. This resulted in high patient satisfaction scores while still ensuring that patients had access to quality, evidence-based information. Our study demonstrates the possible benefits and future uses for technology and patient-centered care in travel medicine.

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### SNAKEBITES IN A RESOURCE POOR AREA ALONG THE SOUTHERN KENYAN COAST: SPATIAL RESULTS AND VICTIM PROFILES

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Snakebites are a serious and often overlooked health problem in developing countries. Victims are principally children from poor households, and bites can result in lifelong debilitation and even death. In 2009, the World Health Organization approved snakebites as a neglected tropical disease (NTD). Nagasaki University, in cooperation with the Kenya Medical Research Institute (NITM-KEMRI), surveys more than 100,000 people in two areas of Kenya on a thrice yearly basis as part of a Health and Demographic Surveillance System (HDSS). Field workers visit households individually and track household population change, pregnancies, deaths and major health events. GPS locations of all households are contained within the database, allowing for detailed spatial analyses. A brief set of questions on past incidence of snakebites was included in a routine round of the HDSS. A follow up survey was conducted of households which reported that someone in the household had been bitten in the past year. Of 8,756 household included, 453 (5.17%) households reported that at least one household member had been bitten in the past year. The mean age of victims was ~31 years of age. Bites occurred mainly on the feet (55%) and occurred near the home (35%). Black (47%) and green mambas (29%) were the most common species of snake. Only half of all victims reported to a hospital after injury. Reporting to a local herbal practitioner was associated with a decreased odds of going to a clinic (OR(.20 (.13,.31)) and that persons who received treatment from a traditional healer were much more likely to have permanent injury than those who did not (OR 9.67 (2.92, 31.99)). Spatial analyses indicated that bites were highly concentrated in a low lying wet region of Kwale indicating that while death (3.8%) and debilitation (8.6%) were uncommon over all, certain populations were at high risk. Future work should include researcher strategies to improve uptake of formal treatment for snakebites to prevent serious injury in resource poor environments.

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### VALIDATION OF A CLINICAL CASE DEFINITION OF ACUTE MESOAMERICAN NEPHROPATHY USING A LARGE RETROSPECTIVE COHORT IN NICARAGUA

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Mesoamerican nephropathy (MeN), a tropical medicine mystery of Central America, affects mostly young agricultural workers who lack traditional risk factors for kidney disease. Although previously known as a chronic kidney disease (CKD), we recently reported that an early stage of acute kidney injury with systemic inflammation can precede CKD and proposed a clinical case definition of acute MeN. Our goals with this analysis were to (1) describe the initial, acute clinical presentation in a large cohort of historic MeN CKD cases and (2) validate the acute clinical case definition in that population. We conducted a retrospective study at a large sugar estate in Nicaragua, a locale disproportionately affected by MeN. We abstracted clinical, demographic, and medical history data from records at the estate's private hospital, which implemented surveillance for MeN CKD in the late 1990's. We documented events associated with the first elevated serum creatinine recorded in the medical record for each patient. Data were available on 746 cases, diagnosed at various stages of CKD: 18% Stage 1, 40% Stage 2, 30% Stage 3, and 12% Stage 4-5. Patients were male (98%) and young (median age 33 years). The mean acute serum creatinine level was  $2.0 \pm 1.3$  mg/dL. Symptoms included back pain (23%), fever (19%), and urinary signs (15%); 17% were asymptomatic. Prior diabetes (2%) and hypertension (8%) were rare. Neutrophilia (53%), leukocytosis (33%), lymphopenia (25%), anemia (68%), and hyperuricemia (51%) were noted. Urine analysis revealed shedding of leukocytes (99%), leukocyte casts (18%), and bacteria (19%). This evidence confirms a systemic inflammatory response in most patients during acute MeN and documents a high frequency of asymptomatic acute MeN. Sensitivity and specificity analyses for the proposed case definition and alternatives are discussed in the context of detecting MeN that later progress to advanced disease. We discuss challenges to using historical data to validate the clinical case definition, propose an alternative definition, and suggest an additional validation study as a planned prospective clinical investigation.

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### IMS, CRP AND PROCALCITONIN TO DIFFERENTIATE CAUSES OF ACUTE INFECTIONS IN INDONESIA

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Early differentiation between different causes of acute febrile illness is important to rationalize antimicrobial prescription. Clinical examination or early microbiological confirmation often fails to differentiate these causes. A point of care screening tool that differentiates viral and bacterial infections is therefore urgently needed. Febrile infectious diseases induce a systemic inflammatory immune response that is often accompanied by changes in circulating blood cells in quantity, composition, function and morphology. This acute systemic inflammatory immune response is different in viral and bacterial infections, which is reflected by distinctive changes in the number, functionality state and morphological changes in the various blood cell subsets. Whereas classical hemocytometry mainly detected changes in the number of cells, e.g. lymphocytosis in viral and neutrophilia in bacterial infections, the newest generation of Sysmex analyzers is also capable of measuring phenotypic changes e.g. functionality state in these circulating cells in real time. Applying these innovative techniques allows a specific characterization of the respective

type of immune response and as such allow a better differentiation between the various causes of infection. Diagnostic algorithms (Infection Manager System: IMS) have been developed using various combinations of cell subsets. A prospective study was carried out in Indonesia to investigate the potential of this approach to differentiate between acute infectious diseases common in tropical regions and compare results with C-reactive protein (CRP) and Procalcitonin (PCT). Out of 466 evaluable patients, a total number of 71 adult patients with proven bacterial infections and 90 with proven arboviral infections (mainly dengue) were enrolled. All cases were flagged as infections. Sensitivity and specificity of IMS to diagnose bacterial or arboviral infection was >95%, outperforming CRP or PCT. Similar results were obtained combining proven and probable cases (n=271). We conclude that IMS may be a valuable and cheap tool to rationalize antimicrobial prescription.

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### SCHISTOSOMA HAEMATOBIIUM EGG EXCRETION IN URINE DOES NOT INCREASE AFTER EXERCISE: IMPLICATIONS FOR DIAGNOSTIC TESTING

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A common practice in many African settings is to have children exercise or jump vigorously shortly before providing a mid-day urine specimen for *Schistosoma haematobium* diagnosis to help increase diagnostic sensitivity. However, the evidence driving this procedure is sparse. We assessed the effect of prior exercise on *S. haematobium* diagnosis in 329 pre-school and school-aged children from five villages in the district of Adzopé, south Côte d'Ivoire. Children provided two urine specimens on consecutive days, collected between 10:00 and 12:00 hours. From each specimen, 10 ml were processed by filtration and evaluated under a microscope by experienced laboratory technicians adhering to WHO guidelines. Children did not jump or exercise on the first day of urine collection. On the second day, children older than 4 years were asked to do 20 jumping jacks prior to providing a urine sample with the exercise performed under the supervision of parents or teachers and our field team. Children under the age of 4 years were gently 'bounced' in a jumping motion 20 times by their parent or guardian. 257 children between ages 1 and 15 years (mean 6.8 years; 39% female) provided urine samples on consecutive days and were included in the analysis. After completion of the study, all study participants received praziquantel (single 40 mg/kg oral dose) at no cost as per national protocols. On the day without exercise, 66/257 (25.7%) urine samples were positive for *S. haematobium*, and on the day with exercise, 59/257 (23.0%) urine samples were positive (McNemar's p-value: 0.31). Among those with eggs present in urine, there were no differences in egg counts between the two days (p=0.62). Age and gender based sub-analyses with revealed no significant differences in egg counts for exercise versus non-exercise days. We conclude that exercise prior to providing a urine specimen for *S. haematobium* diagnosis does not increase the number of eggs excreted.

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### POTENTIALLY SERIOUS DRUG INTERACTIONS RESULTING FROM THE PRE-TRAVEL HEALTH ENCOUNTER

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Medications prescribed during the pre-travel encounter may have interactions with travelers' existing prescriptions. We evaluated travelers seen for pre-travel health encounters at 21 Global TravEpiNet (GTEN) sites from January 2009 to December 2015. Infectious disease pharmacists identified pre-existing medications with potential for serious drug-drug interactions (based on severity, frequency, quality of published reports) associated with six commonly prescribed travel medications: acetazolamide, ciprofloxacin, azithromycin, atovaquone-proguanil, doxycycline, and chloroquine. We characterized the demographics, medical conditions and travel plans of all travelers with serious drug interactions. We evaluated 73,794 travelers seen at GTEN sites. Of these travelers, 2530 (3%) had a potentially serious drug interaction between an existing prescription and a medication prescribed at the pre-travel health encounter. The most common existing prescription medications associated with a potentially serious drug interaction were selective serotonin reuptake inhibitors (SSRIs) such as citalopram (n=428), escitalopram (n=312) and fluoxetine (n=416), the HMG-CoA reductase inhibitor simvastatin (n=1013), and warfarin (n=281). The median age of travelers with potential drug interactions was 55 years. Fifty-six percent of travelers with potential interactions were female, 42% traveled for >=14 days, 68% traveled for leisure, 74% traveled to a country of low/medium human development, and about half had 3 or more medical conditions. In univariate comparisons, travelers with potential drug interactions were more likely to be older, travel for a shorter duration, travel for leisure or service work, and have a pre-existing medical condition. In conclusion, potentially serious drug interactions may occur between existing prescriptions and medications prescribed during the pre-travel health encounter. Travel practitioners should consider the possibility of serious drug interactions, particularly in older travelers and those taking SSRIs and simvastatin.

## 1782

### IN VITRO ANTIFUNGAL ACTIVITY OF *CRYPTOLEPIS SANGUINOLENTA* ON CLINICAL *CANDIDA* ISOLATES FROM GHANA

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*Cryptolepis sanguinolenta* (Linn.) Schltr. (Periplocaceae), is a plant widely used in indigenous traditional medicine for its anti-malarial properties. In Ghana, it has been used as an anti-malarial agent for over 100 years and has recently undergone a clinical study. In this study, the antifungal activity *C. sanguinolenta* was investigated using *Candida* species isolated from female patients presenting with candidal vaginitis from Korle-Bu Teaching Hospital (KBTH), Centre for Plant Medicine Research (CPMR) and Tetteh-Quarshie Memorial Hospitals (TQMH), all in Ghana. Aqueous and 70% ethanol extracts of roots, stems and leaves of *C. sanguinolenta* were evaluated using a modified agar well diffusion assay. Minimum inhibitory concentrations (MICs) and Minimum fungicidal concentrations (MFC) were also determined using standard microbiological methods. Results from the study, showed that the aqueous and ethanol extracts of the leaves, stems and roots of *C. sanguinolenta* have significant antifungal activities against clinical and standard *Candida* isolates with the root extract showing the highest antifungal activity. Average diameter zones of inhibition (DZI) for the aqueous and ethanol extracts ranged respectively from 8.00 ±

0.50 to 30.00 ± 0.10 mm and 8.00 ± 0.10 to 31.00 ± 1.50 mm for the leaves, stems and roots. Minimum inhibitory concentrations (MICs) of the ethanol extracts of the stems and the roots both ranged from 0.15625 to 1.25 mg/mL while the minimum fungicidal concentrations (MFC) ranged from 0.15625 to 1.25 mg/mL for both the ethanol stem and root extracts. Encapsulated *C. sanguinolenta* powder (250 and 500 mg), which contained 25 and 50 mg of the 70% ethanol extracts from the roots both yielded fungicidal activity ranging from 9.00 ± 0.10 to 31.00 ± 0.50 and 8.00 ± 0.10 to 31.00 ± 0.50 respectively. Thus, in addition to the anti-malarial activity, *C. sanguinolenta* has anti-fungal activity. The results obtained may provide valuable information for further detailed studies of the active compounds, necessary for the development of new antifungal drugs in the future.

## 1783

### INCIDENCE AND RISK FACTORS FOR PERIPARTUM FEVER IN PUERTO RICO, OCTOBER 2016 - MARCH 2017

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Peripartum fever, defined as a temperature ≥ 38°C prior to, during, and immediately after delivery, is not uncommon. It has been linked to complications such as early labor, operative deliveries, and may be the consequence of infection with etiologic agents of acute febrile illness (AFI) such as dengue and chikungunya. In some cases peripartum fever can result in neonatal infection, which in turn is associated with increased risk of admission of the neonate to the Intensive Care Unit, low Apgar scores, and sepsis. The reported prevalence of peripartum fever varies widely by region, and has not been reported in Puerto Rico. The aim of the study is to describe the prevalence and outcomes of peripartum fever among women identified by the Sentinel Enhanced Dengue Surveillance System (SEDSS), a facility-based epidemiologic platform established in a tertiary care hospital in southern Puerto Rico. Women reporting fever of ≤7 day's duration before and during active labor from October 2016 to March 2017 were enrolled. Data regarding history of fever ≤7 days prior to delivery was collected in the Delivery Room. Relative and absolute frequencies were calculated for categorical variables, and central tendency and dispersion measures were calculated for quantitative variables. Out of 598 deliveries, a total of 11 women presenting with fever during the peripartum period were enrolled in the study, among whom median age was 27 years (19–36). Preliminary results indicate that the prevalence of peripartum fever is approximately 1.84%. Most cases (36%) occurred in December. Among eight participants with peripartum fever for which medical records have been reviewed, seven (87.5%) had a vaginal delivery and four (50.0%) were positive for Group B *Streptococcus*. Further analysis is being conducted to describe the maternal and neonatal outcomes through medical chart review, and enrollment is expected to continue through at least October 2017. Identifying the causes of fever during the peripartum period could enable targeted interventions in pregnant woman and their infants, and thereby assist in avoiding unnecessary procedures or lengthy medical stays.

## 1784

### ENDOTOXIN LEVELS AT THE MATERNAL-FETAL INTERFACE AND THE ASSOCIATION WITH INTRAUTERINE GROWTH

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Low- and middle-income countries (LMICs) carry a high burden of parasitic diseases that are associated with impaired gut integrity. Children in many

of these countries also experience a high rate of poor intrauterine growth. We hypothesized that factors culminating in microbial translocation (i.e. schistosoma and/or hookworm infection, alcohol consumption) during pregnancy may negatively impact fetal growth by changing the microbial load of the placenta. The placental microbiome is suggested to reflect colonization, in part, via hematogenous spread, and has been linked to poor pregnancy outcomes such as preterm birth. We examined the association between specific risk factors for impaired gut integrity and endotoxin, a key component of the cell wall of gram-negative bacteria, in blood collected from the maternal-fetal interface (MFI) and the umbilical cord at delivery. In addition, we examined the relationship between endotoxin levels and poor intrauterine growth. We utilized samples collected from a recently completed trial, in which women with schistosomiasis were randomized to praziquantel treatment or placebo at 12-16 weeks gestation. None of the risk factors examined had an impact on the endotoxin levels at the MFI, although there were trends for higher endotoxin at the MFI in women who received praziquantel ( $P = 0.08$ ) and those who reported heavy drinking ( $>3$  drinks/week;  $P = 0.06$ ) during pregnancy. Maternal alcohol consumption increased the amount of endotoxin found in cord blood (1.8-fold higher,  $P = 0.01$ ) and cord blood endotoxin levels were associated with higher levels of free amino acids in fetal circulation ( $P < 0.01$ ). In keeping with these data, infants born small for gestational age (SGA) also had higher levels of endotoxin at the MFI ( $P = 0.03$ ). Our findings suggest that maternal alcohol consumption may contribute to gut microbial translocation, increasing levels of endotoxin at the MFI and cord blood. These alterations in the microbial abundance at the MFI and in fetal circulation may contribute to the poor growth *in utero* associated with pre-natal alcohol exposure.

## 1785

### GUILLAIN-BARRÉ SYNDROME IN PATIENTS WITH ZIKA: CLINICAL SERIES WITH LABORATORY CONFIRMATION IN THE STATE OF YUCATAN

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In 2016, over 1700 cases of Zika were confirmed nationwide in Mexico. By the end of the the first half of 2016, autochthonous cases of Zika virus were confirmed in the State of Yucatan. Aedes-borne viral diseases are common in Yucatan, in 2015 a Chikungunya outbreak took place and increased the burden of Aedes-borne diseases in addition to the previous burden. Flaccid palsy (FP) and Guillain-Barre syndrome (GBS) increased their occurrence during 2016, particularly in people under 15 years of age. FP increase its incidence three times between 2015 and 2016 for the same periods. The etiology of GBS and FP in most cases was neither studied nor suspected, Mexican epidemiologic surveillance system is focused exclusively in their relationship with poliomyelitis in young people. In this abstract we present an analysis of the incidence trends in FP among <15 years people and Guillain-Barré syndrome, with emphasis in the specific number of cases that occurred simultaneously with the Zika outbreak in Mexico and in Yucatan, while we analyze a clinical series recorded at a public general hospital. Four patients positive for Zika infection began with clinical traits that suggested GBS 7 days after diagnosis, approximately. Electromyography tests were performed, finding damage compatible with GBS. However, they had different varieties of GBS, two of them had acute inflammatory demyelinating polyneuropathy (AIDP) which is the most common of the GBS subtypes and two of them, acute motor axonal neuropathy (AMAN), this difference was established with the findings in electromyography. Those with AIDP variety, had a favorable outcome after treatment and discharge was achieved. The other two patients with AMAN subtype had a very different evolution. One was a 6 years old male who didn't achieve full recovery even after IVI therapy, and the other was a 15 y/o female who died despite rapid treatment. Zika virus as etiologic agent should have been tested. All patients had had previous positive IgG

test for Dengue, and that could have enhanced the clinical traits or even caused the GBS, this theory corresponds with Halstead's enhancement theory.

## 1786

### MALARIA TRANSMISSION AS MEASURED BY DIRECT SKIN FEEDING OVER A TWO-YEAR PERIOD IN MALI AS AN EFFICACY ENDPOINT FOR A TRANSMISSION BLOCKING VACCINE

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Malaria transmission from human to mosquito is a complex process depending on a number of human and mosquito factors including the density and maturation of gametocytes present in the capillaries, innate mosquito susceptibility to malaria infection and host immunity. Transmission blocking vaccines work by mounting a host immune response that prevents malaria infection developing in a mosquito despite taking a potentially infectious bloodmeal and direct skin feeding (DSF) assays are an ideal way to measure vaccine efficacy in a more natural manner than assays such as the standard membrane feeding assay. Here we will present data from a transmission blocking vaccine trial conducted in Bancoumana, Mali, where DSFs were conducted twice weekly for a six-week period in two subsequent years (post vaccination 3 and post vaccination 4). In total 175 individuals underwent DSFs with a total of 3,849 feeding assays conducted. 88 infected feeds were observed (2.3%) where at least one oocyst was observed seven days post DSF. We will discuss the transmission dynamics across the two years including parasite and gametocyte carriage rates amongst participants with different DSF outcomes, mosquito feeding and survival rates including a change in feeding methodology implemented in the second year and compare between vaccinated and control individuals.

## 1787

### CYTOKINES PROFILES IN PATIENTS WITH HANSEN'S DISEASE AND PARASITIC CO-INFECTIONS IN HYPERENDEMIC AREA OF BRASIL: IMPLICATIONS FOR TRANSMISSION

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While cases of Hansen's disease have decreased over the last 20 years, there is still a steady number of new cases diagnosed each year. Mycobacterium leprae infection has been shown to increase cells producing Th2 cytokines (IL-4/IL-10) in multibacillary (MB) infection. The overall aim of this project is to better delineate the role of parasitic co-infections as well as select micronutrient deficiencies in the transmission and immunologic response of leprosy. Cases of leprosy and controls were enrolled in the rural areas outside GV, MG, Brazil. Structured and pre-

coded questionnaires were applied for demographic and socioeconomic data. PBMC were obtained from cases of leprosy and healthy controls selected for the study. The levels of cytokines in the supernatant of PBMC cultures were determined by CBA Th1/Th2/Th17 Human - BD. Stool was examined for ova and parasites. Preliminary results show a higher production of IL-10 (mean: 252.4 pg/mL specific to ML) by MB patients compared to healthy individuals (mean: 45.3 pg/mL specific to ML). On the other hand, a decreased production of IFN- $\gamma$  was noted by MB patients (mean: 8.0 pg/mL specific to ML) in comparison with healthy individuals (mean: 15.0 pg/mL specific to ML). *S. mansoni* infection has been found in several cases and controls but ova and parasite exams not currently available for all participants. In the sense that the cytokine IL-10 modulates IFN- $\gamma$  levels, our preliminary results confirm that there is a trend of MB patients to display a profile of modulatory response given by an increase of IL-10 and consequently a reduction of IFN- $\gamma$ . Comparison of cases with parasitic co-infections and those with micronutrient deficiencies will further delineate differences in cytokine profiles among cases and controls and increase our understanding of the immune response associated with co-infection. These analyses can help determine whether actors such as co-infections and micronutrients may influence the immune response and propensity for MB disease, and thus lead to ongoing transmission of *M. leprae*. Financial support: FAPEMIG/FNS/MS/EMORY UNIVERSITY/USA

## 1788

### ALTERED FETAL IMMUNE RESPONSES BY PRENATAL EXPOSURE TO MATERNAL CO-INFECTIONS

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In developing countries, parasitic infections are prevalent during pregnancy and approximately 40% percent of these women present with multiple infections. Co-infections are known to alter disease outcome and may affect vaccine and drug efficacy. Since prenatal maternal infections are known to influence fetal immunity, we investigated whether helminth co-infections will have an impact on the neonates' immune response. 331 Kenyan women were tested for the presence of *Schistosoma haematobium* (Sh, n=65), malaria (n=79), filariasis (n=23), Sh co-infection with filariasis (Sh-f, n=31), malaria (Sh-m, n=54) and malaria+filariasis (Sh-fm, n=52) and were compared to non-infected (n=27). Neonates' cord blood lymphocyte responses to Sh worm (SWAP) and egg antigens (SEA) were analyzed for production of IL-2, IL-5, IL-6, IL-10, IL-12, IL-13, IFN- $\gamma$ , TNF- $\alpha$  and GM-CSF. Analysis was done using unpaired t test with Welch's correction. Results showed: i) higher IL-5 to SEA in the Sh and Sh-m groups compared to non-infected (0.012, 0.002) and Sh-f (0.026, 0.023), ii) higher IL-13 to SEA in the Sh (0.006) and Sh-m (0.033) compared to non-infected, iii) higher IL-10 to SWAP and SEA in the Sh (0.023, 0.009) and Sh-m groups (0.015, 0.016) compared to non-infected, iv) lower IFN- $\gamma$  to SEA (0.032, 0.040) and TNF- $\alpha$  to SWAP (0.026, 0.035) in Sh-f compared to non-infected and Sh-m groups, v) higher TNF- $\alpha$  to SEA in the Sh-m group compared to non-infected (0.012), Sh (0.012), Sh-f (0.010) and Sh-mf (0.013) and vi) higher IL-6 to SWAP (0.037) and GM-CSF (0.012) to SEA in the Sh-m group compared to non-infected group. These results indicate that co-infections induce a shift in fetal immune priming with a predominant pro-inflammatory response in the schistosome and malaria co-infection group compared to schistosome only infected. On the other hand, schistosome and filariasis co-infections induce immune suppression on both Th1 and Th2 cytokines. We conclude that maternal co-infections (and type) modifies fetal immune priming. This shift in immune response may influence the child's ability to respond to infections and vaccination later in life.

## 1789

### DOUBLE JEOPARDY: RECURRENT CASE OF DENGUE FEVER

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Case Discussion: 55 year old male with a past medical history DM2, BPH and HLD presented from home with generalized body aches and fevers for one week duration. Associated with headache, nausea, diarrhea, fevers along with joint pains and a diffuse itchy rash across the chest and abdomen. Patient also complained of dysuria without discharge. He denied abdominal pain, chest pain, shortness of breath or cough. He had recent travel to Dominican Republic one week ago but denied any sick contacts. His vitals were significant for low blood pressure of 101/63, tachycardia of 106 and a temperature of 101.8F. On physical, he had a diffuse maculopapular rash on the chest and abdomen without petechiae. Laboratory studies showed anemia and mild hyponatremia. His urinalysis, chest x-ray and EKG were also normal. Supportive care was initiated with intravenous fluids and pain control. ID was consulted, bacteria, viral and febrile infections workup was started. Patient developed a fever, antibiotics were started empirically for bacterial infection. Subsequently, he developed thrombocytopenia with elevated LFTs and AKI. He also developed retro-orbital pain, scleral icterus and abdominal pain. Patient had a similar episode 2 years prior and was diagnosed with dengue fever. His symptoms as well as elevated LFTs and thrombocytopenia resolved. Typhoid, HIV, Hepatitis C, Influenza, Zika, Yellow fever and Chikungunya were ruled out. Patient was discharged with positive antibodies to Dengue IgM/IgG. Conclusion: Dengue fever is a non-contagious vector-borne illness, transmitted by *Aedes aegypti* mosquito. There are 4 different serotypes of dengue fever, recovery from one serotype results in lifelong immunity. However, recurrent infections of dengue fever is usually more severe with different serotypes as it can cause massive internal bleeding. In a patient presenting with fevers, myalgia and joint pains checking for bacterial and viral infections are important. Dengue should always be on top of the list if patient has traveled to an endemic area. Even if patient has had dengue before as recurrent dengue though rare, can happen as in the case we presented.

## 1790

### TRAVEL-RELATED BEHAVIORS OF ADOLESCENTS ON SHORT-TERM INTERNATIONAL SERVICE MISSIONS

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There is an increasing number of adolescents participating in short-term international service missions. Despite this fact, little is known about travel-related behaviors and health risks associated with this age group. We administered an anonymous web-based survey to groups of mixed-age travelers following their short-term international missions in seven countries. All participants attended the same pre-travel educational lecture and participated in similar service activities during their trips. We compared the self-reported travel-related behaviors between adolescent ( $\leq 20$  years old, n=82) and older ( $\geq 21$  years old, n=60) participants. We did not detect any differences between age groups for health-related behaviors such as malaria prophylaxis compliance, hand-cleaning practices, or insect precautions taken. We found that adolescent participants were more likely to report insect bites (P=0.04), experience jetlag (P<0.0001), and sustain injuries (P=0.001) during their trips. We did not find any differences in rates of fever, diarrhea, or animal contact. In conclusion, in a group of travelers that received the same pre-travel health education, younger (adolescent) travelers reported similar rates of compliance with preventive behaviors but these did not protect them from travel-related adverse outcomes such as insect bites, jetlag, and injuries. Our findings suggest

the need to customize pre-travel education for adolescent travelers. Our study is ongoing and we expect to present analysis of a larger number of subjects at the meeting.

## 1791

### IRON, INFLAMMATION AND ERYTHROPOIESIS: ANALYSIS OF FACTORS CONTRIBUTING TO SEVERE ANEMIA IN UGANDAN CHILDREN WITH SICKLE CELL ANEMIA

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African children have a high prevalence of sickle cell anemia (SCA), causing significant morbidity and early mortality. The prevalence of iron deficiency (ID) and the degree to which it contributes to anemia in children with SCA is unknown. The severity of anemia in children with SCA has been previously associated with increases in morbidity and mortality, emphasizing the importance of understanding the various etiologies of severe anemia, including concurrent ID, in this population. Novel use Of Hydroxyurea in an African Region with Malaria (NOHARM) is a Phase III randomized double-blinded placebo-controlled trial of hydroxyurea in children with SCA in Kampala, Uganda. Using plasma from 205 participants collected at enrollment (mean age 27.2 months; 53.7% male), we measured 5 biomarkers of iron status or inflammation by ELISA: ferritin, soluble transferrin receptor (sTfR), C-reactive protein (CRP), hepcidin, and erythropoietin (EPO), in addition to erythrocyte parameters including hemoglobin (Hb), mean corpuscular volume (MCV), and absolute reticulocyte count (ARC). ID was defined as ferritin <12 µg/L or ferritin <30 µg/L if CRP ≥10 mg/L. Median (IQR) values for ferritin, 170.8 µg/L (86.2-329.8), and CRP, 6.6 mg/L (2.5-15.6), were high, collectively reflecting high iron stores in the face of mild inflammation. Low median (IQR) hepcidin, 7.7 mg/L (1.2-20.9), along with high median (IQR) sTfR, 7.2 mg/L (5.4-9.0), is consistent with ongoing gut absorption to support active erythropoiesis. ID prevalence based on ferritin was low (n=7, 3.4%). A larger MCV (OR=1.05, p=0.18), increased EPO (OR=1.2, p=0.003), and increased CRP (OR=1.02, p=0.02) were significantly associated with severe anemia (Hb<7.0 g/dL). Our data reflect a complex picture of iron handling and erythropoiesis in young African children with SCA and demonstrate that inflammation and chronic hemolysis contribute to severe anemia in this population.

## 1792

### THE DEMOGRAPHY, CLINICAL CHARACTERISTICS AND DIAGNOSES OF ACUTE FEBRILE ILLNESS REQUIRING HOSPITALIZATION IN INDONESIA

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Fever is a common cause of hospitalization, particularly in developing countries. Identifying the etiologies of fever influences case management and the success of patient outcomes. Unfortunately, the limited diagnostic capabilities of developing countries leads to both undiagnosed and misdiagnosed illness, resulting in prolonged hospitalization, severe complications, and death. To understand Indonesia's capacity to manage patients presenting with fever, an observational study of acute febrile illness requiring hospitalization was conducted at 8 top-referral hospitals across the country from 2013 to 2016. This study followed subjects as they

received both standard-of-care diagnostic testing and advanced, study-specific diagnostic testing. Demographic and clinical data were collected at enrollment, and blood samples were collected at each study visit for testing. From 5,213 screened patients, 1,486 were enrolled and monitored during enrollment, once during the 14-28 days following enrollment, and at 3 months after enrollment. Based on clinical manifestations, study physicians categorized patient infections as systemic (61.1%), respiratory (19.7%), digestive (8.3%), genitourinary (4.7%), skin/soft-tissue (2.4%), and central nervous system (1.8%). Standard-of-care, molecular, and serological testing identified pathogens in 66% of cases, the majority being dengue virus (44.5%), *Salmonella spp.* (11.7%), *Leptospira spp.* (4.6%), influenza A&B (4.2%), and chikungunya virus (3.3%). Surprisingly, *Rickettsia spp.* were detected in 103 (10.7%) cases, all of which were clinically misdiagnosed. Co-infections were detected in 2.7% of identified cases, and patient mortality was 6.0% overall, mostly in subjects with underlying conditions. Discordance between lab results and clinical diagnoses were found in 325 (33.7%) cases, including cases of rickettsiosis (103), dengue (36) chikungunya (33), HHV-6 (33), leptospirosis (25), and typhoid (15). These findings inform national approaches to the diagnosis and treatment of acute febrile illness and highlight the need for greater diagnostic testing capacity.

## 1793

### WAIT, IS THIS AN ID BOARD QUESTION? CHRONIC HEPATITIS IN A BROADLY EXPOSED LIVER TRANSPLANT PATIENT

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A 32-year-old male with a history of hepatocellular carcinoma, glycogen storage disease, cirrhosis, and successful orthotic liver transplant 11 years ago (on mycophenolate, tacrolimus, and prednisone) was admitted for two weeks of fever and worsening transaminitis. Alanine aminotransferase and aspartate aminotransferase were elevated for the previous six months (173 and 241 U/L, respectively) despite adherence to immuno-suppressive medication. These values continued to up-trend to a maximum of 380 U/L on the day of admission. Previous biopsies did not reveal any evidence of organ rejection, but he was restarted on prednisone (20mg daily) four months ago. The patient reported no significant travel or arthropod bites, however, he had multiple animal exposures within the last few months. He worked as a butcher with occasional blood exposure, he previously worked in the seafood department, handling raw seafood, and at home, he is exposed to a rabbit, dogs, and several parakeets. On arrival to the hospital, the patient was febrile (103.3F), with a white blood cell count and platelet level somewhat decreased from baseline (3,300 and 138,000, respectively). Blood cultures, urinalysis, and chest x-ray were non-diagnostic. A liver biopsy revealed the presence of granulomas with intracellular yeast-like, budding fungal elements, consistent with histoplasmosis. Urine Histoplasmosis antigen also resulted positive (15.7 ng/ml). The patient was started on Ambisome and then transitioned to Itraconazole. He defervesced and had improvement of transaminitis. *Histoplasma capsulatum* is the most common endemic mycoses in the United States and a frequent opportunistic infection among the immuno-compromised. Granulomatous hepatitis has been associated as a cause of fevers of unknown origin in up to 13% of cases and has a broad etiology, including endemic fungal infections, Q fever, tularemia, tuberculosis, viruses, and leptospirosis, among others. Given the patient's exposures to parakeets, as well as increasing steroid dosage, this immune-compromised patient was placed at even higher risk for *Histoplasma* infection and disease progression.

## HOT OR NOT? MANAGEMENT OF UNCLASSIFIED FEVER IN CHILDREN IN SUB-SAHARAN AFRICA

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The World Health Organisation's (WHO) integrated Community Case Management (iCCM) strategy states that febrile children seen by community health workers (CHW) without a diagnosable cause of illness and without danger signs are advised to return to the CHW for a follow-up visit on day 3, regardless of symptom resolution. Yet, fever is common in children in sub-Saharan Africa and generally resolves by itself. The day 3 follow-up visit might therefore be unnecessary and there is limited evidence of whether caregivers of children complete their follow-up assessment visit promptly. The outcome of the febrile children from whom treatment was withheld is also unknown. The safety of a conditional three-day reassessment visit only in cases where symptoms did not resolve, was compared with a universal follow-up visit at day 3 for all children. A two-arm cluster-randomised controlled non-inferiority trial was conducted in Southwest Ethiopia with 25 health facility clusters. All 282 CHWs (in Ethiopia, Health Extension Workers - HEWs) in these clusters enrolled children 2-59 months with fever and no malaria, no pneumonia, no diarrhoea or danger signs. Caregivers of children received advice to come back on day 3 (control), or to only come back on day 3 if fever persisted (intervention), according to cluster assignment. The primary outcome was treatment failure by day 7. We did a per-protocol analysis with a non-inferiority margin of 4% for treatment failure rate using generalised linear models with binomial distribution and identity link using robust variance estimator, treating cluster as a random effect. From Dec 1, 2015, to Nov 30, 2016, 4626 children were enrolled. Of these, 1953 (87.7%) children in the universal follow-up group and 1993 (84.1%) children in the conditional follow-up group had a day 7 ( $\pm 1$  day) follow-up visit. The conditional follow-up advice was non-inferior to the universal follow-up advice in terms of treatment failure (-3.81% [95% CI  $-\infty$  to 0.65%]). There were no deaths recorded by day 28. Conditional follow-up of children with non-severe unclassified fever in a low malaria setting in Ethiopia was concluded to be safe in children 2-59 months.

## IMPACT OF FIVE YEARS OF CDTI ON ENTOMOLOGICAL TRANSMISSION INDICATORS OF ONCHOCERCIASIS BY SIMULIUM DAMNOSUM S.L. IN THE CASCADES REGION OF BURKINA FASO

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**Abstract :** Onchocerciasis or river blindness is a dermal filariasis due to the development in human subcutaneous tissue of a nematode called *Onchocerca volvulus*. This parasite is transmitted to humans through the bites of blackflies of the genus *Simulium*. Mass ivermectin administration to populations of endemic areas is the strategy recommended by WHO in the elimination of onchocerciasis. Since 2011, this strategy has been applied in the form of Community-directed treatment with ivermectin (CDTI) to the Cascades Region of Burkina Faso following a resurgence

of the disease in the aforesaid region. Evidence of the interruption of onchocerciasis transmission through CDTI campaigns has been demonstrated in the past. In this study, we evaluated the impact of 5 years of CDTI treatments on parasite loads and infestation rates of *Simulium damnosum s.l.* in order to evaluate the first phase of elimination of onchocerciasis in Burkina Faso. For this purpose, we collected blackflies in the breeding sites near the four villages which had the highest prevalences of the disease during the recrudescence. We carried out the first collection of blackflies at the beginning of CDTI treatments and a second collection after 5 successive years of annual treatments. Flies were dissected and any larvae found were identified molecularly. The infective larvae of 2012 have been identified and the identification of the 2016 infective larvae is ongoing. Finally, infective rates of flies and the annual potential of transmission for each village will be calculated at the completion of the molecular analysis. Comparison of the entomological indicators of onchocerciasis transmission from 2012 to 2016 will allow us to better understand the dynamics of the infectivity of blackflies as a function of CDTI treatments.

## ONCHOCERCIASIS IN CAMEROON: A SYSTEMATIC REVIEW OF HISTORY AND IMPACT OF CONTROL INTERVENTIONS

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Onchocerciasis or river blindness remains a major public health problem, especially in Africa where more than 99% of infected population reside. This parasitic disease is responsible of severe skin disease with unsightly lesions and intense itching, the main complication being severe eye disease that can lead to blindness. Also, the huge burden of river blindness has a great impact on socio-economic development of infected population. Many control methods had been implemented to tackle river blindness and great successes had been achieved, so that the paradigm has shifted from control to complete elimination. However, high discrepancies have been observed in prevalence and intensities of the disease, and different scenarios to explain this persistence of infection have been evoked. The mandate of the African Programme for Onchocerciasis Control (APOC) ended in 2015 and endemic countries need to move toward elimination by themselves. In Cameroon, an elimination committee is being putting in place and data on the situation of onchocerciasis are highly needed. In this context, the present study aimed at systematically review available data on prevalence of onchocerciasis and different control interventions in order to provide pertinent information that will be useful to defeat onchocerciasis in Cameroon. To this end, all published and unpublished data were gathered from different sources, and inspected for inclusion in the study. Environmental (climate, season, vegetation ...), and socio-demographic factors have also been collected. A countrywide map was generated to provide the dynamics of river blindness prevalence according to MDA status, as well as environmental and socio-demographic factors to guide the decision of the Cameroon elimination committee.

## LYMPHATIC FILARIASIS IN MAINLAND SOUTHEAST ASIA: A SYSTEMATIC REVIEW AND META-ANALYSIS OF PREVALENCE AND DISEASE BURDEN

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Accurate prevalence data is essential for the elimination of lymphatic filariasis as a public health problem. Despite bearing one of the highest burdens of disease globally, there remains limited reliable information on the current epidemiology of filariasis in mainland South-East Asia. We conducted a systematic review and meta-analysis of available literature to assess the current prevalence of infection and morbidity in the region.

56 journal articles and reports containing original prevalence data were identified including over 512 010 participants. Data were summarised using percentage prevalence estimates and a subset compared using a random effects meta-analysis by country and year. Pooled estimates for microfilaraemia, immunochromatographic card positivity and combined morbidity were 2.64%, 4.48% and 1.34% respectively. Taking into account pooled country estimates, grey literature and the quality of available data, we conclude that North-East India, Lao PDR and Myanmar demonstrate ongoing evidence LF transmission that will require multiple further rounds of MDA. Bangladesh, Malaysia, Thailand and Vietnam appear close to eliminated LF, whilst Cambodia has already achieved elimination status. We estimated that the burden of morbidity is likely high in Bangladesh and Northeast India, moderate in Cambodia, Myanmar and Thailand and low in Vietnam. There was insufficient evidence to accurately estimate the disease burden in Lao PDR or Malaysia. The results of this study will assist policy makers to advocate and budget for future control programs, and highlights the significant need for further filariasis prevalence studies in the region.

## 1798

### LYMPHATIC FILARIASIS SERO PREVALENCE IN MOMBASA COUNTY

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**Background:** Lymphatic Filariasis (LF) is a neglected tropical disease targeted for elimination in Kenya. In Kenya's coastal region, LF affects about 3.5 million people. We aimed to determine the sero-prevalence and distribution of LF in Mombasa County. **Methods:** This was a cross-sectional survey involving multistage sampling in which one village was randomly selected from each of the six sub-counties. In each village, a minimum of 300 individuals were randomly selected from 233 randomly selected households (HHs). Persons aged  $\geq 3$  years, a resident of the county between January and December 2016 was included in the study. Persons on anti-filarial chemotherapy were excluded. We collected blood samples from participants and collected demographic information using questionnaires. We used Filarial test strip (sensitivity = 98% and specificity = 99%) for detecting circulating LF antigens in blood. We analyzed data using Microsoft Excel and Epi-Info, calculated proportions for and used chi-square ( $\chi^2$ ) to compare sero-prevalence with demographic characteristics. **Results:** A total of 1,826 individuals from 233 HHs were sampled. The overall sero-prevalence was 1.1% (95% CI: 0.7-1.7). There were 14 (6%) HHs with at-least a positive case. The sero-prevalence in males was 1.4% [(1/1772); (95% CI: 0.8-2.5)] compared to females 0.9% [(9/1056); (95% CI: 0.4-1.6)]; [ $\chi^2 = 0.87$ ; p-value=0.352]. The mean age of the sero-positive individuals was  $17.4 \pm 9.8$  years compared to sero-negative which was  $21.8 \pm 15.6$  years (t Stat=1.28; p-value=0.201). All positive cases were from Jomvu sub-county where 14 (35%) of 40 HHs had at-least one positive case. Clustering was seen in 4 (29%) of 14 HHs; 2 HHs had 3 cases each while 2 HHs had 2 cases each. **Conclusion:** Sero-prevalence estimates show presence of LF in Jomvu Sub-County. We recommend intensification of anti-filarial chemotherapy in Jomvu. The findings provide baseline information for future epidemiologic investigations and response to LF in Mombasa County.

## 1799

### EFFECT OF A SINGLE DOSE OF IVERMECTIN ON LOA LOA MICROFILAREMIA 18 MONTHS AFTER TREATMENT

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Implementation of ivermectin-based community treatment for onchocerciasis or lymphatic filariasis elimination has been delayed in Central Africa because of the severe adverse events (SAEs), including death, in people with high levels of circulating *Loa loa* microfilariae (mf). Between August and October 2015, *L. loa* microfilaremia was measured in 16,205 individuals living in an area of central Cameroon that is also co-endemic for onchocerciasis. This testing was performed as part of a "Test and (not) Treat" (TNT) with ivermectin strategy for onchocerciasis elimination based on excluding from ivermectin treatment those with  $>20,000$  mf/ml deemed at-risk for SAEs. Among those tested in 2015, 342 (2.1%) did not receive ivermectin because of an excessive *L. loa* density, whereas 15,469 persons were safely treated with ivermectin. A second TNT campaign is currently being conducted 18 months after the first TNT campaign in the same population. Interim results based on the  $>9000$  individuals that have already been tested so far show that none of the individuals treated with ivermectin in 2015 (those with  $<20,000$ /ml) have *L. loa* mf levels that would preclude safe ivermectin treatment in 2017. Interestingly, mf densities of 21 individuals that were unable to receive ivermectin in 2015 had mf levels that had decreased below the 'at-risk' threshold and were therefore treated with ivermectin in 2017. Finally, mf densities of 24 individuals that could not receive ivermectin in 2015 were still above the threshold in 2017. Results for the entire district of  $>16,000$  people will be available in May, 2017. These interim results suggest that, in onchocerciasis/loiasis co-endemic areas, pretreatment testing for *L. loa* microfilaremia is necessary only before the very first treatment with ivermectin and that ivermectin can be safely administered to all individuals treated within the previous 18 months.

## 1800

### MODELLING ALTERNATIVE STRATEGIES FOR ONCHOCERCIASIS ELIMINATION: THE CASE FOR MOXIDECTIN

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African onchocerciasis control and elimination programmes rely predominantly on annual mass drug administration of ivermectin. Phase II and III clinical trials have indicated that moxidectin, a registered veterinary anthelmintic, is a more efficacious treatment. Both drugs, given as a single oral dose, suppress skin microfilarial (mf) loads, but ivermectin does this for a shorter period and with greater variation among individual responses than moxidectin. The influence of the duration of mf suppression and the distribution of individual pharmacodynamic (PD) responses on the projected impact of community-directed treatment with ivermectin (CDTI)

or moxidectin (CDTM) has not been investigated. Using phase II and III clinical trial data, and our individual-based analogue of the EPIONCHO transmission model (EPIONCHO-IBM), we capture inter-individual PD variation for standard dose ivermectin and ascending (2mg (n=45), 4mg (n=44), 8mg (n=1,016)) doses of moxidectin. We model interventions based on annual and biannual CDTI and CDTM, comparing how long it takes to eliminate onchocerciasis under these different strategies with and without variation in PD responses. Annual CDTM is similar to biannual CDTI (in the absence of PD variation) but biannual CDTM always eliminates onchocerciasis more rapidly. Persistent infection and transmission with multiple treatment rounds of CDTI may be due to inter-individual and inter-treatment round variability in PD responses. Inter-individual variation in responses is substantially lower for moxidectin, and with biannual CDTM there would be minimal opportunity for inter-treatment transmission to vectors, which contributes to its potential for accelerating elimination. The cost-effectiveness implications of the various strategies modelled will be discussed.

## 1801

### ARE WE ON THE RIGHT TRACK? STOPPING CRITERIA FOR ENDING SOIL-TRANSMITTED HELMINTHS RANDOMIZED CLINICAL TRIALS

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Throughout the last decade, the World Health Organisation's (WHO) efforts with regards to soil-transmitted helminths (STH) have been focused on morbidity control in children. However, there now exists a shift towards elimination beyond 2020. Models indicate that with a high coverage level and good compliance, elimination may be achievable with mass-drug administration (MDA) alone; however, this has not been verified. Trials to investigate the possibility of elimination are ongoing (e.g. the TUMIKIA and DeWorm3 studies). Achieving high coverage levels may be difficult in areas with high migration or low compliance in the study population. If target coverage is not achieved or prevalence is not coming down, it may be better to stop an ongoing trial and to investigate the reasons. In this work, we investigate the stopping criteria which would be necessary to make this decision. We adopt a stochastic simulation model to simulate the DeWorm3 randomized trial. In short, this trial compares two arms, including a control arm (treating school-aged children with MDA once a year) and an intervention arm (biannual treatment of the whole community with MDA). The DeWorm3 trial lasts for three years and the sites are closely monitored for the following two years. At the end of year five, a cross-sectional survey is performed to investigate if the prevalence thresholds for interruption of transmission (<2%) have been achieved. As the whole process is costly, it may be more efficient to stop the trial when interruption of transmission can be ruled out, to determine why it was not achieved in order to inform future trials. In this study, we investigate if stopping criteria can be defined *in situ* where interruption of transmission is unlikely, resulting in higher prevalence levels than expected during the study period. This may occur when high coverage levels needed to reach this goal are not achieved or when immigration results in re-infection of the study-site. We investigate different time points and the effects of varying prevalence thresholds that can determine the stopping criteria.

## 1802

### ASSESSMENT OF TWO DENSITOMETRIC READERS TO MEASURE RESULTS OF FILARIASIS TEST STRIPS IN THE DEMOCRATIC REPUBLIC OF CONGO

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The Alere Filariasis Test Strip (FTS) is a point-of-care diagnostic tool that detects *Wuchereria bancrofti* circulating filarial antigen (CFA) in blood. The Global Program to Eliminate Lymphatic Filariasis (GPELF) employs the FTS for mapping filariasis-endemic areas and assessing the success of elimination efforts. Recently, we demonstrated that the quantitative reading of FTS results (qFTS) using a densitometric reader (FD5, Konica-Minolta) provides additional information because the intensity of the test-line is correlated with CFA titers. In June 2016, we conducted a study in the Kwilu Province (DRC) to compare the performance of the FD5 reader with a new an Android-based ratiometric image analysis software application (mReader) operating on a low-cost Amazon Fire (2015 Tablet), which is more adapted for the field than the FD5. We tested 586 individuals and assessed the results by visual reading (vFTS), qFTS, and reading with mReader (mFTS). 152 (25.9%) individuals were positive by vFTS. Both qFTS and mFTS results were correlated with the vFTS scores (Spearman's coefficients 0.72 and 0.72 respectively, for all the subjects, and 0.82 and 0.78 respectively for only the positive vFTS), and were correlated between themselves ( $p = 0.55$  and  $0.78$  respectively, for total population and positive vFTS). The quantitative ROC analysis showed that the areas under the curves (AUC) were similar for qFTS ratios and mFTS values (96.5 and 96.4, respectively;  $P = 0.880$ ). Following the sensitivity analysis, we defined cut-offs of 0.46 and 16.5 for the qFTS and mFTS, respectively. For the qualitative performances, AUC were 92.3 and 91.2 for qFTS and mFTS, respectively ( $P = 0.396$ ). In conclusion, the new tablet-based ratiometric image analysis application tool, mReader has similar performance to qFTS, and we hypothesize that results are similarly correlated with CFA titers. Although minor problems with misclassification remain, our preliminary testing demonstrates that it is possible to extract a quantitative indicator from the FTS. Such an indicator could provide the GPELF with greater information on which to base stopping MDA and surveillance decisions.

## 1803

### EMPIRIC TESTING OF A MODEL TO IDENTIFY DISTRICTS ELIGIBLE FOR SAFE IVERMECTIN-BASED MASS TREATMENTS FOLLOWING INTEGRATED MAPPING FOR ONCHOCERCIASIS, LYMPHATIC FILARIASIS AND LOIASIS

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Onchocerciasis (Oncho) and lymphatic filariasis (LF) are among neglected tropical diseases (NTDs) targeted for elimination by preventive chemotherapy (PC-NTD). As a consequence of post-ivermectin (IVM) SAEs

occurring in individuals harboring very high *Loa* microfilarial (mf) densities, this drug is not recommended in areas hypo-endemic for *Oncho* or endemic for LF, that are co-endemic for loiasis. However, to achieve global elimination of *Oncho* and LF, PCs are also needed in areas where loiasis is endemic, regardless the infection rate of the targeted filarial disease. Recent MDA campaign using the Test and Not Treat strategy in Cameroon has demonstrated that *Loa* mf load of 20,000 mf/ml is a safe threshold for MDA. From existing data, we hypothesized using a mathematical model that IVM-based PC can be safely implemented in community with probability to have < 1% of *Loa* mf load >20 000mf/mL is above 95%. To test this model and define a cost effective and accurate method to map IVM-naïve health districts (HD) for *Oncho*, LF and loiasis, and therefore identify HD eligible for safe IVM-based MDA, two community-based cross sectional surveys were carried out in the Garoua Boulai HD (Cameroon) where no IVM MDA was ever performed. (1) a cluster survey using the probability proportionate to estimated size strategy, and (2) a systematic random survey in communities that pass mathematical model predictions. To do this, finger-prick blood samples were collected for loiasis (LoaScope), *Oncho* (IgG4 antibody to human Ov16 antigen) and LF (filaria test strip, FTS). The prevalence of loiasis was 8.0% (95% CI: 7.2 - 9.0), with high heterogeneity between clusters (Min: 0.0%; Max: 43.5%). *Oncho* was found to be hypo-endemic (Ov16 prevalence: 4.9%; 95% CI: 4.2 - 5.7), and LF infection rate was quite low (prevalence: 0.4%; 95% CI: 0.2 - 0.7). The likelihood to find >1% of individuals harboring *Loa* mf densities > 20,000 mf/mL in a cluster seems to be positively associated with the prevalence of loiasis. Systematic sampling allow to validate data from cluster survey, both for loiasis prevalence and percentage of individuals harboring more than 20,000 mf/mL.

## 1804

### STOPPING IVERMECTIN DISTRIBUTION IN ONCHOCERCIASIS AND LYMPHATIC FILARIASIS CO-ENDEMIC FOCI. WHAT IS THE WAY FORWARD?

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In Tanzania, in communities where onchocerciasis (OV) is co-endemic with lymphatic filariasis (LF), treatment programs have been achieving high mass drug administration (MDA) coverage rates and reduction of infection levels. Tukuyu is one of the co-endemic foci, where over 800,000 inhabitants, spread across four districts, have received 15 rounds of annual ivermectin and albendazole treatments. In 2015, all districts achieved the criteria for stopping LF MDA, but OV transmission status was not known so treatment has continued. At mapping/baseline, clinical manifestations included skin lesions and nodule prevalence was up to 70%. After initiation of MDA four focus-wide assessments of human onchocerciasis infection have been conducted. First, an APOC phase 1a epidemiological assessment conducted in 2012 after 12 effective treatment rounds, in 10 villages, indicated 0% (n=1861) microfilaridemia among the general population. Second, an APOC phase 1b epidemiological assessment in 2013 in 20 villages indicated again microfilaridemia level of 0% (n=3146) in the general. An entomology assessment was not done at the time. Third, an independent assessment (n=948) in 2015, carried out skin exam, skin snips for microfilaria count (≥5 years-old only) and skin snip PCR, and blood draw for the rapid OV16 antibody test (RDT). OV16 ELISA and daytime blood smears reported overall prevalence 0.9% among children 0-10 years of age and 5.5% overall on OV16 RDT. Finally, in December 2016, an epidemiological evaluation using OV16 RDT and OV16 ELISA among children below 10 years of age, (mean age 7.5 years), reported

1 child positive for OV16 antibodies 0.03% (n=3198). An entomological study was also conducted in 2015 where O-150 PCR was carried in in 8000+ *simulium spp* flies collected from all active breeding sites across the focus area. The PCR results reported zero infected black flies. It would appear to that the Tukuyu focus has interrupted onchocerciasis transmission. However, since the Tanzania program aims at elimination, in line with the new WHO guidelines, (despite zero infective vectors and one positive child) has transmission really been interrupted?

## 1805

### RESPONSES OF *ONCHOCERCA VOLVULUS* AFTER THE INTRODUCTION OF BIENNIAL TREATMENT WITH IVERMECTIN IN GHANA

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Despite many years of onchocerciasis control in Ghana, the disease remains persistent in pockets of endemic areas. Due to reports of sub-optimal responses in parasite population, treatment strategy was changed from annual to biennial in 2010. This study assessed the impact of the first three years of biennial strategy on the microfilarial loads in hosts' skin and the embryostatic effect on the adult female worm. A community-wide skin snipping was performed on 956 consenting adults aged ≥20 years from 10 sentinel communities. A cohort of 217 participants who were microfilaria (mf) positive and/or had palpable nodules at baseline were followed up over the first two rounds of biennial treatment to estimate the rates of microfilarial repopulation. Nodules were performed on consenting participants and adult worms were isolated using the collagenase technique. Embryogram analyses were performed on intact female worms and classified into three response groups (good, intermediate and poor). The baseline mf prevalence was 22.7% (1-45%). The biennial treatment substantially reduced infection intensity in most communities, although infections were detected in all communities even after 4 or 5 rounds of treatment. The 6-month repopulation rates were generally higher than 10% in all the communities. Three communities previously identified as sub-optimal to ivermectin were observed to have statistically significantly high microfilarial repopulation rates. A total of 225 nodules were excised from 106 participants with an average of 1.4 female worms and 0.7 male worms per nodule. Twenty eight percent of the adult female worms isolated were observed with normal intra-uterine mf whiles 72% were observed with either degenerated or no stretched mf three months after treatment. In all 10 communities, there were female worms observed with normal intra-uterine mf three months after treatment. Constant monitoring of parasite response to ivermectin treatment is necessary to ascertain any resistance developing in parasite population. A study is ongoing to identify any genetic changes that may be associated with these response phenotypes.

## 1806

**ALL FOR ONE, ONE FOR ALL: ACROSS BORDER LYMPHATIC FILARIASIS TRANSMISSION CAN COMPROMISE NATIONAL ELIMINATION PROGRAMS IN SOME SETTINGS**Joaquin M. Prada<sup>1</sup>, Lisa J. Reimer<sup>2</sup>, Deirdre Hollingsworth<sup>1</sup><sup>1</sup>University of Warwick, Coventry, United Kingdom, <sup>2</sup>Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Lymphatic filariasis (LF) is one of the neglected tropical diseases (NTD) with ambitious global targets for elimination as a public health problem. Most LF elimination programs involve multiple rounds of mass drug administration (MDA) and a post campaign evaluation at some defined sentinel sites. Since progress in each country occurs independently, we explored the across border transmission between neighbouring countries where one of them is further in their elimination program. However, the extent and rate of spatial spread of LF transmission across a landscape of variable suitability for LF is poorly understood. Using a well-described mathematical model of transmission publicly available, we investigated under what circumstances can foreign countries reduce or compromise, at least locally, elimination of LF in some regions, which could give rise to hotspots of transmission. This work helps better understand border effects and illustrates that combined efforts for LF elimination could potentially be more effective.

## 1807

**LESSONS LEARNED FROM IMPLEMENTING LYMPHATIC FILARIASIS TRANSMISSION ASSESSMENT SURVEYS IN THE FIRE BELT OF NORTH DEPARTMENT IN HAITI**Carl Renand Fayette<sup>1</sup>, Franck Monestime<sup>1</sup>, Alain Javel<sup>1</sup>, Luula Mariano<sup>1</sup>, Ellen Knowles<sup>2</sup>, Sarah Craciunoiu<sup>2</sup>, Cudjoe Bennett<sup>2</sup>, Abdel Direny<sup>3</sup>, Jean-Frantz Lemoine<sup>4</sup><sup>1</sup>IMA World Health, Port Au Prince, Haiti, <sup>2</sup>IMA World Health, Washington, DC, United States, <sup>3</sup>RTI International, Washington, DC, United States, <sup>4</sup>Ministry of Public Health and Population, Port Au Prince, Haiti

To achieve global goals to eliminate lymphatic filariasis (LF) by 2020, the Haiti NTD Control Program follows WHO's recommendations to implement 5 rounds of annual mass drug administration (MDA) of diethylcarbamazine (DEC) and albendazole among at risk populations to halt LF transmission. MDA is followed by a transmission assessment survey (TAS) to determine if the commune has met the criteria for stopping MDA (<2% antigenemia). Efforts to halt transmission have been successful in two-thirds of the country. However, prevalence remains high in five contiguous communes in the North department (Quartier Morin, Milot, Acul-du-Nord, Limonade, and Plaine du Nord) that had prevalence between 28-45% at baseline. By 2014, they received five rounds of MDA before conducting pre-TAS through use of immunochromatographic card test (ICT). Three communes failed: a total sample size of 1,513 were tested across Quartier Morin, Milot and Acul-du-Nord, of which prevalence was 4.2%, 3.35%, and 2.35% respectively. The communes were subsequently given two additional rounds of MDA before another pre-TAS was conducted in 2016 using a different test: filariasis test strip (FTS). The three communes failed again with an increase in positive cases at 9.2%, 11%, and 2.35%, respectively. The remaining two communes barely passed, so were recommended to implement TAS using FTS in 2016, which subsequently failed. A pattern within the five communes has emerged: the *ceinture de feu filarirose*, or LF Fire Belt appears to fail or succeed together. Sustained high prevalence, despite high MDA coverage, may require alternative strategies. A study in Quartier Morin is using a triple drug combination (adding ivermectin) to test the safety and acceptability of the drug combination and its effects on LF prevalence. If successful, it represents a new approach that could be used in communes with high baseline prevalence. Additional combined interventions, such as intensified

vector control, increased communication, door-to-door strategies during MDA and advocacy with local leaders should also be applied across all 5 communes, instead of individual ones, in order to stop transmission.

## 1808

**THE ROAD MAP TO LF ELIMINATION IN TANZANIA - THE CHALLENGING END GAME**Upendo Mwingira<sup>1</sup>, Andreas Nshala<sup>2</sup>, Maria Chikawe<sup>1</sup>, Lousie Kelly Hope<sup>3</sup>, Charles Mackenzie<sup>4</sup>, Sarah Craciunoiu<sup>5</sup>, Delali Bonuedi<sup>6</sup>, Mwele Malecela<sup>7</sup><sup>1</sup>Tanzania NTD Control Program; National Institute for Medical Research, Dar es Salaam, United Republic of Tanzania, <sup>2</sup>Tanzania NTD Control Program; IMA World Health, Dar es Salaam, United Republic of Tanzania, <sup>3</sup>Liverpool School of Tropical Medicine, Liverpool, United Kingdom, <sup>4</sup>Michigan State University, East Lansing, MI, United States, <sup>5</sup>IMA World Health, Washington, DC, United States, <sup>6</sup>RTI International, Washington, DC, United States, <sup>7</sup>National Institute for Medical Research, Dar es Salaam, United Republic of Tanzania

Lymphatic filariasis (LF), caused by *Wuchereria bancrofti*, is a leading cause of morbidity in Tanzania. The Tanzania NTD Control Program (TZNTDCP) has a target to eliminate LF by 2020. When treatment started, the entire country was considered endemic. Elimination efforts by the TZNTDCP have largely targeted interruption of transmission through mass drug administration (MDA) and alleviation of morbidity for those already affected. Since 2001, ivermectin and albendazole have been distributed in affected communities as funding has allowed, with increasing geographical coverage reaching all endemic communities by 2015. In 2016, 41 million treatments were distributed to 20.6 million people at an epidemiological coverage rate of 74% (where the minimum coverage rate is 65%). Recently, programmatic and independent disease monitoring have recorded reduction in prevalence levels of LF. The reduction has resulted in scaling down of MDA as more and more communities now live in transmission free zones. Re-mapping assessments in 2015 in 65 districts, indicated prevalence levels of below 1% circulating filarial antigen thus not warranting MDA. This brought the LF endemic district count down to 121 (out of 186). In 2017, via transmission assessment surveys (TAS), 74 districts recorded circulating filarial antigen levels below 1% among children 6-7 years age, thus meeting the criteria for stopping LF MDA. It is the remaining 47 districts which are still conducting MDA that pose a challenge in reaching elimination targets. Most of these districts are along the Indian Ocean coast where the vector is abundant and mapping/baseline circulating filarial antigen (CFA) levels ranged from 7.5% to 74%. Routine disease monitoring in these districts has indicated declining trends of CFA levels, but not to a level where MDA can be stopped. For instance in Mafia district, LF prevalence levels have decreased from 49% at mapping/baseline to 4% after 11 treatment rounds. Additional treatment rounds as well as alternative strategies might be required to achieve the 2020 elimination targets in Tanzania.

## 1809

**EPIDEMIOLOGY OF FILARIASIS IN ZAIRE PROVINCE, ANGOLA**Célio C. Njinga<sup>1</sup>, Filipa Vaz<sup>1</sup>, Rossely C. Paulo<sup>2</sup>, Pedro Van Dunem<sup>3</sup>, Miguel Brito<sup>4</sup><sup>1</sup>CISA, Luanda, Angola, <sup>2</sup>Liverpool School of Tropical Medicine/CISA, Luanda and Liverpool, Angola, <sup>3</sup>National Coordinator at Public Health Department, Luanda, Angola, <sup>4</sup>Lisbon School of Health Technology, Lisbon, Portugal

Filariasis are known to be endemic in Angola and contributes to outpatient morbidity and mortality of the country with over 12 million people at risk of infection. As so, little is known about the geographical distribution and co-endemicity of three filarial parasites such as *Onchocerca volvulus*, *Wuchereria bancrofti*, and *Loa loa*. Hence, it is important to understand the distribution in co-endemic areas with multiple species of Filariasis with the assess of the prevalence of lymphatic filariasis (LF), onchocerciasis

and loiasis. The 6 municipalities of Zaire were surveyed and 7 communes were selected based on the geographical location-river proximity and swampy areas of plantation or forest. The random sampled respondents were interviewed using the Rapid assessment procedure (RAPLOA) for *Loa loa* and the Rapid Epidemiological Mapping of onchocerciasis (REMO) surveys to assess LF clinical signs (hydrocele and/or Lymphedema). A total of 157 respondents took part in the survey. The nested Polymerase Chain Reaction (PCR), followed by electrophoresis and visualization with staining with ethidium bromide was performed to detect *Loa loa* infection. For *Wuchereria bancrofti* (LF), Real-Time PCR with specific Taqman probes, Dried Blood Spot (DBS) samples was used. In addition, for each subject an onchocerciasis rapid test and dried blood spot (DBS) was performed between 8 a.m. - 3 p.m. The results of molecular testing were combined with conventional epidemiological approaches to determine the spatial-temporal distribution of loiasis and population-level risk factors for reported diseases. Moreover, the nested PCR assay showed 0% prevalence for loiasis. Still, the prevalence for LF is also found to be 0%, according to the Real-Time PCR. Conversely, with the rapid tests for onchocerciasis infection, it was found that males have higher prevalence (6.3%) compared to females (3.8%). The molecular biology did not find co-endemicity but it provided insights for the importance for innovative approaches and alternative strategies for filarial infections required for the elimination targets established by the World Health Organization.

## 1810

### THERAPEUTIC POTENTIAL OF *WITHANIA SOMNIFERA* IN FILARIAL INDUCED SECONDARY LYMPHEDEMA

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Lymphatic filariasis is a major parasitic disease caused by nematodes *Brugia malayi* and *Wuchereria bancrofti*. Our present study aimed at evaluating the bacterial killing, free radical scavenging and cytokine inhibiting potential of thymol derived from *Trachyspermum ammi*. Thymol shows a clear zone of inhibition against *Bacillus cereus* and *Staphylococcus epidermidis* associated with filarial lymphedema. Further, thymol scavenges the DPPH and Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) free radicals at a dose dependent manner, indicating its inherent anti-oxidant property. In-vitro lymphangiogenic activity of thymol was evaluated by 2D matrigel and Nitric Oxide (NO) production in human endothelial cells in response to TNF- $\alpha$  (10ng/ml). These studies showed that thymol favours the formation of the tubular network and interestingly the NO production is elevated with increasing concentrations of thymol. Bacterial Infection Assay and Viable cell count analysis were carried out to evaluate the internalization of bacteria into the host cells and the viability of host cells after infection respectively. RT-PCR analysis and Confocal Imaging studies are under progress.

## 1811

### BARRIERS TO CONTROL AND ELIMINATE LYMPHATIC FILARIASIS IN ZANZIBAR: TACKLING THE REALITY OF THE MASS DRUG ADMINISTRATION PROGRAM

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In 2001, Zanzibar started the mass drug administration (MDA) programme as part of Global Programme to Eliminate Lymphatic Filariasis (LF). The programme stopped MDA in 2006 after showing a significant reduction in prevalence, however, in 2012 the Transmission Assessment Survey

(TAS) highlighted areas of ongoing transmission. This resulted in a further two MDA rounds, which recorded low coverage rates in some areas that geographically overlapped with areas of ongoing transmission. The island of Unguja had low transmission/high MDA coverage, whereas Pemba Island had high transmission/low MDA coverage. This study aimed to identify the barriers in achieving LF elimination by comparing the contrasting areas between islands; specifically, i) health care workers' knowledge, attitudes, practices and challenges during MDA, ii) community members' perceptions of LF and MDA participation. Cross-sectional surveys, interviews and focus group discussions (FGD) were conducted. The community drug distributor (CDD) surveys indicated that participants in Pemba were less satisfied with the training and MDA campaigns. CDDs returning to partially empty households was significantly less frequent in Pemba ( $p=0.053$ ), and was correlated to the number of years working on MDA campaigns. The FGD highlighted inadequate training and poor salaries as challenges for CDDs. Community member surveys found that reasons for non-compliance included lack of information and poor awareness. Community members also reported that direct observation of drug intake was significantly lower in Pemba ( $p=0.004$ ), which correlated with what the CDDs reported ( $p=0.001$ ). Factors related to low coverage and low compliance geographically coincided in northern Pemba where evidence of ongoing transmission was found during TAS. In order for the LF programme to move forward and achieve elimination, selected problem areas need to be provided with specific CDD training on MDA 'best practice', increasing motivation and monitoring drug consumption, together with improved community awareness of the disease and benefits of the medication.

## 1812

### COMPARISON OF THE *IN VITRO* SUSCEPTIBILITY TO EMODEPSIDE OF MICROFILARIAE, THIRD STAGE LARVAE AND ADULT WORMS OF RELATED FILARIAL NEMATODES

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Current efforts to eliminate onchocerciasis are hampered by the lack of a drug that can be used for mass drug administration and either has macrofilaricidal efficacy or leads to a permanent sterilization of female adult worms. Emodepside has been shown to have a broad spectrum efficacy against gastrointestinal nematodes and is approved for use in veterinary dewormers for cats and dogs. Emodepside is under evaluation as an adulticide treatment of human onchocerciasis by a joint collaboration with academia, DNDi and Bayer. Therefore, the *in vitro* potency of emodepside against a range of filarial life-cycle stages and filarial species was compared. In general, emodepside was capable of reducing the motility of adult filariae, third-stage larvae and/or microfilariae of *Onchocerca gutturosa*, *Onchocerca lienalis*, *Brugia pahangi*, *Litomosoides sigmodontis*, *Dirofilaria immitis*, and *Acanthocheilonema viteae* in a concentration-dependent manner. Emodepside had a broad *in vitro* activity against the microfilarial stage of related filarial species, although differences in the susceptibility were detected (*A. viteae* > *D. immitis* > *L. sigmodontis* > *O. lienalis*). Similarly, infectious L3 larvae of *D. immitis* had an increased emodepside susceptibility compared to *L. sigmodontis*. With regard to adult worms, emodepside failed to completely inhibit motility of *B. pahangi* adults at concentrations up to 12.5  $\mu$ M, whereas *L. sigmodontis* adult female worms and *O. gutturosa* adult male worms were completely paralyzed by low to sub  $\mu$ M emodepside concentrations. Those results demonstrate that emodepside has a broad range of activity against the microfilarial, L3 and adult worm stage of related filarial species. Based on these findings, emodepside is validated as promising candidate for treatment of human onchocerciasis.

## INVESTIGATION INTO THE EFFECT OF HOST MIGRATION ON THE TRANSMISSION OF *ONCHOCERCA VOLVULUS* USING A PATCH MODEL

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Despite the elimination of onchocerciasis in some regions, infection remains endemic in many African countries despite decades of concerted control efforts such as large-scale mass drug administration (MDA) of ivermectin. Furthermore, there is documented evidence of *Onchocerca volvulus* recrudescence in populations of Burkina Faso; ongoing transmission in Senegalese foci in which the infection was deemed to have been eliminated, and rapid reappearance of skin microfilariae following ivermectin treatment in Ghana. Resurgence could be due to (and difficult to distinguish from): i) premature cessation of control efforts (affected by the duration and/or coverage of MDA) particularly in areas with high vector biting rates; ii) re-introduction of infection from neighbouring foci (via vectors or infected persons), or iii) possible emergence of sub-optimal ivermectin responses. Two mathematical models for onchocerciasis transmission and control (EPIONCHO and ONCHOSIM) have been used extensively to evaluate the feasibility of eliminating onchocerciasis under a variety of scenarios, but both models consider transmission within single, isolated populations. It is however clear from geostatistical modelling that infection prevalence (and intensity) is unevenly distributed and that transmission should be considered as a spatial mosaic of infection "patches". By extending the population-based version of EPIONCHO into a spatial model framework, we investigate the effect of parasite migration (via vectors or humans) on *O. volvulus* transmission between neighbouring patches or regions. Results from different scenarios will be discussed based on varying the coupling strength (rate of migration) between two regions with different levels of endemicity (hypo-, meso-, or hyperendemic). Our work provides further insight into the underlying driving factors that may contribute to the sustained transmission of *O. volvulus* in areas where ivermectin MDA has been unsuccessful, particularly regarding decisions on cessation of treatment where elimination may have been achieved on one "patch" but persists in a neighbouring "patch".

## FOLLOWING 11 ROUNDS OF IVERMECTIN DISTRIBUTION, HOW CLOSE IS INTERRUPTION OF ONCHOCERCIASIS TRANSMISSION IN THE TUNDURU FOCUS IN TANZANIA?

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In Tanzania, over 6 million people are at risk of infection with onchocerciasis (OV), a parasitic filarial infection caused by *Onchocerca*

*volvulus*. The disease is spread by black flies, in poor rural communities, that breed in fast flowing rivers and streams. Control efforts, focusing on annual ivermectin preventive chemotherapy started in the 1990s. The Tunduru OV transmission focus, comprised of 1 district with 330,942 people, has received 11 rounds of ivermectin plus albendazole treatment due to co-endemicity with lymphatic filariasis (LF). In 2015, LF transmission was declared interrupted but ivermectin treatments for OV continued with sufficient coverage. A cross-sectional assessment of the prevalence of antibodies to OV16 protein of *Onchocerca volvulus* was conducted in December 2016 using OV16 RDT. The Tanzania Neglected Tropical Disease Control Program (TZNTDCP) purposively sampled 10 villages representative of the hypo-, meso- and hyperendemic transmission zones. In each of the selected villages, systematic sampling was used to select households where all consenting residents, over 5 years were tested for antibodies to the OV16 protein using the OV16 rapid diagnostic tests. A total of 2,732 individuals were tested (560 below 10 years of age) and only 11 (0.4%) had positive results. All positive individuals were above 15 years old. Reactivity rates increased with age. At 50 years and over, OV16 antibody prevalence was at 1.53% (95CI 0.6-2.53). Results from this assessment provide a clear signal that new transmission could have been halted. These findings are in line with the results from the APOC phase 1a epidemiological evaluation carried out in 2013 after seven rounds of treatment that indicated 0% *Onchocerca volvulus* microfilaridemia in the general population aged 5 years and above. With no phase 1b or entomological study done in the past, this rapid assessment provided useful insight. A full epidemiological and entomological evaluation is now warranted to evaluate interruption of transmission in line with WHO guidelines.

## DEMONSTRATED CAPACITY BUILDING OF LOCAL SURGEONS TO PERFORM HYDROCELECTOMY IN REMOTE HOSPITALS IN TANZANIA

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In Tanzania, lymphatic filariasis (LF) affects millions of people leading to a high burden among men of LF associated hydrocele; a fluid-filled enlargement of the tunica vaginalis sac around the testes. The economic, physical, and psychosocial impact of hydrocele can be devastating not only for the individual, but also for the family and community. In the Lindi and Mtwara regions alone, hydrocele backlog was estimated at 6000 men awaiting hydrocele surgery in 2014. Some hospitals perform routine hydrocelectomy but for only a few patients compared to the backlog. Typically surgeons use sac eversion technique under spinal or general anesthesia which requires lengthy hospital stays. In 2014, the Tanzania NTD Control Program (TZNTDCP) with support from partner NGOs and international donors introduced a safer technique and built capacity of local surgical teams. In 2015 and 2016, the TZNTDCP carried out surgeries in 3 districts in Mtwara and Lindi regions including patients who had hydrocele with no signs of scrotal skin involvement, no need for scrotoplasty, and an absence of medical comorbidities that could affect surgery. Two consultant surgeons trained 16 district level surgeons to perform the surgeries. All surgeries were done under local anesthesia; only in few cases was ketamine added for hernioraphy. The technique included: median raphe incision; draining of hydrocele fluid; its volume, and report on color; report on state of testes, sac and epididymis; partial excision and hemostatic vicryl 2/0 sutures; layered closure of hemiscrota; and subcuticular skin closure using catgut 3/0. Scrotal support was applied and patients had one night stay post operation with follow up after two weeks. A total of 1641 patients received surgery (age range 10 – 99 years). In only 1.9% of the patients, the hydrocele fluid color contained

necrotic tissue, blood, or pus. In conclusion, the new technique of partial sac excision under local anesthesia was easily adapted by local surgical teams through pre-camp training, demonstration, direct observation and consistent support and mentorship.

## 1816

### MULTIPLE PATHS TOWARDS LOSS OF DRUG SENSITIVITY: WHOLE-GENOME SEQUENCING OF *ONCHOCERCA VOLVULUS* INDICATES GENES UNDER SELECTION ARE DEPENDENT ON TRANSMISSION ZONE

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Onchocerciasis is a disease caused by the filarial nematode *Onchocerca volvulus*, targeted for elimination in many of the 31 African countries in which it is endemic. The drug deployed for mass treatment and elimination is ivermectin, which kills microfilariae and suppresses adult female fertility. In several African communities, a sub-optimal response to ivermectin has been observed. Our goal is to identify genetic markers associated with variation in drug response which control programmes could use to monitor presence and change in frequency of sub-optimal responders. We have scored female worms from Ghana for variation in drug response based on uterine embryograms. Whole-genome sequencing identified windows that are genetically differentiated between good and poor responders to ivermectin. Ivermectin response in *O. volvulus* involves multiple genes, with contributions from changes in both protein sequence and expression likely. However, the genetic variants that are associated with variation in drug response differ among populations, highlighting the importance of defining transmission boundaries when determining the possible effects of any genetic association. We discuss strategies for using these markers to screen African communities for *O. volvulus* with sub-optimal response to ivermectin and define transmission zones.

## 1817

### COMPARISON OF WET MOUNT MICROSCOPY, MINI-FLOTAC AND PCR FOR THE DIAGNOSIS OF *ASCARIS LUMBRICOIDES*

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Single wet mount microscopy of stool samples is the usual tool for the diagnosis of soil-transmitted helminths in many resource-limited areas. However, sensitivity is imperfect. Mini-FLOTAC is a rather novel, low-cost method, which does not require extensive equipment, and PCR assays presumably show highest sensitivity. The clinical significance of additional infections detected by these more advanced techniques is, however, not well established. In an area of predominant *Ascaris lumbricoides* infection in southern highland Rwanda, stool samples from 845 schoolchildren were examined for this helminth in a blinded fashion by wet mount microscopy, mini-FLOTAC, and PCR. The prevalence of *A. lumbricoides* infection detected by wet mount microscopy, mini-FLOTAC and PCR was 25%, 32%, and 37%, respectively. Agreement was moderate for wet mount microscopy and mini-FLOTAC or PCR, and good for mini-FLOTAC and PCR. Setting *A. lumbricoides* detected by any of the three methods as reference, the sensitivities (and 95% confidence intervals) of wet mount microscopy, mini-FLOTAC, and PCR were 56.4% (51.3-61.4), 71.9% (67.1-76.3), and

81.9% (77.6-85.6), respectively. *A. lumbricoides* infections diagnosed by mini-FLOTAC but not by wet mount microscopy showed clear associations with clinical manifestation whereas this was hardly the case for infections detected by PCR exclusively. Wet mount microscopy missed almost half of the *A. lumbricoides* infections actually present. Mini-FLOTAC showed superior sensitivity including otherwise missed, clinically relevant infections while PCR had highest sensitivity. These findings argue for the extended use of mini-FLOTAC in patient management in endemic regions and for the application of PCR in assessing actual prevalence and epidemiology.

## 1818

### INSIGHTS FROM MATHEMATICAL MODELS OF SOIL TRANSMITTED HELMINTH (STH) TRANSMISSION INTO POLICY FOR THEIR CONTROL AND ELIMINATION BY MASS DRUG ADMINISTRATION (MDA)

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The soil transmitted helminths (STH) include three important gut nematode parasites; the round worm *Ascaris lumbricoides*, the hookworms (*Ancylostoma duodenale* and *Necator americanus*) and the whip worm *Trichuris trichiura*. There is a requirement for well-validated policy recommendations on strategies for control and elimination of these diseases. We discuss the contributions modelling work can make in this area and set these findings in the context of prior modelling work. Examining current treatment strategies based on prevalence monitoring, we evaluate the likely impact of WHO recommendations on communities in a range of transmission settings across the three diseases. Following this up we propose new guidelines improving the chances of achieving target outcomes particularly on medium and heavy infection burdens and with a view toward elimination where appropriate, and demonstrate the impact of the guideline recommendations.

## 1819

### EFFICACY OF ANTHELMINTHIC DRUGS AND DRUG COMBINATIONS AGAINST SOIL-TRANSMITTED HELMINTHS: A SYSTEMATIC REVIEW AND NETWORK META-ANALYSIS

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The mainstay of soil-transmitted helminth (STH) control is regular distribution of benzimidazole anthelmintics through mass drug administration campaigns, which have seen a dramatic global scale-up in the past five years. Benzimidazoles, particularly albendazole, have high efficacy against *Ascaris lumbricoides* and hookworm; however, efficacy against *Trichuris trichiura* is poor and control of this parasite remains a significant challenge. Given the commitment of the WHO and endemic countries implementing deworming programs and of pharmaceutical companies donating drugs, it is important to reflect on whether we are currently using the most adequate drug regimen. Previous meta-analyses of anthelmintic efficacy have been limited to placebo-controlled studies and a small number of standard anthelmintic drugs. In order to allow examination of a broader evidence space, we conducted a network meta-analysis to compare the efficacy of a wide variety of both standard and novel anthelmintic drugs. Studies reporting efficacy of individual drugs or drug combinations against STH were systematically identified. Analysis involved comparing available drug efficacy data to standard treatment (single-dose albendazole), using an automated generalized pairwise modeling framework for network meta-analysis to generate mixed treatment effects against a common comparator. Preliminary results from the analysis of 102 included studies showed that for *A. lumbricoides* and

hookworm, single-dose albendazole was equally or more efficacious than other drugs and drug combinations. However, for *T. trichiura*, several drug combinations - albendazole plus ivermectin, mebendazole plus ivermectin, and albendazole plus oxfantel pamoate - were significantly more efficacious than single-dose albendazole. These results suggest that combining benzimidazole drugs with other anthelmintics is a promising strategy that may represent a feasible alternative in areas where *T. trichiura* is prevalent. Use of drug combinations may also decrease the likelihood of emerging drug resistance, although economic implications must also be considered.

## 1820

### SEASONAL INFLUENCERS FOR ASCARIS TRANSMISSION: WHAT COULD THEY MEAN FOR PUBLIC HEALTH PROGRAMS AND THE 2020 GOALS?

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*Ascaris* eggs are exposed to the environment during maturation, leaving them at the mercy of the seasons. There is clear empirical evidence that temperature can influence egg development, but rainfall and moisture availability are also important contributing factors to parasite survival and transmission. Previous studies suggest that in temperate climates there will be sufficient moisture in the feces and surrounding soil, but settings with extremely high temperatures and low rainfall will see retarded development and viability. Rainfall is also associated with greater sequestration of eggs through the soil and studies have shown that soil samples during rainy seasons often produce the highest yield of viable ova. Settings with extreme fluctuations in rainfall or temperature will hence exhibit strong seasonal transmission patterns that may be partially masked by the longevity of *Ascaris* infections. With the 2020 goals, laid out in the World Health Organization neglected tropical disease roadmap, calling for a scale up in control and hundreds of millions of doses donated, there is a need to maximise their impact by assessing potential obstacles and limitations of current programs. Incorporating rainfall into a previous model that used temperature to predict optimal timing of mass drug administration (MDA) for temperate climates allows for a more generalizable template for a wide range of settings. This is then used to demonstrate how seasonally timed MDA could increase the probability of meeting WHO targets in Africa and make comparisons between best and worst case scenarios. Bi-annual MDA instead of annual MDA results in a less marked seasonal effect, with a higher chance of elimination, but timing of treatment can still impact the predicted outcome and could be important for onward surveillance as well as elimination.

## 1821

### INTESTINAL POLYPARASITISM IN PAMPA DEL INDI, CHACO PROVINCE, ARGENTINA

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The prevalence on intestinal parasites in Argentina is largely unknown and available data is from a restricted number of research studies that have been conducted in different localities depending on the interest of each research team or according to institutional affiliation. Few data from the Province of Chaco has been publicly available and therefore this study was conducted in the locality of Pampa del Indio, Department of General San Martín where Mundo Sano has a regional office and a long standing collaboration with the local hospital. Since there is no record of prevalence for either STHs or other intestinal parasites in this area, the aim of this study was to determine the prevalence of intestinal parasites in a community from a peri-urban neighborhood of Pampa del Indio. Parque Industrial is composed of 22 blocks and at least five houses from each block were randomly selected for participation, a total of 109 houses

were visited and 528 containers distributed for the collection of stool samples. A total of 315 samples (59.7% participation) were received for coprological analysis through sedimentation and flotation techniques. Houses were georeferenced and a questionnaire with socioeconomic and housing characteristics was filled out. Age of participants ranged from 1 to 76 years old (mean = 19) and 57.1% of them were female. Many of the individuals, concretely 11.1% were polyparasitized and 25.4% were infected with parasites of waterborne transmission. The most prevalent parasite was *Giardia lamblia* (12.7%) and the only STH found was *Strongyloides stercoralis* with a prevalence of 2.5%.

## 1822

### DIFFERENTIAL EXPRESSION OF MEMBRANE AND MEMBRANE-BOUND PROTEINS FROM FILARIFORM LARVAE AND ADULT FEMALE OF *STRONGYLOIDES VENEZUELENSIS*

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Human strongyloidiasis has assumed great importance in public health, in the context of neglected diseases of Brazil and worldwide. It is known that the rodent species, *Strongyloides venezuelensis*, has been used for the development of molecular and serological diagnostic methods of human strongyloidiasis. The present study aims to identify the differential proteome found in the membrane fraction from filariform larvae (L3) and adult female of *S. venezuelensis*. Larvae L3 were obtained from charcoal culture of feces of experimentally infected *Rattus norvegicus* and adult females recovered from the infected intestines. Firstly, a Tris HCl soluble supernatant were prepared after homogenization of the larvae and female parasites. Membrane fractions from both stages were obtained by centrifugation of the recovered homogenates. After digestion with trypsin, the recovered peptides from both fractions were analyzed by mass spectrometry using an Ultimate 3000 UHPLC in line with a Q Exactive mass spectrometer. Protein identification was performed using the Proteome Discoverer platform using the *Strongyloides ratti* database as a reference proteome. The differential proteomic analysis revealed the majority of proteins upregulated in the female involved in biogenesis of ribosomes, translation machinery, protein phosphorylation and transport across membranes. In contrast, in the L3 larvae stage, proteins related to motor activity and ATP binding were among the most upregulated molecules. Our preliminary data revealed a membrane proteome associated to a high metabolic state in the female whilst the major processes operating in the active migrating larvae are intimately associated to muscle activity. We believe this differential membrane proteome of *S. venezuelensis* can contribute to the elucidation of proteins involved in the parasite-host relationship and direct new studies aimed at the identification of diagnostic targets. Financial Support: FAPESP (2016/06185-0)

## 1823

### DIAGNOSIS OF *ASCARIS LUMBRICOIDES* INFECTIONS IN ETHIOPIAN CHILDREN AND ADULTS BY THREE COPROLOGICAL TECHNIQUES AND TWO NOVEL SEROLOGICAL TESTS

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The nematode parasite *Ascaris lumbricoides* is estimated to infect over 800 million people and is considered to be an important neglected tropical disease pathogen. Ascariasis has a substantial impact on public health with routine diagnosis still relying on the detection of eggs in stool. This technique has important limitations in terms of both application and interpretation. Recently, two different ELISA tests were developed in our lab to measure exposure to *Ascaris* infection. In this study, we aim to compare established coprological techniques with these novel serological tests for the detection of *Ascaris* infection and exposure. Stool and serum samples were collected from 600 children and 600 adults from Jimma town in the Western part of Ethiopia. In addition, dried blood spots (DBS) were also collected from 95 individuals. Parasite eggs in each of the stool samples were detected by Kato-Katz, McMaster and Mini-FLOTAC. The collected sera and DBS samples were evaluated for anti-*Ascaris* IgG4 antibody levels using two serological tests. The first ELISA detects antibodies against purified adult *A. suum* haemoglobin (AsHb), while the second detects antibodies directed against an extract of the lung stage larvae of *A. suum* (As-Lung-L3). When the results of all three coprological techniques were combined, a total of 35.2% of children had *Ascaris* eggs in their stool. The highest percentage of infected children were detected by Kato-Katz (31.5%) followed by the Mini-FLOTAC (26.3%) and McMaster (23.7%) technique. Using serology, 24.3% of the children were seropositive on the AsHb ELISA whereas double the amount (48.3%) were positive on the As-Lung-L3 ELISA. No correlations were found between eggs per gram and antibody response. Currently, collection of samples from the adult population and evaluation of DBS samples is still ongoing. All data is expected by this summer and will also be presented in detail. Serology using the As-Lung-L3 ELISA was able to detect a much higher percentage of individuals exposed to ascariasis than when traditional coprology is used. Serology could be a useful tool during certain stages of control programs.

## 1824

### PREVALENCE OF INTESTINAL HELMINTH INFECTION IN EQUATOGUINEAN INFANTS, CHILDREN, ADOLESCENT AND ADULTS AND ITS IMPACT ON IMMUNOGENICITY TO A LIVE, ATTENUATED, WHOLE SPOROZOITE MALARIA VACCINE

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Helminth infections can negatively impact vaccination outcomes. Additionally, infants born to mothers that are helminth-infected during pregnancy show reduced vaccine take and responsiveness. Data on prevalence of soil-transmitted helminth infections (STH) in Equatorial Guinea in all age groups are limited. We are currently conducting a randomized, double-blind placebo controlled trial to evaluate the safety, tolerability, and immunogenicity of a whole sporozoite PfSPZ candidate vaccine (PfSPZ Vaccine) in 135 healthy Equatoguineans within an age range of 6 months to 65 years. Since ongoing STH infection was an exclusion criteria for trial participation, stool samples were collected from more than 250 potential volunteers during screening. Stool samples were analyzed using the Kato-Katz technique to identify type of helminth infection and to quantify parasite burden. STH-positive individuals were treated with oral mebendazole and repeat stool samples were analyzed to assess treatment success. We will report on the prevalence of *Ascaris lumbricoides* and *Trichuris trichiura* in our volunteers stratified by age groups and the potential impact of recent STH infection on PfSPZ Vaccine immunogenicity.

### DETECTION OF SOIL TRANSMITTED HELMINTH DNA IN STOOL SAMPLES DRIED ON FILTER PAPER

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In order to facilitate integrated assessment of helminth control and elimination programs better methods of the detection of soil transmitted helminthes (STH) are needed. To improve sample preservation and archiving of stool samples for PCR detection of STH we selected 103 STH positive stool samples collected in rural Liberia. Duplicate Kato Katz smears were performed on fresh samples and equal aliquots were preserved in RNA later (-20C) and dried on FTA cards (room temperature, RT). Kato Katz detected 31 hookworm, 95 *Ascaris*, and 30 *Trichuris* positive samples. qPCR revealed more positive samples for RNA later and FTA card preserved samples: 34 and 56 for *Necator*, 97 and 99 for *Ascaris* and 30 and 45 for *Trichuris*. To examine whether storage duration of FTA cards affects the results of DNA extraction and qPCR, we compared qPCR results from another set of 69 samples that was extracted and tested directly after collection in 2014 and another aliquot was extracted and tested 36 months later. Similar results were obtained for both time points for *Necator* (46 vs 49), for *Ascaris* (22 vs 21), for *Trichuris* (15 vs 15) and for *S. mansoni* (39 vs 43). The average Ct value for positive samples was also very similar at both time times. Our results confirmed other studies that show that qPCR is more sensitive than the traditional Kato Katz smear for the detection of for STH infection. Furthermore, the results show that the preservation of dried stool samples on FTA cards provides suitable templates for qPCR analysis and samples can be stored at RT for at least 36 months without significant degradation of target DNA. The novel storage protocol for stool samples may facilitate assessment of STH by qPCR during integrated assessment of helminth infections.

### TESTING FOR STH ELIMINATION: MODELLING THE IMPACT OF DIFFERENT DIAGNOSTICS TOOLS

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Technological advances and falling costs are making new diagnostic tools available for field use, offering improved sensitivity and specificity. For the soil-transmitted helminth diseases, this includes the use of PCR and qPCR techniques for egg detection in stool samples. Although new techniques offer greater accuracy at low disease prevalence levels, it is important to understand how they correspond statistically to the host's worm burden. Additionally, most monitoring and evaluation (and the WHO treatment guidelines based on them) are currently expressed in terms of prevalences and intensities as measured by the Kato-Katz egg counting method. A means of conversion between old and new diagnostic measures are necessary to ensure consistency and continuity. We attempt to answer these questions using a dataset comprising matching sets of Kato-Katz readings, qPCR readings and worm expulsions for each individual. We fit models of egg production and qPCR reading per female worm. These allow us to compare the sensitivity and specificity of the two tests as prevalence measures against each other. We examine the relationship between light, medium and heavy infection categories under the two diagnostic approaches. We show how using qPCR rather than Kato-Katz affects the measured prevalence in a population as it approaches elimination and the thresholds used to detect it.

### COMPARISON OF KATO-KATZ, MINI-FLOTAC AND MULTI-PARALLEL REAL-TIME POLYMERASE CHAIN REACTION TECHNIQUES FOR DETECTION OF SOIL-TRANSMITTED HELMINTHS IN FEIRA DE SANTANA, BRAZIL

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Soil-transmitted helminth (STH) infections, primarily caused by the roundworm *Ascaris lumbricoides*, the hookworm species *Necator americanus* and *Ancylostoma duodenale*, and the whipworm *Trichuris trichiura*, affect over 1 billion people, with billions more at risk, especially in warm, moist climates. Current STH control efforts in Brazil are conducted using passive surveillance and incidental case finding, such as by the Schistosomiasis Control Program, which is limited to schistosomiasis endemic areas, leaving STH infection prevalence under-notified. Diagnostic testing for the STH relies mainly on the World Health Organization (WHO) recommended Kato-Katz thick smear method, a quantitative technique that has been shown to lack sensitivity. Other economical, feasible, and higher-accuracy diagnostic methods are needed to detect and combat STH, especially in areas of low endemicity. In the city of Feira de Santana, Brazil, we collected human stool samples and analyzed them using the WHO recommended Kato-Katz thick smear method, as well as with two more recently developed diagnostic methods, the mini-FLOTAC method and a multi-parallel quantitative polymerase chain reaction (qPCR) method. The mini-FLOTAC method allows for quick analysis of fresh or preserved feces with minimal equipment needed. The qPCR method allows for detection and quantification of parasites with a high degree of specificity and sensitivity, and is optimized to allow for inexpensive analysis of each sample. All three diagnostic methods are being analyzed and compared for both parasite detection and quantification. Analysis of the qPCR samples is currently underway, and all comparative analyses will be complete by the conference. Both the mini-FLOTAC and qPCR methods offer feasible, higher-accuracy diagnostic alternatives to the standard Kato-Katz method, and will be useful in enabling a shift away from the current STH morbidity control approach and towards an elimination approach.

### NA-GST-1/ALHYDROGEL HOOKWORM VACCINE CO-ADMINISTERED WITH CPG 10104 IMPROVES IMMUNOGENICITY IN HEALTHY, HOOKWORM NAIVE ADULTS

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*Necator americanus* glutathione S-transferase-1 (Na-GST-1) is a 24-kDa protein produced by adult hookworms that is thought to play a role in detoxifying heme and other breakdown products of the hookworm blood digestion pathway. Recombinant Na-GST-1 was expressed in *Pichia pastoris* and formulated on Alhydrogel. A series of Phase 1 trials have been conducted in the USA, Brazil, and Gabon of Na-GST-1/Alhydrogel in combination with an aqueous formulation of glucopyranosyl lipid A, a synthetic Toll-like receptor (TLR)-4 agonist. In these studies, the vaccine was safe, well tolerated and induced anti-Na-GST-1 IgG antibodies; however, induced IgG did not significantly inhibit native GST enzymatic activity. To improve immunogenicity, Na-GST-1/Alhydrogel was tested

with or without the point-of-injection addition of CPG 10104, a synthetic oligodeoxynucleotide and TLR-9 agonist, in a Phase 1 trial in Washington, DC. Hookworm-naïve healthy adults (n=24) were vaccinated with 30 or 100 µg Na-GST-1 coadministered with 500 µg CPG 10104 (n=8 per dose), or 100 µg Na-GST-1/Alhydrogel (n=8). Subjects received 3 intramuscular injections at 2-month intervals. Common adverse events included mild to moderate injection site pain and tenderness, headache, and nausea. No significant differences were observed in adverse events between dose groups or formulations, although arthralgia and myalgia were more common in those receiving CPG 10104. Anti-Na-GST-1 IgG antibody levels as measured by indirect ELISA were detected in all subjects, with a peak 2 weeks after the 3rd vaccination. Geometric mean antibody levels at this time point were higher in the two groups that received CPG 10104 compared to the non-CPG group, although differences were not statistically significant: 49.4 Arbitrary Units (AU) for 100 µg Na-GST-1/Alhydrogel, 300.7 AU for 30 Na-GST-1/Alhydrogel/CPG (p=0.08 for comparison to non-CPG group), and 389.5 AU for Na-GST-1/Alhydrogel/CPG (p=0.067). Addition of CPG 10104 to Na-GST-1/Alhydrogel improves IgG responses but does not compromise safety. Further clinical testing of this vaccine formulation in endemic areas is justified.

1829

### EXPLORING CHAGAS DISEASE ECO-EPIDEMIOLOGY IN CENTRAL PANAMA

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Chagas disease, a neglected tropical disease affecting millions of people throughout the world, is transmitted by multiple species of triatomine “kissing bug” (Hemiptera: Reduviidae). In Panama, species that transmit the disease are sylvatic (ie: wild-living), and the risk factors that govern human transmission are not well characterized. This study investigates the factors potentially contributing to Chagas disease exposure risk across a human land-use gradient in central Panama. Epidemiological surveys and sixteen months of in-home kissing bug collection were performed in nine communities across three urban to rural land-use gradients. Household surveys explored social and behavioral factors, such as living conditions, education level, socioeconomic status, and knowledge of both kissing bugs and Chagas disease. Reported presence of domestic and wild animals around the home was positively correlated with having seen kissing bugs around the home and with greater average kissing bug capture during the study. Entomological surveys captured five common species of kissing bug across the gradients, with a greater diversity of kissing bug species in more rural areas. Significantly more kissing bugs were captured on average in semi-urban and rural areas as compared to urban areas, and houses that kept chickens captured significantly more kissing bugs than houses without chickens. Preliminary testing has indicated presence of *Trypanosoma cruzi* in kissing bugs; further tests will reveal if infection prevalence differs across the land use gradient. Better understanding of these risk factors is integral to successful control efforts.

1830

### QUANTIFICATION OF INFECTION RESERVOIRS IN HUMAN VISCERAL LEISHMANIASIS BY XENODIAGNOSIS

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Visceral leishmaniasis (VL) is the most fatal form of leishmaniasis caused by protozoan parasite *Leishmania donovani* complex and transmitted between people by the bites of blood sucking vector sand fly *Phlebotomus argentipes*. An important factor influencing the transmission dynamics of disease is the unknown contribution of human host with different parasitemia to infected sand fly population. The aim of present study is to investigate the infectiousness profile of VL patients by xenodiagnosis during repeat exposure of colony reared sand flies. Direct xenodiagnosis was performed on 25 active VL patients using 10 male and 30 female pathogen free colonies of *P. argentipes* sand flies. After 60 hrs of post blood meal, fed sand flies were dissected and infections of sand flies were evaluated by microscopy. Repeat xenodiagnosis was performed at same site after 24hrs to investigate the effect of biting response on parasite transmission. We found varying sand fly feeding response (3-94%, Mean: 57.2%) with VL patients, and transmission of *L. donovani* to *P. argentipes* female sand flies was low (Mean: 30% of fed sand flies). No significant effects on transmission of parasites were seen during repeated xenodiagnosis. In conclusion, the study shows first time on human that VL patients harbored parasites in sufficient numbers to promote infections in vector sand flies.

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### A LEISHMANIA SKIN TEST SURVEY OF CUTANEOUS LEISHMANIASIS IN THE HUMAN POPULATION OF DIEMA DISTRICT, WESTERN MALI

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Cutaneous leishmaniasis (CL) has been reported as an endemic disease in the western region of Kayes, Mali. The aim of this study was to determine the prevalence and annual incidence rates of *Leishmania major* exposure by leishmanin skin test (LST) in 3 villages on the District of Diema (Nafadji, Guemou, Debo Massassi), Kayes region. LST was performed among 2466 subjects aged 2 to 18 years old residents of the study villages. Of those, 2020 negative LST subjects were followed up and re-tested one year later for the development of CL to estimate the annual incidence of *Leishmania* infection. The prevalence of LST positive individuals was 18.73% and 18.53% in Nafadji and Debo Massassi, respectively and lower in Guemou (8.51%). The prevalence increased by age varying from 5.37%, to 19.69% and 39.20% among 2 to 6 years old, 7 to 12 years and 13 to 18 years respectively. Moreover, we observed a higher annual incidence of *Leishmania* infection with 23% in Nafadji, 26.22% in Guemou and 23.45% in Debo Massassi, respectively. This high incidence of LST reflects a stable high endemicity of *Leishmania* transmission in the district Diema.

1832

### BIOGEOGRAPHY OF *TRYPANOSOMA CRUZI* IN AREQUIPA, PERU

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Chagas disease is caused by the zoonotic protozoan parasite *Trypanosoma cruzi*. *T. cruzi* is endemic in 21 countries throughout the Americas, and has migrated to countries around the globe. Despite causing more than 12,000 deaths annually, and despite 65 million people currently at

risk of infection, the epidemiological patterns of *T. cruzi* remain poorly understood. *T. cruzi* and its insect vector thrive in urban environments and have spread rapidly across urban landscapes throughout South America. In an increasingly urban world, it is important to understand how disease systems that thrive in urban environments migrate through them. Determining migration patterns and the processes that affect migration of such populations in urban environments requires evaluation at fine spatial and temporal scales (<1km and annual). Population genetic and coalescent approaches are powerful tools to assess population patterns and processes, but their power scales with the genetic variation per sample analyzed. We therefore sequenced and assembled complete genomes of ~150 *T. cruzi* isolates collected in Arequipa, Peru. Arequipa, the second largest city in Peru, is a rapidly urbanizing city that is experiencing an epidemic of *T. cruzi*. We used Bayesian methods to reconstruct the phylogenetic history and to determine migration patterns and colonization history of *T. cruzi* within the urban environment of Arequipa. The migratory patterns of *T. cruzi* suggest a single introduction into Arequipa followed by multiple "micro-epidemics" in which rare long-distance migration events seed local epidemics that are limited to neighboring city blocks. Between-district migration is beyond the migratory capabilities of the insect vector, suggesting that human migration and the trading of small domestic mammals are responsible for long-range migration, while within-district migration is facilitated by the vector.

### 1833

#### **TRYPANOSOMA CRUZI ECOLOGY AT FACILITIES HOUSING NATURALLY INFECTED NON-HUMAN PRIMATES IN TEXAS, USA**

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*Trypanosoma cruzi*, agent of Chagas disease, is a vector-borne protozoan parasite that infects a wide range of mammalian hosts throughout the Americas. Spillover from wildlife in sylvatic cycles results in local transmission to humans, domestic dogs, and non-human primates (NHPs) in the southern US. This region, particularly Texas, is home to many biomedical research facilities housing NHPs with outdoor access. Locally-acquired *T. cruzi* infection has been reported in these animals since the 1970s, with negative impacts on the use of infected animals in research and breeding programs. *T. cruzi* transmission at NHP facilities is poorly characterized, specifically the infectiousness of seropositive NHPs to vectors or other NHPs; the importance of local wildlife reservoirs; and the presence, infection status, and feeding behavior of local triatomine vectors. We investigated *T. cruzi* infection in NHPs, vectors, and wildlife at NHP facilities in Texas. We hypothesized that robust sylvatic transmission cycles would exist in proximity to NHP housing, characterized by highly infected wildlife and triatomines. Further, we expected variation in infection prevalence, infectiousness, and *T. cruzi* strain type among wildlife species and NHPs, with relevance to reservoir status. *T. cruzi* was detected via PCR in the blood of 33/45 (73.3%) seropositive rhesus macaques (*Macaca mulatta*) and 23/34 (67.6%) seropositive cynomolgus macaques (*Macaca fascicularis*) with intermittent or low level parasitemia in many animals. Strain types TcI and TcIV were detected in NHPs in nearly even proportion ( $p=0.94$ ), with 4 mixed TcI+TcIV infections. While none of 182 rodents were infected, 8/10 wild mesomammals were PCR positive in heart and blood. Infected opossums harbored only TcI whereas raccoons harbored TcIV. Of 78 triatomines collected at NHP facilities, 45% were infected: 62% with TcI, 24% with TcIV and 14% with mixed TcI+TcIV infection. Blood meal analysis of some bugs ( $n=11$ ) shows they fed on diverse species, including human, dog, raccoon, deer, and gecko. These ecological data will be used to define parasite transmission pathways and targets for intervention.

### 1834

#### **ANTIMONY SENSITIVITY OF LEISHMANIA BRAZILIENSIS PROMASTIGOTES VARIES ACCORDING TO THE FORM OF LEISHMANIASIS THEY DERIVE**

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Antimony is the first line drug for treating American tegumentary leishmaniasis (ATL) in Brazil. In this country, *Leishmania braziliensis* causes at least three distinct forms of disease: localized cutaneous (CL), mucosal (ML) and disseminated leishmaniasis (DL). All forms can be found in Corte de Pedra, Northeast Brazil. ML and DL respond poorly to antimony, in contrast to CL. The *L. braziliensis* population causing ATL in Corte de Pedra is complex, with strains of the parasite associating with form of leishmaniasis. We tested the hypotheses that antimony refractoriness is associated with *L. braziliensis* genotypes, and that parasites from ML and DL present greater *in vitro* resistance to antimony than *L. braziliensis* from CL. Comparison of geographic coordinates of living sites between antimony responders and non-responders by Cusick and Edward's test showed that refractoriness and responsiveness to the drug were similarly wide spread in the region ( $p>0.05$ ). Parasites were then genotyped by sequencing a locus starting at position 425,451 on chromosome 28, which is polymorphic among *L. braziliensis* of Corte de Pedra. Haplotype CC- in CHR28/425451 was associated with risk of treatment failure among CL patients (Fisher's exact test,  $p=0.03$ , odds ratio=4.65). This haplotype could not be found among parasites from ML or DL. Finally, sensitivity to antimony was evaluated exposing *L. braziliensis* promastigotes to increasing concentrations of meglumine antimoniate *in vitro*. Parasites from ML and DL were more resistant to antimony at doses of 2 mg/100  $\mu$ L and beyond than those isolated from CL (Fisher's exact test,  $p=0.02$  and  $p=0.004$ , respectively). The intrinsically lower susceptibility of *L. braziliensis* from ML and DL to antimony parallels what is observed for patients' responsiveness in the field. This finding reinforces that ML and DL patients would benefit from initiating treatment with drugs currently considered as second line, like amphotericin B.

### 1835

#### **LYMPHATIC FILARIASIS MASS DRUG ADMINISTRATION COVERAGE, COASTAL REGION, KENYA, 2015 AND 2016**

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Lymphatic Filariasis (LF) is one of the diseases targeted for elimination. It has affected over 120 million in the world and about 3.5 million people in Kenya. We aimed to compare the reporting rates and coverage of LF in 6 counties within the coastal region. This was a programmatic operation where mass drug administration (MDA) of albendazole (ALB) and diethyl carbamazine (DEC) was distributed in 5 and 6 counties in 2015 and 2016 respectively in the endemic coastal region of Kenya. A register of all residents living in the area was developed just before the MDA by community health volunteers (CHV). The registered data was used to estimate the number of tablets needed by each of them. The CHVs distributed the tablets in houses they had registered. The target population were aged two years and above but excluded pregnant women. However, coverage denominator was the total population in the target counties and sub counties. Data was collected and aggregated using registers and tally sheets then analyzed using Microsoft Excel and Epi-Info. A population of 2,447,764 was targeted in 2015 and 3,486,730 in 2016. County reporting rates were above 93% in both years. County coverage ranged from 49% in Lamu to 99% (Taita Taveta) and 57% (Mombasa) to 76% in 2015 and 2016 respectively. Of the 16 sub counties dewormed in 2016, 11 (68%) recorded 100% reporting rate with 8 (50%) recording coverage above 65% in 2015. In 2016, 20 (87%) out of the 23 sub counties dewormed

recorded reporting rate of 100% while 13 (57%) recorded coverage above 65%. In conclusion, there was a general improvement in the proportion of counties and sub counties recording 100% reporting rate as well as those recording coverage above 65%.

## 1836

### ECO-BIO-SOCIAL DETERMINANTS OF HUMAN INFECTION WITH *TRYPANOSOMA CRUZI* IN RURAL COMMUNITIES IN THE ARGENTINE CHACO

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Neglected tropical diseases (NTDs) are complex systems composed of multiple biological, ecological, socio-economic, and cultural factors acting at different scales in variable environments. The multivariate association between these factors and human infection with *Trypanosoma cruzi*, the causative agent of Chagas disease, has rarely been studied. Here we assessed the spatial patterns of human and vector (*Triatoma infestans*) infection with *T. cruzi* and identified the eco-bio-social determinants of human infection in a well-defined rural area of Pampa del Indio, northern Argentina, 10 years after the last community-wide insecticide spraying campaign. Most residents were indigenous (90%) and a creole minority. A cross-sectional serosurvey aiming at full coverage was conducted over 2012-2015 using two ELISA tests (Wiener). The seroprevalence of *T. cruzi* infection was 25.3% (n=1929, ≥8 months old), increased with age, and was nearly twofold higher among indigenous people (26%) than creoles (15%). The risk of human infection increased 60% with each additional *T. cruzi*-infected vector collected by timed-manual searches in human sleeping quarters; 40% with each infected household resident, and also increased with increasing household social vulnerability (a multidimensional index of poverty), and decreased with domestic host availability (humans, cats, dogs and chickens). We found a significant negative interaction between household social vulnerability and the relative abundance of infected *T. infestans*, indicating that residents from vulnerable households were exposed to a greater risk of infection at low vector abundance than less vulnerable residents. Although local *T. cruzi* transmission occurs at a household level, spatial analysis identified local hot-spots of human and vector infection. Integration of these results into a disease risk map revealed high-risk areas that would benefit from targeted vector surveillance and control combined with etiologic treatment. This approach is useful to develop cost-effective strategies oriented to reduce the burden of Chagas disease and other NTDs in the affected areas.

## 1837

### SEROLOGICAL EVIDENCE OF *TRYPANOSOMA CRUZI* INFECTION AMONG BLOOD DONORS IN MARICOPA COUNTY, ARIZONA, 2007-2016

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In the United States (US) the voluntary testing of blood donors for antibodies to *Trypanosoma cruzi* was initiated in January 2007. Since April 2010 the US Food and Drug Administration has approved several tests to allow blood, tissue, and organ donors to be screened for serological evidence of *T. cruzi* infection. Since 2007 Maricopa County Department of Public Health (MCDPH) in Phoenix, Arizona, USA has been notified of any blood donor with antibodies to *T. cruzi*. We analyzed retrospective data on all blood donors reported to MCDPH who were positive for serological

evidence of *T. cruzi* infection. Demographic information, travel history, *Triatoma* species (kissing bug) exposure and bite history, and symptoms of Chagas disease were collected when available. From 2007 to 2016 there were 31 blood donors reported to MCDPH with serologic evidence of *T. cruzi* infection. Of these, 58% were male, 83.8% were of Hispanic ethnicity; ages ranged from 17-69 (median 36) years, with 16% (5/31) ≤18 years. United blood services reported 28 donors and the American Red Cross reported 3 donors. Twenty-nine donors (93.5%) were reactive using the *T. cruzi* enzyme immunoassay (EIA) and confirmed with either *T. cruzi* radioimmunoprecipitation analysis (27/29) or ABOIT<sup>®</sup> ESA Chagas (2/29). Two donor results were reactive using the *T. cruzi* screening EIA but were not confirmed. Seventeen donors (55%) were unable to be contacted for follow-up or questioning. Among the 14 (45%) donors interviewed, all reported travel outside of the US, 13 (93%) were either born or spent > 2 weeks in Mexico, and 1 donor vacationed in Argentina. Only 1 donor remembered kissing bug exposure and bites; 3 (21%) donors reported symptoms consistent with chronic Chagas disease. Arizona blood donor screening for *T. cruzi* infection initiated in 2007 has led to the identification of more than 30 cases of probable Chagas disease in Maricopa County. Although all cases available for interview reported travel to a Chagas endemic area, this screening program provides a surveillance mechanism to identify new cases and endemic spread of Chagas disease in Maricopa County and Arizona.

## 1838

### CHAGAS DISEASE. A SYSTEMATIC REVIEW OF CASE REPORTS THROUGH THE LAST 50 YEARS

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Chagas disease is a parasitic disease caused by *Trypanosoma cruzi*, a protozoan which is transmitted to humans primarily vector-borne. The clinical spectrum of the disease is widely broad. During the acute phase patients present unspecific manifestations and during the chronic phase they may develop life-threatening complications. Chagas disease is frequently underdiagnosed and untreated, increasing morbimortality of those who suffer from it. The objective of our review was to describe demographics, mechanisms of transmission, clinical manifestations, diagnosis and treatment of the disease. A systematic review was conducted following the "Preferred Reporting Items for Systematic Reviews and Meta-Analyses" guideline. PubMed database was searched using the search terms "*Chagas disease*" or "*American trypanosomiasis*" and limited to case reports published without time frame in English or Spanish. Full text articles were assessed for relevance and data extraction was performed as an iterative process. During initial search, 227 items were obtained. Until now, 134 items have been included, describing 149 patients with Chagas disease from which 54% were females. Human Immunodeficiency Virus was the most common associated comorbidity observed. Non vector-borne transmission was reported in 38 cases (25%) and vector-borne transmission in 10 cases (7%). The route of transmission was unknown in 101 cases (68%). Fever, asthenia and headache were the most common initial clinical manifestations during the acute phase; and dyspnea, chest pain and tachycardia during the chronic phase. For diagnosis, Polymerase Chain Reaction, Enzyme-linked Immunosorbent Assay and Indirect Immunofluorescence were used in 51 cases (34%). Benznidazole was the treatment of choice in 73 cases (48%), Nifurtimox in 16 cases (11%) and Itraconazole in 1 case (1%). Mortality rate was of 22%. The conducted systematic review provides valuable information about Chagas disease reported cases that have been published in the literature. It demonstrates how its trends and behaviors have been changing through the last 50 years and enriches previous published data.

## TRYPANOSOMA CRUZI INFECTION AND CARDIAC OUTCOMES IN GOVERNMENT WORKING DOGS ACROSS THE UNITED STATES

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Infection with the protozoan parasite *Trypanosoma cruzi* can cause Chagas disease in humans and animals, and in some ecological settings canines may be sentinels of human disease risk. Chagas is increasingly recognized in the southern US, where triatomine vectors transmit the parasite among diverse wildlife and domestic dogs in sylvatic transmission cycles. The Department of Homeland Security trains thousands of working dogs to provide detection and security functions across the country. We hypothesized that *T. cruzi* infection would be greatest in dogs that work in the southern states- coinciding with established sylvatic cycles- but that dogs in northern states may also be exposed owing to required training in the south. In 2015-16, we sampled working dogs from the US-Mexico border in Texas and New Mexico, and expanded our study in 2017 to include dogs across the country. Additionally, we opportunistically collected kissing bugs from canine environments. While cross-country collections are ongoing, the working dogs in Texas and New Mexico showed significant variation in seroprevalence across management areas 18.3-26.7% (n=528; P=0.02-0.04) compared to 11.6% seroprevalence in young dogs at a training center based on immunochromatographic tests and indirect fluorescent antibody testing. Using multiplex rt-PCR of the spliced leader intergenic region (SL-IR) to determine parasite discrete typing unit (DTU), we found three dogs (0.6%) had parasite DNA in the blood, including TcI and TcI/TcIV mix. Nine of 20 (45%) *T. gerstaeckeri* and *T. rubida* from the canine environments were infected with TcI and TcIV; insects analyzed for bloodmeals (n=11) fed primarily on canine (54.5%) but also human and wildlife. Ongoing clinical assessments of infected dogs include the use of 24-hour Holter monitors for electrocardiogram assessment of the heart, and abnormalities in electrical activity of the heart will be analyzed in relation to parasite DTU. Epidemiological and clinical assessment of *T. cruzi*-infection in these high value working dogs will provide a basis for quantifying the economic impact of the disease and implementing control measures.

## 1840

### VISCERAL LEISHMANIASIS IN SYRIA: A SILENT KILLER UNCOVERED

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Historically, Syria is listed as one of the top six countries in the world for cutaneous leishmaniasis, but is not a country that has been associated with visceral leishmaniasis. Between 2004 and 2008 the World Health Organization recorded a national average of just 14 cases of visceral leishmaniasis per annum, rising to 36 cases in 2014. Across MENTOR's areas of activity in the north of Syria alone, notably in Idlib, MENTOR had 88 confirmed cases reported between 2014 and 2016 - a large escalation compared to nationwide pre-war figures. In May 2016 MENTOR conducted an RDT survey in the two villages of Betya and Tellemar, Salqin, Idlib. An awareness campaign coupled with the support of local authorities produced a turnout of 90%, with 4,226 people tested. MENTOR disclosed a high prevalence of visceral leishmaniasis infection, with 8 people in the two villages testing positive (a rate of 19/10,000 population, compared to WHO's national estimate of 0.02/10,000). This survey has exposed the possibility of a hidden lethal epidemic. MENTOR has raised awareness among health workers about the existence of the disease and provided them with the easy-to-use RDT. Since the survey there has been a further increase in the diagnosis of visceral leishmaniasis in local MENTOR supported health facilities, as health workers now include the disease as

a differential diagnosis for symptoms such as fevers of unknown origin, anemia, lethargy and organ involvement. This suggests that many deaths in this area previously attributed to organ or heart failure may have actually been caused by visceral leishmaniasis. The survey is an important first step towards a new understanding of visceral leishmaniasis epidemiology and control in Syria, which could ultimately save many lives. More research remains to be done but the data accumulated so far indicates that there is, at least locally, a high rate of undetected infection which could be more widespread across the country. It is imperative that efforts are now made to capitalize on the momentum gained by the first survey, and MENTOR intends to implement further surveys in 2017 to verify this apparent silent epidemic.

## 1841

### HEALTH SECURITY DURING ERA OF CONFLICT AND FORCED DISPLACEMENT: LEISHMANIASIS AS A CASE STUDY

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The Syrian conflict reports the worst forced displacement after the Second World War as a consequence of the increasing violence and escalations by the Government of Syria and their allies. Approximately, nine million people have been displaced internally as well as five million who have been recorded as refugees in neighbouring countries. Health infrastructure suffered immensely from the increased attacks on health facilities- three quarters of health service facilities have been destroyed. Over the course of 2016, 101 attacks on hospitals and health care centres across Syria have been recorded, with 14 healthcare workers killed and 40 injured. Moreover, a severe collapse in public services has been reported affecting rubbish accumulations and water supplies. Since 2013, terrorist networks have gained a foothold within the fragile states of Syria and Iraq. Consequently, the terrorist group ISIL ruled Deir ez-Zor, Al-Raqqa and Al-Hasakah in Syria as well as Ninewa, Anbar, Salah ad-Din and Kirkuk in Iraq. Leishmaniasis outbreaks have been reported throughout the period of the conflict, with an unprecedented increase as the proxy-war unfolds. The intervention of the international community affected disease dynamics in areas such as Al-Hasakah Syrian governorate which is situated on the Iraqi-Syrian border. Human movement driven by attacks on civilian populations, absence of immunity to leishmaniasis and poor access to the health services were all found to play a key role in the current outbreak.

## 1842

### VISCERAL LEISHMANIASIS IN THE URBAN AREA OF TWO MUNICIPALITIES OF SAO PAULO STATE, BRAZIL: A KEY TO UNDERSTAND THE ROLE OF THE STREET LEVEL BUREAUCRATS

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The street level bureaucracy can be a key to understand how some diseases spread in urban areas and how to block it in some contexts, because it means the way the workers represent the public health programs they work for. In Brazil, the surveillance and control program for Visceral Leishmaniasis (SCPVLeish) is structured at the national level, accompanied at the state level by different protocols. However, it is on the local scale, in the municipalities, that actions are triggered in the territory. This study presents itself as an excellent opportunity to test the hypothesis that the street level bureaucracy can promote a better understanding of the intra-urban actions for VLeish. The objective was to analyze, from

the point of view of the set of actions of the Program, how the frontline intervention can influence the spatial distribution of human and canine VLeish cases in two endemic municipalities: Dracena and Tupi Paulista. We performed statistical descriptive and geospatial analysis of serum samples collected in the field. The patterns of practice, routines and simplifications of the local-based SCPVLeish personnel were also evaluated, in order to compare their behavior with stipulations of SCPVLeish. There were 145 cases of human VLeish, with 5 deaths in the city of Dracena and in Tupi Paulista, 36 cases of human VLeish with 3 deaths. From 2006 to 2015, a total of 27,586 canine serum samples from Dracena were analyzed, identifying prevalence of 26.3% (7,268), accounting 58.5% (4,489) of euthanasia and 4.9% (373) with refusal. In the same period in Tupi Paulista, a total of 8,317 serum samples were analyzed for canine VLeish, identifying prevalence of 22.1% (1,877), accounting 80.6% (1,757) of euthanasia and a refusal of 13.5% (310). The Municipality of Dracena can be considered in intense transmission and Tupi Paulista in moderate transmission. The spatial distribution of canine VLeish presented in the urban area of Dracena and Tupi Paulista allowed us to infer on the occurrence of the increase of the number of human cases. Hot spots of VLeish have to intensify SCPVLeish practices, in continuous work and specialized team.

### 1843

#### EPIDEMIOLOGY OF *TRYPANOSOMA CRUZI* IN URBAN DWELLING OPOSSUM (*DIDELPHIS VIRGINIANA*) AND FERAL CAT (*FELIS CATUS*) POPULATIONS OF THE RIO GRANDE VALLEY, TEXAS

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*Trypanosoma cruzi* is a zoonotic protozoan parasite known to infect a wide range of mammals but minimal information is known about the infection of urban dwelling animals in the United States. In the Rio Grande Valley of TX, our ongoing epidemiological investigations have documented anti *T. cruzi*-antibodies in 1.8% of people and >21% of their pet dogs. We hypothesized that urban wild and feral animals from the region serve as infectious reservoirs that may serve as a bridge between sylvatic and domestic transmission cycles. In early 2017, samples were collected from urban dwelling opossums (*Didelphis virginiana*) and feral cats (*Felis catus*) of Hidalgo Co., TX, an area with documented human and canine Chagas disease and from where we regularly collect infected triatomine vectors. After euthanasia (performed by animal control for reasons unrelated to our study), whole blood and cardiac tissue were collected from feral cats and wild opossums. Additionally, anal gland secretions were collected from opossums because previous research has documented *T. cruzi* epimastigotes in possum reservoirs with uncertain ecological and epidemiological relevance. Through serological testing of 120 cats using two immunochromatographic tests and indirect fluorescent antibody testing, 14.2% individuals on at least two independent antibody detection platforms. All cardiac tissue, whole blood, and anal gland samples were subjected to qPCR for parasite detection and discrete typing unit (DTU) determination. None of the seropositive or negative cats tested positive for parasite DNA. In contrast, of 56 opossums sampled, parasite DNA was found in matched whole blood and cardiac tissue of two (3.6%) individuals, and in 6 (10.7%) anal gland secretions, including the one with positive heart and blood. The three typed anal secretion samples were all TcI- the DTU previously associated with human disease in the US. Ongoing histopathology evaluation of cardiac tissue will identify if cardiac pathology is associated with infection. Given the high abundance of feral cats and opossums in urban foci, it is likely these species serve as wild reservoirs around urban dwellings.

### 1844

#### PILOTING WORKSTATIONS TO IMPROVE HYGIENE PRACTICES AMONG POULTRY WORKERS DURING POULTRY PROCESSING IN A LIVE BIRD MARKET IN BANGLADESH

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In Bangladesh, influenza A/H5N1 has become endemic in poultry and there are 8 human infections reported since 2008. Live bird markets have been implicated as a source of H5N1 and identified as lacking infrastructure required to maintain biosecurity. To reduce risk of environmental contamination and improve handwashing practices, we designed and piloted portable workstations (including a worktop and handwashing facility) in 13 shops in a live bird market in June 2015 and explored their functionality, acceptability and feasibility. We recommended the workers to use the worktop to process birds and wash hands with soapy water after processing each batch of birds. During June-November 2015, we conducted 4 hours of structured observation in 6 shops at each of 3 time points (before intervention, and at 2 weeks and 2 months after intervention) and 8 in-depth interviews to collect users' feedback. On average, we observed 45 batches of birds processed by 2 persons per batch in each 4 hours. Before intervention, we observed 90 handwashing opportunities; handwashing with soap was not observed. Handwashing with soapy water increased to 21% (22/104) at 2 weeks after intervention but then decreased to 2% (1/67) at 2 months after intervention. In all shops, workers used water and soapy water to clean utensils and the worktop to process birds after intervention. Rinsing hands with clean water increased after intervention: 12% (12/104) at 2 weeks and 24% (16/67) at 2 months, whereas rinsing hands with blood-mixed water was observed before intervention. Major barriers identified were difficulty to manage increased water cost by individual shops and inability to frequently wash hands during busy hours. As motivation, workers mentioned that customers preferred shops with workstation and that workers did not have to leave the shop to go to the public toilets for handwashing anymore. The workstations were acceptable, functional, and improved handwashing practices and the use of clean water. However, handwashing decreased over time. A complete solution at the shop level has feasibility concerns; hence a market level slaughtering arrangement may be more appropriate.

### 1845

#### DOG OWNERS' ATTITUDE, A RISK FACTOR FOR HUMAN RABIES IN NIGERIA

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Rabies is an endemic zoonotic disease in Nigeria occurring in dogs, other domestic animals and sometimes man. Elimination of rabies in humans is dependent on effective control in dogs. Diagnostic records of the National Veterinary Research Institute Vom, Nigeria, 2011- 2016 showed 686 rabies positive of 1,226 canine case samples submitted for laboratory investigation. Of the positive cases, 635 involved human exposures. We conducted a field investigation and interviewed 743 dog owners in November 2016 using structured questionnaires and random selection of dog owners to ascertain factors responsible for the high incidences of rabid dog bites, and to assess dog owners' knowledge, attitude and practice towards rabies and dog vaccination in Jos South administrative area of Plateau State (LAT: 9.728; LONG: 8.794); which had the highest number of reported cases. Associations between demographic variables

and knowledge, attitude or practice scores were also assessed using Chi squared analysis. Of the population interviewed, 698 (94%) had knowledge of rabies and 622 (89%) of them know about anti-rabies vaccine. Among those who know about anti-rabies vaccine, 396 (63.7%) know about yearly vaccination while 385 (61.9%) claimed to vaccinate their dogs. From the group that claimed to vaccinate dogs, only 116 (30%) had evidence of vaccination history. Knowledge of yearly anti-rabies vaccination, length of dog keeping and dog management systems were found to be significantly associated with dog vaccination. Independent Population attributable risks associated with non-vaccination of dogs were ignorance (22.4%), carelessness (9.7%) and financial constraints (0.09%). Illiteracy and wrong perception about rabies and dog vaccination are factors that could impact global human deaths due to rabies of which Africa and Asia are known to contribute significantly. Considering that 627 (84.4%) of the survey group did not vaccinate dogs, awareness campaign on rabies, enforcement of responsible dog ownership, and government intervention by mass vaccination of dogs are essential for the control of rabies and to reduce risk of infection in humans in Nigeria.

## 1846

### MARINE MAMMAL STRANDINGS IN PERUVIAN COAST: AN ELEVEN YEARS STUDY

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Marine mammals are considered to be excellent sentinels of environmental health and monitoring reflects the state of the ecosystem in which they inhabit. The presence of marine mammal's stranding are a reflection of the ecosystem situation and an alert of human being, connecting interdependently animal, human and environmental health as a one health. The aim of the study was to describe and analyze the epidemiological characteristics as well as the annual trends of stranding events reported along the Peruvian coast between 2006-2016. The variables analyzed were the number of beached specimens, type of beached specimen, type of stranding and localization where the stranding took place. Stranding reports were found through the Google search engine through algorithms in English and Spanish; and the review of official documents available on the IMARPE (Peruvian Institute of the Sea) website. A total of 22 reported stranding events were found, of which 14 were single individuals and 8 mass events. There were 1746 animals that included Odontocetes (77.3%), Pinnipeds (22.3%) and Mysticetes (0.4%). The largest number of strandings occurred on the north coast of Peru, with a total of beached animals ( $n = 1739$ ) and stranding events ( $n = 15$ ). Observing the seasonality of these events, the greatest number of animals has been reported in the summer months, with a total of beached animals ( $n = 1737$ ) and stranding events ( $n = 13$ ). All mass stranding events were reported in summer and on the north coast. All the years included in the study presented at least one stranding event, except for 2007 and 2016. Reports with the largest number of beached animals occurred in 2009 ( $n = 266$ ), 2012 ( $n = 965$ ), 2013 ( $n = 98$ ) and 2014 ( $n = 410$ ). The data obtained from the mentioned websites were consistent with the data of the IMARPE reports, for each stranding event. Understanding the patterns of temporality of the occurrence of these events will be useful to establish future conservation and prevention strategies. This work is an important step in future research to monitor and associate the consequences of climate change on marine ecosystems and human populations.

## 1847

### RESEARCH ON ZOOONOTIC AND NEGLECTED DISEASES IN CHAD: CASE OF TUBERCULOSIS, RIFT VALLEY FEVER, BRUCELLOSIS AND Q FEVER

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In 2012, Cross-sectional studies were conducted by staffs from Chadian Animal and Public health systems in order to point out the importance of TB and RVF, Brucellosis, Q fever. From 478 TB suspected patients, sputum samples were collected on Cethyl pyridinium (5%) and sent to IRED for bacteriological and molecular diagnostic. Seventy seven were identified as Acid Fast Bacilli (AFB) infection cases, from which, 71 were from genus mycobacteria: 42 *M. tuberculosis*, 1 *M. bovis*, 1 *M. avium* and 30 non tuberculosis mycobacteria. Ten suspected cases were due to other mycobacteria. From 332 carcasses suspected as TB cases, 236 were identified as AFB infection cases within which only 66 were characterized as belonging to the genus Mycobacterium: one case of infection was due to *M. tuberculosis*, 44 to *M. bovis*, and 11 to Non Tuberculosis Mycobacteria. From 924 sera collected in Lake Chad region, ELISA test showed that RVF apparent prevalence of 32.9% (29.9 - 35.9) at 95% CI was identified among ruminants. The prevalence by species was 37.8% (34.2%-41.3%) in cattle, 18.8% (12.3%-25.3%) in sheep and 10.8% (3%-18.5%) in goats. For a total number of 561 cattle, 7.8 % (5.6 - 10.1) of Q fever and 11.9% (9.3 - 14.6) of Brucellosis cases were detected. Our main findings were the isolation of *Mycobacterium bovis* from human and *Mybacterium tuberculosis* from animal. A co-infection *M. bovis* - *M. avium* in human was identified highlighting the importance of the zoonotic aspect of mycobacterial diseases in Chad. Importance of other mycobacteria as causes of lung infections in human and animal have to be more investigated. Suspicions of RVF, Q fever and Brucellosis from domestic animal are at risk for human population in Lake Chad region.

## 1848

### EMERGING INFECTIOUS DISEASES PREDICTION: A STUDY ON IxODES SCAPULARIS-BORNE PATHOGENS

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Vector-borne diseases (VBDs) are the most common types of emerging infectious diseases and constitute major threats to public health. Understanding the interplay between the environment, vectors, pathogens, and humans that expedite pathogen population growth or range expansions remains a challenge. The rapid increase in availability of ecological information has led to "big data" opportunities and challenges in disease surveillance. The goal of this study is to build geospatial models to predict disease risk by accounting for the interaction of human characteristics and ecological factors, using multiple analytical frameworks. We have previously identified some of the environmental features that have influenced population dynamics on *I. scapularis*, but not on the pathogens specifically, using conventional regression methods. In this study, we focus on pathogens transmitted by the black-legged tick, *Ixodes scapularis*, including human granulocytic anaplasmosis (HGA), babesiosis, and Lyme disease. We are investigating the entomological, environmental, and human demographic factors that determine tick-borne disease risk using multiple regression, stochastic search variable selection (SSVS), and boosted regression trees (BRTs) analysis. Using SSVS, a big-data Bayesian analytic approach, allows us to identify that factors that influence the population dynamics of tick-borne pathogens due to its high precision and statistical power. SSVS addresses many of the limitations of multiple regression methods to identify variables of both practical and statistical significance. Preliminary coarse-scale analyses suggest that human incidence rates of each pathogen are linked to both age and gender. BRTs are highly effective for pathogen prevalence and disease predictions in addition to the identification of important environmental factors. We will

present these results as well as a comparison of the predictive power of these models at the ASTMH meeting in November 2017. Our preliminary studies demonstrate that these models effectively forecast disease risk over time and space with greater accuracy than conventional methods.

## 1849

### CATTLE-ASSOCIATED RISK FACTORS FOR HUMAN TUBERCULOSIS IN RURAL LIVESTOCK KEEPING COMMUNITIES, UGANDA

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Tuberculosis (TB) is a leading infectious cause of human death worldwide. TB can also infect cattle, resulting in productivity losses, trade barriers, and zoonotic transmission via milk, meat, or direct contact. While the majority of TB cases are non-zoonotic, an unknown proportion are acquired from cattle; in Africa, this proportion is estimated to be 0.4% to 10%. We conducted a cross-sectional study in rural communities in southeastern and northwestern Uganda between 2014 and 2016 to evaluate the association between tuberculosis skin test (TST) positivity in humans and cattle-associated risk factors. Human and cattle skin testing was performed in communities followed by a survey of household practices. TST data are available on 493 humans, 250 men and 243 women; 184 individuals in total—111 men and 73 women—tested positive. Separate log binomial models were fit to estimate relative risks (RR) for herd TST positivity stratified on gender and for raw milk consumption, using generalized estimating equations. Having at least one TST positive bovid in the household's herd was significantly associated with decreased risk of TB among men (RR 0.61, 95% CI 0.47, 0.79) but was not significantly associated with TB among women (RR 1.26, 95% CI 0.80, 1.97), contrary to the hypothesized higher risk among men, the primary caretakers of cattle. This apparent protective effect may be the result of residual confounding by socioeconomic status: wealthier individuals may be less likely to be TB positive, but more likely to have TST positive herds by virtue of larger herd sizes, ability to purchase new and possibly infected stock, and propensity to keep more TB-susceptible European breeds. No significant effect was found for raw milk consumption, and adjustment for confounders or pathways mediated by non-zoonotic TB exposure did not change effect estimates. The importance of cattle-associated risk factors for human TB burden may be setting-specific, as suggested by the lack of consensus reached by prior research. In settings where bovine TB prevalence is low, such as Uganda, cattle-associated zoonotic transmission may be rare.

## 1850

### ANTIMICROBIAL RESISTANCE TRANSMISSION ASSOCIATED WITH SMALL-SCALE FOOD-ANIMAL PRODUCTION IN PERI-URBAN COMMUNITIES OF QUITO, ECUADOR

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Small-scale food animal production is widely practiced around the globe, yet it is often overlooked in terms of the public health risks. Evidence suggests that small-scale food animal producers regularly employ the use of antimicrobials to improve the survival and growth of their animals, and that this practice leads to the development of antimicrobial resistance (AMR) that can potentially spread to humans. We conducted an analysis of AMR among *E. coli* isolates present in fecal samples from young children (n=64) and domestic animals (n=174) living in the same neighborhood. In this analysis, we selected one dominant *E. coli* isolate from each fecal sample to assess AMR transmission. Thirty-five children (56%) and 46 domestic animals (26%) carried MDR *E. coli* (defined as resistance to  $\geq 3$  classes of antimicrobials). Three children and 5 food-animals carried *E.*

*coli* that were resistant to more than 9 of the 12 antibiotics tested using CLSI guidelines. In the relatively small set of *E. coli* isolates analyzed, we found evidence that animal and human *E. coli* isolates are recently linked clones that have changed slightly in resistance phenotype. Three isolates (one from a child and two from animals) were resistant to eight of the same drugs, but among those only two animal isolates shared the same sequence type, suggesting that horizontal gene transfer could be driving AMR gene movement. These same three isolates were resistant to cefotaxime, a third-generation cephalosporin. This study elucidates the mechanisms of AMR transmission (i.e. clonal spread versus horizontal gene transfer) and provides an understanding of the driving factors for use of antimicrobials in small-scale food-animal production. This research will have important implications in low- and middle-income countries where small-scale food animal production is often promoted to reduce poverty and food insecurity.

## 1851

### "LET'S GET THIS TICKING TIME BOMB!"

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Tick borne diseases cause high morbidity and mortality in dogs in the Caribbean. *Rhipicephalus sanguineus*, is known to be a main vector for *Ehrlichia canis*, *Babesia canis* and may also be implicated in the transmission of *Bartonella henselae*, *B. vinsonii berkhoffii* and *B. clarridgeiae*. These organisms cause a myriad of non-specific clinical signs in dogs and humans including pyrexia, anorexia, joint pain and swelling. Bartonellosis is an emerging and potentially hidden epidemic affecting dogs and humans and is considered especially important as opportunistic pathogens. It is also associated with cardiac arrhythmias and endocarditis in dogs. *Borrelia burgdorferi*, the vector for Lyme Disease is transmitted by *Ixodes scapularis*. Erythema Migrans-like Illness have been documented in Caribbean islanders however to date there has been no comprehensive study to detect these agents in both dogs and humans. We suspect that cases of dogs which were unsuccessfully treated for Ehrlichiosis or Babesiosis, were possibly undiagnosed Bartonellosis and that veterinarians, owners and animal handlers have been exposed to tick borne diseases. This study aims to detect the presence of *E. canis*, *A. phagocytophilum*, *Babesia spp.*, *B.v.berkhoffii*, *B. burgdorferi* in dogs and humans. High risk groups in Trinidad and Tobago, Barbados, St Maarten and Jamaica will be sampled for TBD, in addition all dogs with endocarditis will be investigated for tick borne illnesses. In human cases, a questionnaire will be administered and a blood sample taken to test for these organisms. We suspect that these organisms will be definitively diagnosed in humans and dogs in the Caribbean Islands.

## 1852

### THE ROLE OF ANTHROPOGENIC LAND-USE CHANGE IN DRIVING DISEASE EMERGENCE IN HIGHLY-COUPLED VECTOR-HOST SYSTEMS: ZONOTIC CUTANEOUS LEISHMANIASIS AS A CASE SYSTEM

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The role of the reservoir host is often neglected when studying vector-borne zoonoses. The classical Ross-MacDonald model predicts positive effect of vector density on disease spread rate but inverse relationship with respect to host density. This model, originally developed in the context of Malaria, assumes that with the exception of obtaining a blood-meal, vector's and host's life-cycles are largely independent of one another. However, in systems where the host either constitutes or provides a habitat for the vector (hereafter "coupled vector-host systems") more complex relationship are expected. In this talk, we present the results of an individual-based simulation model that evaluates the effect of the vector's host-dependence on epizootiological patterns within the host

population. We demonstrated that, in contrast to the predictions of the Ross-MacDonald model, in coupled systems hump-shaped or positive association between host abundance and infection prevalence could occur. This mechanism suggests that anthropogenic land-use change affecting reservoir host density could drive disease emergence in competent ("source") or sub-competent ("sink") reservoir host systems. Empirical evidences from Zoonotic Cutaneous Leishmaniasis in Israel support this prediction: Infection occurrence in the fat sand rat (*Psammomys obesus*) was found to be positively correlated with its density. Epidemic emergence in the new sub-competent host system of *Meriones tristrami* is also suggested to be associated with such a density-dependent effect. Therefore, in vector-host systems characterized by high degree of coupling host-culling becomes a viable control option. But even more exciting, in this kind of system, systemic control becomes a particularly promising control alternative. We report results of a systemic control in a lab study with *P. obesus* and in the field in Israel with *M. tristrami*.

## 1853

### MATHEMATICAL MODELLING OF DOG RABIES TRANSMISSION IN AN AFRICAN CITY

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Rabies is a viral disease that affects the central nervous system of humans and other mammals. The disease is transmitted by bite and is fatal after the onset of symptoms. Rabies is endemic in many countries, where the domestic dog is the primary vector, and causes approximately 59,000 human deaths worldwide annually. Two mass vaccination campaigns of dogs were conducted in N'Djamena, Chad, in 2012 and 2013, reaching a vaccination coverage of more than 70% in both years. The campaigns interrupted transmission for several months, but recently there has been a resurgence in cases, earlier than suggested by previous homogeneous models of rabies transmission. We developed new population-based and individual-based models to explore possible causes for this resurgence and to simulate the effects of different interventions on dog rabies transmission. We calibrated model parameters to four years of geolocated weekly incidence data of dog rabies and subsequent human exposure. For the individual based models, we used additional data on dog movement and contact patterns from GPS collars. We analysed the models to derive threshold conditions of vaccination coverage and importation of latent dogs, and conducted simulations to assess the long term effects of different vaccination scenarios. Our analysis suggests that underreporting of rabies cases is unlikely to lead to the early resurgence seen in N'Djamena. Incorporating heterogeneity showed that the reproductive ratio was underestimated in the previous homogeneous models, but our simulation results suggest that while spatial heterogeneity and the decrease of the vaccination coverage due to population turnover seemed to facilitate the fast re-establishment of rabies in N'Djamena, the main cause for the recent cases was the importation of latent dogs. Therefore, to sustain elimination of rabies after the vaccination campaigns, reintroduction of rabid dogs from the surroundings of the city would need to be prevented through alternative strategies, such as the vaccination of surrounding areas.

## 1854

### NEEDS ASSESSMENT AND ALTERNATIVE STRATEGIES TO ACHIEVE THE ELIMINATION OF DOG-MEDIATED HUMAN RABIES DEATHS BY 2030 BASED ON DOG VACCINATION

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Every year, rabies kills about 60,000 people globally; most (~99%) originate in domestic dogs. The WHO and collaborating agencies have set the goal of eliminating dog-mediated human rabies deaths globally by 2030. This goal could be achieved by providing massive post-exposure prophylaxis, with ever increasing costs; through mass dog rabies vaccination, requiring a sustained effort and long-term planning; or by a combination of both strategies. Drawing from multiple datasets and using a country-level analysis, we estimated the years and resources required to achieve global dog rabies elimination based on four factors: country development status, dog vaccination costs, availability of dog rabies vaccine, and estimated number of vaccinators. Following WHO, we assumed that vaccinating 70% of the dog population for 7 consecutive years would eliminate dog rabies. Using 2015 dog vaccination rates, we estimated a cumulative global shortage of ~7.5 billion doses to eliminate dog rabies in endemic countries. We estimated a vaccination cost of \$6,300 million to eliminate dog rabies in all endemic countries, or a \$3,900 million gap, compared to current spending. The estimated vaccination workforce may suffice to eliminate dog rabies, only if all public health veterinarians in endemic countries dedicated 3 months to dog rabies vaccination annually. We discuss implications of plausible technology improvements, including population management, vaccine price, and increases in dog-vaccinating capabilities. We suggest pragmatic and feasible options toward dog rabies elimination by 2030, while identifying several benefits and drawbacks of specific approaches. In addition to providing global estimates, we created a user-friendly tool to assist countries in their long-term planning efforts. The tool allows users to estimate the required resources to achieve rabies elimination using country-specific input values. Comparable, systematic, quantitative estimates are important to inform the discussion about global and regional strategic planning and resource mobilization towards the goal of dog rabies virus elimination.

## 1855

### THE COST-EFFECTIVENESS OF A NOVEL INTEGRATED BITE CASE MANAGEMENT PROGRAM FOR THE CONTROL OF DOG-MEDIATED HUMAN RABIES, WEST DEPARTMENT, HAITI, 2014-2015

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Haiti has the highest burden of rabies in the Western hemisphere, with 130 estimated annual deaths. As in many developing countries, most human and dog rabies cases are not recognized and are not reported to health authorities, thus limiting rabies awareness, funding, and prevention efforts. We present the cost-effectiveness evaluation of an integrated bite case management program combining community bite investigations and passive animal rabies surveillance, using a governmental perspective. In collaboration with the CDC, the Haitian government initiated in 2013 the Haiti Animal Rabies Surveillance Program (HARSP), a form of integrated bite case management combining community bite investigations and passive animal rabies investigations to provide tailored rabies risk assessments for persons potentially exposed to the rabies virus. The

Haiti Animal Rabies Surveillance Program (HARSP) was first implemented in three communes of the West Department, Haiti. Our evaluation encompassed all individuals exposed to rabies in the study area (n=2,289) in 2014-2015. Costs (2014 US dollars) included diagnostic laboratory development, training of surveillance officers, operational costs, and post-exposure prophylaxis (PEP). We used estimated deaths averted and years of life gained (YLG) from prevented rabies as health outcomes. Our results suggest that HARSP had higher overall costs (range: \$39,568-\$80,290) than the no-bite-case-management (NBCM) scenario (\$15,988-\$26,976), partly from an increased number receiving PEP. But HARSP had better health outcomes than NBCM, with estimated 11 additional annual averted deaths in 2014 and 9 in 2015, and 654 additional YLG in 2014 and 535 in 2015. Overall, HARSP was more cost-effective (US\$ per death-averted) than NBCM (2014, HARSP: \$2,891-\$4,735, NBCM: \$5,980-\$8,453; 2015, HARSP: \$3,534-\$7,171, NBCM: \$7,298-\$12,284). HARSP offers an effective human-rabies prevention solution for countries transitioning from reactive to preventive strategies (i.e., comprehensive dog vaccination).

## 1856

### ONE HEALTH APPROACH TO COST-EFFECTIVE RABIES CONTROL IN INDIA

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Over 20,000 rabies deaths occur annually in India, representing one-third of global human rabies. The Indian state of Tamil Nadu has pioneered a "One Health" committee to address the challenge of rabies in dogs and humans. Currently, rabies control in Tamil Nadu involves post-exposure vaccination of humans following dog bites, while potential supplemental approaches include canine vaccination and sterilization. We developed a data-driven rabies transmission model, fit to human rabies autopsy data and human rabies surveillance data from Tamil Nadu. Integrating local estimates for canine demography and costs, we predicted the impact of canine vaccination and sterilization on human health outcomes and evaluated cost-effectiveness according to WHO criteria for India, which correspond to thresholds of \$1582 and \$4746 per DALY for very cost-effective and cost-effective strategies, respectively. We found that highly feasible supplemental strategies focused on stray dogs, vaccinating as few as 7% of dogs annually, could very cost-effectively reduce human rabies deaths by 70% within five years, and a modest expansion to vaccinating 13% of stray dogs could cost-effectively reduce human rabies by almost 90%. Through integration over parameter uncertainty, we find that for a cost-effectiveness threshold above \$1400 per DALY, canine interventions is at least 95% likely to be optimal. If owners are willing to bring dogs to central-point campaigns at double the rate that campaign teams can capture strays, expanded annual targets become cost-effective. This case study of cost-effective canine interventions in Tamil Nadu may have applicability to other settings in India and beyond.

## 1857

### THE CONTROL OF ZONOTIC VISCERAL LEISHMANIASIS IN EUROPE

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Zoonotic visceral leishmaniasis (ZVL) is a tropical infection transmitted by female sandflies that may infect dogs, humans, and wildlife. In the last decade the disease prevalence has increased fivefold in several parts

of southern Europe, where currently an estimated 2.5 million dogs are infected. The main drivers of transmission are the increase in sandfly distribution due to climate change and the traveling and migration of dogs. Many international organisations have created guidelines, providing protocols describing intervention strategies to control the spread of this tropical infection into Europe. In this study, we research whether these guidelines are being implemented in the field to control the further spread of ZVL. We conducted a survey consisting of 24 questions that we distributed via many online platforms and through multiple mailing lists to reach practicing veterinarians in Spain and France. The questions tested their 1) awareness of the spread and public health risks of ZVL in Europe, 2) awareness of the international guidelines, 3) type of protocol used when suspecting and confirming a ZVL case, and 4) their reporting of confirmed cases. 889 veterinarians participated in the survey of which 616 were Spanish (64%) or French (36%). Even though 60% of them were aware of the current increase of ZVL in Europe, 70% were not aware of any guidelines. However, most of their preventive and treatment actions were in line with the intervention strategies as advised in the guidelines' protocols, apart from the fact that only 12% of veterinarians would report a confirmed case, including notifying colleagues within the same practice. In conclusion, we suggest that an easy online international network is founded where both veterinarians as well as general practitioners report confirmed cases of ZVL. This will be crucial for monitoring, control and preventing the further spread of this tropical zoonotic infection into Europe at the regional, national and international level.

## 1858

### HOUSEHOLD PARTICIPATION IN PIG AND HUMAN INTERVENTIONS FOR CONTROL OF *TAENIA SOLIUM* AND LIKELIHOOD OF CONTINUED TRANSMISSION A YEAR LATER IN NORTHERN PERU

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*Taenia solium* is one of the leading causes of adult onset epilepsy in the world. Mass screening and treatment interventions in humans and pigs are core components of strategies to control this zoonosis, but participation is rarely if ever complete. Few studies have explored whether participation influences future risk of parasite transmission at the household level. In this analysis, we take advantage of the sequential inclusion of 4 communities in two studies to evaluate the relationship of household participation in control interventions and risk of continued *T. solium* transmission 16 months later measured by seropositivity in pigs using LLGP EITB. Mass screening for taeniasis and purchase of seropositive pigs was conducted in August 2015 in 4 communities to end an education-based intervention, and in December 2016 all pigs in these communities were serotested using EITB as part of the baseline for a new intervention. Household data from the two studies were matched resulting in a total of 99 households that raised pigs in 2016. We evaluated the effect of prior participation in 2015 on household pig seropositivity in 2016 expressed as an odds ratio. Although results were not statistically significant, point estimates suggest that those with incomplete participation in mass screening for taeniasis were more likely to have a seropositive pig (OR=1.79[.76, 4.19]). The magnitude of this association was greater in those with incomplete participation in mass screening who also retained a seropositive pig (OR=2.33[.47, 11.46]). These results suggest that there may be a decreased risk of exposure for households participating fully in intervention, and that full household participation should be encouraged. This preliminary finding warrants future studies that are sufficiently powered to describe this possible risk.

### REEMERGENCE OF CANINE RABIES IN COMPLEX URBAN ENVIRONMENTS: LESSONS FROM AN OUTBREAK IN AREQUIPA, PERU

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The city of Arequipa, Peru is in the midst of an urban rabies epidemic that was identified in March 2015. This is a rare case worldwide of reintroduction of canine rabies into an area that had previously been declared free of transmission. Continued transmission in dog populations puts the one million inhabitants of the city at risk of infection. During the last two years we have conducted dog GPS-tracking and community-based studies to understand the ecological and social drivers of the reemergence and persistence of the rabies virus. We found that the urban layout dictates the presence of rabid dogs: cases are detected closer to some urban structures—dry water channels—than expected by chance (mean distance = 335m;  $p=0.027$ ). Also, movement of free-ranging dogs is highly heterogeneous, temporally and spatially, suggesting that the ring containment practiced by health authorities might not be effective. Mass dog rabies vaccination campaigns have been conducted but do not reach the appropriate coverage. We coupled a qualitative study and a quantitative survey on more than 5,400 houses to determine factors associated with vaccination coverage. The survey obtained demographic variables of dog owners, household level variables (e.g. age of the house), dog care practices within the house, and knowledge about the vaccination campaign. All houses were georeferenced. Importantly, our spatial analysis shows significant pockets of unvaccinated dogs threatening to maintain transmission of the virus and these pockets aggregate significantly the further the campaign is located. Distance to the vaccination campaign reduces greatly the odds of dogs being vaccinated (OR = 0.71 for every 100m;  $p<0.001$ ), after controlling for other variables. In addition, the level of urbanization and the city layout also influence people's preferences on the location of the vaccination campaign. The reemergence of rabies in Arequipa is the result of complex urban socio-ecological drivers that call for the implementation of a One Health approach to halt rabies virus transmission.

### ANTIMYCOBACTERIAL EFFECT OF VARYING CONCENTRATIONS OF E559: A NATURAL PLANT PRODUCT IN NIGERIA

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The global tuberculosis (TB) report for 2016 revealed that Nigeria is listed among the high burden countries (HBCs) for TB, HIV/TB and MDR-TB (multidrug-resistant TB). In a bid to proffer an in-house solution with global relevance, the current study was carried out to explore the potential of a plant extract, E559 at varied concentrations against local and standard strains of *Mycobacterium tuberculosis* (MTB). The golden standard procedure for drug susceptibility testing on Löwenstein Jensen (LJ) media using the proportion method was employed in a Biosafety Level-3 facility for antimycobacterial screening. Phytochemical screening was also carried out. Phase I extract concentrations was between 1 and 250 µg/ml while that of phase II was > 1000 µg/ml. The extracts were prepared with the LJ media and subsequently inoculated with 10-3 and 10-5 suspensions of both local (MTB-584) and standard (H37Rv) strains

of *Mycobacterium tuberculosis*. Rifampicin served as the standard drug for positive control while inoculated media was taken as negative control. The inoculated LJ media and control slants were incubated at 37°C and observed every seven days for six weeks. The antimycobacterial screening result showed that both local and standard strains displayed resistance (> 200 colonies) to the extracts at Phase I concentrations (between 1 and 250 µg/ml) evidenced by the ample growth with typical creamy non-pigmented morphology on all the LJ media prepared with extracts. However, there was no single growth (zero colony: 0%) up to six weeks on all the LJ media prepared with > 1000 µg/ml of E559; compared to the substantial growth observed on the negative control (150 and 500 colonies respectively for MTB-584 and H37Rv: 100%). Alkaloids, phenols, steroids, tannins and terpenoids were present in the extract. It can therefore be inferred that elevated crude concentrations of E559 possibly possess strong antimycobacterial activity which can give rise to pure lead bioactive compounds against MTB while activity is minimal at minute concentrations.

### EVALUATION OF A LOW-COST AIR SAMPLING SYSTEM FOR THE DETECTION OF MYCOBACTERIUM TUBERCULOSIS IN COUGHING PATIENTS

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*Mycobacterium tuberculosis* (TB) aerosol droplets pose significant occupational risks, particularly to healthcare workers in low and middle-income countries. Improvement of airborne TB detection could have broad impacts on global TB control. Our study evaluated an air sampling system to assess active TB aerosols from coughing patients with active pulmonary TB. We enrolled subjects with 2+ or 3+ auramine sputum smear in Lima, Peru, and collected paired sputum samples and cough frequency data. Air-sampling was performed over a 15 minute period of maximally induced cough via a portable 2L/minute vacuum pump onto a PTFE filter with 0.3µm pores. Samples were taken on day 0 and 14 of treatment and at 1m distance from the patient's face in an airborne infection isolation room. In parallel, we performed serial dilutions of 1 McFarland TB and fungal contaminant suspension overlaid on filter material, to define the thresholds of detection of TB on this medium. The filters were subsequently tested with GeneXpert PCR, culture using Middlebrook 7H9 broth, culture with a trisodium phosphate decontamination, and culture with NaOH decontamination. Cultures were assessed up to 8 weeks or until positivity. Our final cohort included 7 patients, of which 5 were 3+ auramine smear and 1 was multi-drug resistant. Clinical samples revealed no positive GeneXpert PCR nor positive culture. A validation lab study revealed threshold of detection of the filters by GeneXpert was 1 in 10 colonies of TB, while by culture was 1 in 100 colonies of TB, suggesting our clinical samples were below this threshold of detection. Growth of TB on filters notably decreased over time, compared to control, suggesting PTFE filters were inhibitory to growth. Decontamination of was also less effective on filters. Our study supports existing hypothesis that PCR can be used to determine TB microbial load from air filters. Assessment of active infectious TB requires culture, which remains difficult due to issues of fungal contamination and poor TB growth on PTFE filters. Future studies will assess broader options for low-cost aerosol filters to assess patient infectivity and occupational risk.

## 1862

**CO-INFECTION AS A RISK FACTOR FOR DISEASE SEVERITY AMONG PATIENTS WITH ADENOVIRUS INFECTION**

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Adenovirus (AdV) infection results in a broad range of clinical manifestations, with illness severity ranging from asymptomatic/mildly symptomatic to severe/life-threatening. AdV-infected individuals with co-infections (AdV-CO) may have more severe illness, but relevant data are limited. To analyze co-infection as a risk factor for more severe AdV infection, we analyzed data from an acute febrile illness surveillance system in southern Puerto Rico which enrolled patients presenting with fever for <7 days. Naso/oropharyngeal samples were tested for AdV and up to 12 other respiratory viruses. Serum samples were tested for dengue, chikungunya, and Zika viruses. This analysis included all subjects enrolled between May 2012 and May 2016 in whom AdV was detected by PCR in naso/oropharyngeal secretions. Of 497 patients with AdV infection, 92% were <18 years old and 48% were male. In total, 43 (9%) patients had AdV-CO, most of whom (75%) were <14 years of age. All co-infections were viral: influenza A (n=10; 33%), respiratory syncytial virus (n=8; 26%), dengue (n=7; 16%), chikungunya (n=6; 14%), human metapneumovirus (n=3; 10%), parainfluenza-3 (n=3; 10%), human coronavirus HKU1 (n=2; 7%), influenza B (n=2, 7%), and parainfluenza-1 (n=2, 7%). The proportion of patients with rhinorrhea, chills, conjunctivitis, nausea and anorexia did not differ significantly between the two groups ( $\geq 0.08$ ), but a higher proportion of AdV-CO participants (83% vs. 63%) reported cough ( $p=0.007$ ). Patients with AdV and AdV-CO did not differ significantly according to proportion hospitalized [115 (25%) versus 9 (21%), respectively ( $p=0.52$ )] or duration of hospitalization (3.7 days versus 4.3 days, respectively ( $p=0.56$ )). In summary, with the exception of cough occurring more frequently in AdV-CO patients, AdV and AdV-CO patients had similar clinical manifestations. Similarly, AdV and AdV-CO patients did not differ significantly in terms of the proportion hospitalized and the duration of hospitalization. Additional analyses are underway to further evaluate co-infections as well as other potential risk factors for severe AdV infection.

## 1863

**MEASUREMENT OF POA EFFLUX PUMPS RATE IN MYCOBACTERIUM SMEGMATIS STRAINS OBTAINED BY GENE KNOCKOUT**

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Pyrazinamide (PZA) is a first-line drug used in the treatment of tuberculosis. Inside the cytosol, the bacterial nicotinamidase (PZAse) transforms the PZA into pyrazinoic acid, POA is expelled from the bacteria by an uncharacterized efflux-pump to the external environment causing cytoplasm acidification and lethal disruption of the membrane transport. We evaluated 9 potential candidate genes to encode POA efflux pump in *Mycobacterium tuberculosis*, based on a bioinformatic study. For this purpose, we knocked-out homologous genes in *M. smegmatis*, which has a highly active POA efflux pump. Knockout of these genes were done using a two-step strategy using p2NIL/pGOAL vectors. POA efflux rate were estimated by measuring extracellular POA after incubation with PZA (3 repetitions were performed). Finally, mutant of *Mycobacterium smegmatis* strains were analyzed for its PZA level of resistance by minimum inhibitory concentration (MIC). Knockout of the POA efflux pump decreased the rate of pumping pyrazinoic acid outside the *M. smegmatis* below its normal value only in genes MSMEG 3815 (Gene Rv1634 in MTB)

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and MSMEG 0410 (Gene Rv 1183 in MTB). Rates were 1023.82; 1162.40 and 2307.805 nmol POA/mg protein/min for MSMEG 3815, MSMEG 0410 and Wild Type *M. smegmatis* strain, respectively. These rate differences were statistically significant. *M. smegmatis* mutant strains with altered POA efflux rate below its normal value (MSMEG 3815 and MSMEG 0410) did not show changes for its PZA level of resistance. We obtain viable mutant strains with deletion of individual POA efflux pump genes from the chromosome of *M. smegmatis* with changes in their POA efflux rate below its normal value (MSMEG 3815 and MSMEG 0410).

## 1864

**CARETAKERS PERSPECTIVES OF PEDIATRIC TUBERCULOSIS AND IMPLICATIONS FOR CARE SEEKING BEHAVIORS IN SOUTHERN MOZAMBIQUE**

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Tuberculosis remains an important public health problem, especially in poorly resourced settings. Lack of knowledge and awareness of the disease compromises prompt diagnosis and treatment compliance. To gain insights regarding caretakers knowledge of the aetiology and prevention of paediatric TB, to describe their care seeking behaviours and to assess the acceptability of diagnostic procedures. This study was carried out in Manhica and Macia district in Mozambique using qualitative methods. The qualitative data were collected through an interview guide. All underwent the same semi-structured interviews, the results of which were analysed and compared using content analysis. 11 were caretakers of children diagnosed with TB at the health facility, 11 of children for whom TB was discarded at the health facility and 13 of children with TB compatible symptoms identified in the community. The first 2 groups took part in a TB incidence study, while the third group did not. All underwent the same semistructured interviews, the results of which were analysed and compared using content analysis. Even when confronted with signs suggestive of TB, most caretakers never suspected it or misinterpreted the signs, even among TB positive caretakers and those with TB contacts. There was limited knowledge of TB, The transgression of social norms was often presented as an explanation for TB. The use of traditional care for prevention is widespread, but it varied for treatment purposes. TB diagnostic procedures were considered painful but were unanimously tolerated. Caretakers have low knowledge of TB, seldom suspect paediatric TB, which is often confounded by other illnesses. Moreover, the causes of TB are also mixed with traditional concepts. Health seeking is complex and alternates between conventional and traditional health care providers. TB diagnostic procedures were accepted and tolerated by caretakers regardless of the transitory feelings of fear they elicited. Misconceptions of paediatric TB, associated complex care seeking itineraries and negative feelings of the diagnostic procedures may result in delays, and lost to follow up.

## 1865

**EFFECTIVENESS OF PCV-10 VACCINE AGAINST VACCINE TYPE IPD IN PAKISTAN: IMPACT ASSESSMENT AFTER INTRODUCTION OF PCV-10 IN ROUTINE IMMUNIZATION PROGRAM**

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Pakistan is the first country in South Asia to introduce Pneumococcal Conjugate Vaccine (PCV) in its National Expanded Program on immunization (EPI). Impact assessment of this program is essential for its continuity and to guide other countries in the region regarding introduction of PCV in their EPI programmes. The primary objective of

this study is to assess PCV-10 effectiveness on invasive pneumococcal disease (IPD) due to vaccine serotypes of pneumococcus. A matched case-control study is enrolling children eligible to receive PCV-10 vaccine who present with meningitis and/or radiologically-confirmed pneumonia at 15 hospitals serving low and middle income population in Sindh province of Pakistan. In order to detect IPD proportion of these cases, *lytA* gene is tested by PCR in blood (for radiologically proven pneumonia) and CSF (for purulent meningitis). The proportion of IPD due to vaccine type serotypes is determined through serial multiplex PCR. Five controls are enrolled for each case of vaccine type IPD, matched by age, catchment and season. Enrollment started in July 2013. Of the required sample size of 28 vaccine type IPD cases, 24 cases have been enrolled so far. This includes 17 cases of meningitis and 7 cases of pneumonia. 134 controls have been enrolled for these cases. Estimated PCV-10 vaccine effectiveness (VE) against vaccine type IPD was 70.1% with at least one dose of vaccine and 77.0% for at least two doses of vaccine. The study points towards high effectiveness of PCV-10 vaccine against vaccine type IPD in children in Pakistan.

## 1866

### IMPACT OF PNEUMOCOCCAL CONJUGATE VACCINE (PCV-10) ON PNEUMOCOCCAL NASOPHARYNGEAL CARRIAGE IN CHILDREN IN PAKISTAN: RESULTS OF SERIAL SURVEYS PRE AND POST INTRODUCTION OF VACCINE IN ROUTINE IMMUNIZATION PROGRAM

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Pakistan introduced PCV-10 vaccine in EPI programme in April 2013 in Sindh province. We hypothesized that with increase in coverage of PCV-10; there will be decrease in nasopharyngeal carriage of pneumococcal serotypes which are included in PCV-10. Aim of the study was to determine if there is serial decline in carriage among children of age less than five years, after introduction of PCV-10 in routine immunization programme. Yearly cross sectional surveys were planned over the past five years, one before and four after the introduction of PCV-10 in EPI programme. Nasopharyngeal swabs were obtained from representative randomly sampled healthy children in one urban and one rural district of Sindh. Swabs were collected in STGG media and Pneumococci were identified through routine microbiology. Serotypes were identified through real-time sequential multiplex PCR using Centre of Disease Control (CDC) scheme. Each year 220 children of age 3-12 months were enrolled from one rural and one urban district. In rural district, PCV-10 serotypes reduced from 34% in first round to 16% in fourth round among children of age 3-12 months. While in urban district, it remained stable from 30% in first round to 35% in fourth round. Most common PCV-10 serotypes in rural district included 23F, 14, 9V and in urban included 6B, 19F and 23F. While there has been significant reduction in nasopharyngeal carriage of PCV-10 serotypes in rural district, there has been no significant change in urban district after introduction of vaccine. Data from subsequent round may provide better understanding about impact of PCV-10 on nasopharyngeal carriage of serotypes.

## 1867

### FOOD SECURITY AND DIETARY INTAKE OF TUBERCULOSIS PATIENTS IN LIMA, PERU

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Food insecurity and malnutrition are risk factors for contracting tuberculosis (TB), and for failing to complete treatment. In Lima, Peru, TB is often perceived by the community as a nutritional disease. It has been reported that people are more likely to seek screening for tuberculosis because of weight loss than because of cough, and contracting TB is often attributed to poor dietary habits. However, relatively few studies have investigated how these beliefs impact patients' dietary preferences during treatment. To characterize the dietary preferences of recently diagnosed TB patients, we conducted a mixed-methods pilot study in the east of Lima. Structured questionnaires were administered to 45 patients to characterize food security and socio-economic status at baseline and after one and two months of treatment. At one month, twenty-four hour dietary recalls were administered to determine average daily intake of macro and micro-nutrients. Qualitative key informant interviews were also conducted with a subgroup of 9 participants. An equal proportion of patients were overweight, as were underweight, at baseline (14%). Potential micro-nutrient deficiencies, estimated based on the percent of patients eating below estimated average requirements, were common. Qualitative results suggest that patients made active efforts to improve their diets during treatment, were receptive to nutritional information received through their participation in the national TB program, and actively sought out additional nutritional information from other sources. However, misinformation and increased spending on un-evidenced "nutritional" products was also common. Recall data suggested that patients ate an average of approximately 1000 calories/day in excess to what was needed to maintain their current weight, and that most of these additional calories came from carbohydrates. Our results suggest that TB patients in Lima have both positive dietary preferences, that should be reinforced, but are also at risk of misinformation. Dietary guidelines should promote strategies to improve dietary quality through low-cost and locally available foods.

## 1868

### ANTIMICROBIAL RESISTANCE PATTERNS OF COLONIZING STREPTOCOCCUS PNEUMONIAE AMONG YOUNG CHILD-MOTHER PAIRS IN THE RURAL HIGHLANDS OF THE PERUVIAN ANDES

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Pneumococcus is a cause of pneumonia among children and adults, despite the use of pneumococcal conjugate vaccines (PCVs). We saw a high prevalence of nasopharyngeal colonization with pneumococcus resistant to multiple antibiotic classes (including tetracyclines) among children in rural Peru, prior to PCV use. We sought to confirm whether this resistance pattern persisted in the post-PCV era. We enrolled subjects from the same households that participated in our previous study, which currently had children <3 years of age in the province of San Marcos - Cajamarca, in rural Peruvian Andes. Nasopharyngeal samples were collected from children, mothers, cows, guinea pigs, and dogs, when available. Samples were cultured for pneumococcus and disk susceptibility testing was performed for several antibiotic classes. Drinking water and milk samples were tested for the presence of beta-lactam and tetracycline residues using the IDEXX Snap-Duo Beta-Tetra testing kit (Westbrook, ME, USA). 47 households were enrolled, encompassing 50 children and 47 mothers. The median (interquartile range) age of children was 1.2 years (0.6-2.2), and the median number of household members was 5 (4-6). Among children, 16/50 (32%) had received an antibiotic course in the prior 6 months, while 7/47 mothers (15%) reported recent antibiotic exposure. Pneumococcus was detected in culture from 31/50 (62%) children, 9/47 (19%) mothers, and 1/31 (3%) guinea pigs. Pneumococci

were not detected in any dogs or cows. Resistance to several classes of antibiotics was common among both children and adults. No beta-lactam or tetracycline residues were detected among drinking water (n=41) or milk (n=7) samples. In conclusion, pneumococcal colonization was common among young children, less prevalent among adults, and minimal among animals. The prevalence of resistance to trimethoprim-sulfamethoxazole, oxacillin, macrolides, and tetracycline was high among children and adults. Additional studies are needed to determine if antimicrobial-resistant pneumococci in this rural area occurs because of local prescribing practices or unintentional environmental exposures.

## 1869

### THERAPEUTIC DRUG LEVELS OF FIRST-LINE TUBERCULOSIS MEDICATIONS AMONG CHILDREN FROM RURAL TANZANIA

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Although newer pediatric dosing recommendations for tuberculosis (TB) have been issued by the World Health Organization (WHO), the number of pharmacokinetic studies in children is limited. Understanding plasma drug concentrations is important to minimize toxicity and maximize successful outcomes. We evaluated plasma drug levels of Rifampin (RIF), Isoniazid (INH), Pyrazinamide (PZA) and Ethambutol (EMB) among children undergoing TB treatment in Haydom, Tanzania. Two months after starting TB therapy, phlebotomy was performed two hours after a witnessed medication administration to estimate peak drug concentrations (C<sub>2h</sub>). Plasma drug concentrations were measured using validated high performance liquid chromatography or liquid chromatography-mass spectrometry methods. Risk factors for low drug levels were analyzed. Between January 2014 and January 2016, 52 children were enrolled (median age 5.3 years, range 0.75-14), including 8 (16%) who were younger than 2 years. All were HIV negative and 24 (46%) were female. Moderate/severe malnutrition affected 40 (77%) participants; persistent diarrhea was reported by 7 (14%) participants. Pulmonary involvement was found in 34 (66%) children; 20 (39%) had microbiologically-confirmed TB. Mean drug concentrations to all TB drugs were significantly below target ranges (RIF: 1.68 mcg/mL, target range 8-24 mcg/mL; INH: 2.03 mcg/mL, target range 3-6 mcg/mL; PZA 19.22 mcg/mL, target range: 20-50 mcg/mL; EMB 1.03 mcg/mL, target range: 2-6 mcg/mL). Children receiving the newer WHO dosing recommendations had higher drug levels for RIF, PZA and EMB, but not for INH; however overall, very few (16%) achieved levels within the target range. Multivariate regression analysis identified malnutrition as a significant risk factor for low RIF and INH levels. In this cohort, estimated peak two-hour drug concentrations of TB medications fell below the target values. The prevalence of malnutrition in this setting may be contributing to suboptimal drug exposure. Maximizing the weight-based doses according to the revised WHO guidelines holds promise for improving drug levels.

## 1870

### DRUG RESISTANT TUBERCULOSIS

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Multidrug-resistant tuberculosis (MDR-TB) control is adversely impacted by delay in diagnosis and treatment. This study was undertaken to identify operational factors contributing to ongoing delay from MDR-TB diagnosis

with molecular methods to treatment initiation. Mixed qualitative and quantitative approaches were utilized within randomly sampled districts from high burden regions during the fourth quarter of 2016. District TB and Leprosy Coordinators (DTLCs), and District AIDS Coordinators (DACs) were interviewed, along with all laboratories within the selected districts where molecular diagnostics tests for MDR-TB were performed. Furthermore, the 2015 registers for retreatment TB cases were audited as these patients were targeted for molecular diagnostic testing within the Tanzania National TB and Leprosy program (NTP). Twenty-eight TB Districts from 5 Regions provided 53 clinicians' interviews; 27 (51%) DTLCs and 26(49%) DACs. DTLCs and DACs with intermediate and no knowledge on the clinical application of XpertMTB/RIF was 5(19%) and 11(42%) [p=0.02] while GenotypeMTBDRplus was 19(70%) and 25(96%) [p=0.04] respectively. Of the 399 retreatment TB cases, only 160 (40%) had specimens collected for drug-susceptibility testing (molecular or phenotypic methods), and of those specimens only 120 (75%) had results communicated back to the clinic. Additionally, 11 (100%) of the laboratories surveyed only 4-module XpertMTB/RIF equipment was available, while 5 (45%) and 6 (55%) were maintained by the government and private donors respectively. As a consequence, the median number of specimens received for XpertMTB/RIF was 12 (Min/Max 10 - 30) per day but the median number processed was only 12 (IQR 6 - 24) per day. Reported challenges for molecular diagnostics maintenance included equipment out of service for a median duration of 2 months (IQR; 1 - 4) and periodic stock-out of cartridges found in 9 (82%) of laboratories. In Conclusion, innovative programmatic interventions are required before the full potential of molecular diagnostics for MDR-TB can be realized in Tanzania.

## 1871

### MYCOBACTERIUM TUBERCULOSIS PREVALENCE IN A MILITARY POPULATION

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About one third of the world population is estimated to be infected with *Mycobacterium tuberculosis* (TB) and this proportion is expected to be higher in countries with a high TB burden. The Philippines is considered as both high TB burden (incidence  $\approx$  324,000 with 14,000 deaths) and high MDR-TB ( $\approx$ 17,000 cases) burden country (WHO Global tuberculosis report 2016). In military populations, TB has been historically responsible for causing considerable burden of disease due to unique factors such as working and living in confined environments and deployment to areas with high TB rates. Though TB has been extensively addressed in the Philippines civilian population, the burden of disease among military personnel has not yet been characterized. In 2015, the Armed Forces of the Philippines Medical Center (AFPMC), Manila was designated as a TB Xpert testing facility with the capability to detect TB and rifampicin (RIF) resistance or multi-drug resistant TB (MDR-TB) via real-time reverse transcriptase polymerase chain reaction. From July 2015 to March 2017, patients seen at AFPMC underwent testing for *M. tuberculosis* using a combination of direct sputum smear microscopy (DSSM) and real-time RT-PCR: 40/278 (14%) were TB positive by real-time RT-PCR and RIF resistance (MDR-TB) was detected in 8/40 (20%). Age range of the TB positive cases was 1 to 72 years and male to female ratio was 3:1. While there were more confirmed TB and MDR-TB cases in the civilian vs the military population, the proportion of TB positive cases was higher in the active duty military [OR 1.54] versus the civilian population. This initial report describes TB prevalence among active duty military personnel in a high TB and MDR-TB burden setting and indicates that this communicable disease poses a substantial force health protection threat.

## 1872

**MAPPING LOWER RESPIRATORY INFECTIONS IN SPACE AND TIME IN AFRICA**

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Lower respiratory infections (LRI) are some of the leading causes of death in the world, particularly in lower and middle income countries. Much of the burden of LRIs are felt in Africa, where they were responsible for more than 790,000 deaths and more than 70,000,000 infections in 2015. Moreover, LRIs were the 2nd leading cause of death and disability-adjusted life-years (DALYs) in Africa across all age groups as well as for children under the age of 5. Despite a growing population in Africa, mortality and morbidity associated with LRIs have decreased in the last 25 years. However, these changes vary significantly across both regions and countries. Assessing progress and identifying locations most in need of further interventions requires an accurate understanding of spatial variation at the scales on which public policy acts. Past efforts have focused on estimating burden either for a single country at a fine spatial resolution, or continent-wide at very coarse spatial resolution (country by country). Here we present the first effort to model yearly all-cause LRI prevalence and mortality at a 5km<sup>2</sup> resolution across all of Africa. Our model-based geo-statistical approach captures significant spatiotemporal variation in prevalence and mortality. Further, it exposes substantial heterogeneity in both of these metrics of burden, as well as spatial inequality in gains made against them through time. Finally, this Bayesian approach also propagates uncertainty through the model to expose not only locations with high or low burden, but also understudied locations whose burden may or may not be considerable but require further investigation. These results will guide targeted interventions within or between countries and regions as well as assess the effectiveness of current strategies.

## 1873

**DOES THE ABSENCE OF HYBRIDIZATION WITH THE WILD-TYPE PROBE IN THE GENOTYPE MTBDRPLUS ASSAY MEAN THE MYCOBACTERIUM TUBERCULOSIS ISOLATE IS RIFAMPICIN RESISTANT?**

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Effective management of drug-resistant TB including Rifampicin-resistant TB (RR-TB) relies on accurate and rapid diagnosis. In 2008, WHO endorsed the Genotype MTBDRplus assay for the rapid diagnosis of drug-resistant TB including RR-TB. This assay detects resistance to RIF by identifying mutations in the *rpoB* gene of *Mycobacterium tuberculosis* (Mtb) from codons 507 to 534. Mutations that enable Mtb to be resistant to RIF are located within this region. However, not every mutation in this region is associated with resistance to RIF. A recommendation in interpreting the Genotype MTBDRplus assumes that the detection of any mutation in this region based solely on the absence of hybridization to an assay probe should be considered as resistant. Some of the mutations in *rpoB* gene region could be silent mutations or missense mutations with unclear association with resistance to RIF. Here, we screened 275 Mtb isolates from naïve and previously treated Cameroonian TB patients with the Genotype MTBDRplus assay and identified 6% of Mtb isolates classified as resistant to RIF based on this recommendation. Sequencing of these Mtb isolates revealed that impaired hybridization to the assay probes was due to the presence 'disputed' RIF mutations (511Pro, 513Pro, 516Tyr, 526Arg, 526Leu, 526Asn, 531Trp, and 533Pro). These mutations are called 'disputed' because not all Mtb isolates bearing them have a RIF-resistant phenotype. As such, using the recommend interpretation for the Genotype MTBDRplus assay will lead to the over-diagnosis of very

few RIF resistant cases. Considering these few cases have been reported to result in adverse treatment outcome, they should be reviewed and treated as 'true' Rifampicin resistant cases. Thus, the recommendation of the Genotype MTBDRplus assay to assume resistance based solely on the absence of hybridization to wildtype probe allows the identification of clinically significant Rifampicin-resistant Mtb isolates.

## 1874

**AFFINITY NANOCAGES ENABLE DETECTION OF MYCOBACTERIUM TUBERCULOSIS LAM AND PROTEIN ANTIGENS IN THE URINE OF HIV NEGATIVE PULMONARY TB PATIENTS**

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A non invasive urine test for pulmonary TB patients who are not co-infected with HIV (85% of 9.6 million with active TB) is critically needed for worldwide surveillance, and treatment management. We have created a novel multifunctional nanoparticle buoyant cage that contains a new class high affinity copper dye which binds carbohydrate and glycoprotein TB antigens with an affinity hundreds of times higher than existing antibodies or lectins. We used this technology in one step, in solution, to affinity capture, concentrate, and displace from interfering biomolecules, TB antigens present in patient's urine. Past attempts failed to reliably detect the mycobacterial glycan antigen lipoarabinomannan (LAM), or ESAT6, well characterized TB antigens, in the urine of HIV negative pulmonary TB infected patients' urine. Our technology can increase the analytical sensitivity of downstream analytical techniques greater than 100 fold, and reduce the background, with a yield close to 100 percent to achieve a new class of urinary TB immunoassay. Our technology was applied to study pretreatment urine from hospitalized Peruvian patients, all negative for HIV, with microbiologically-confirmed, culture positive, active pulmonary TB. LAM was quantitatively measured in the urine via immunoblot with a sensitivity >95% and a specificity >80% (p<0.00001, N=101) in a concentration range of 14 to 2000 picograms per mL, as compared to non-TB, healthy and diseased, age matched controls (ROC AUC = 0.95, 95% CI: 0.9005-0.9957). Moreover, urinary LAM was significantly (p<0.05) elevated in patients with a higher mycobacterial burden, or a higher diseases severity (cachexia, cough rate). Finally, we used our nanotechnology and mass spectrometry to identify unique peptides specific for *Mycobacterium* proteins in the urine of a subset of patients harboring active pulmonary TB. Our technology, coupled to immunoassay or mass spectrometry analysis, has broad implications for pulmonary TB screening, transmission control, and treatment management for HIV negative patients.

## 1875

**QUALITATIVE AND QUANTITATIVE ANALYSIS OF CRYPTOSPORIDIUM PARVUM GROWTH IN POLARIZED INTESTINAL EPITHELIAL CELLS**

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*Cryptosporidium parvum* is an obligate intracellular parasite that can cause life-threatening diarrhea among children and immunocompromised adults. Optimal *in vitro* models of long-term *C. parvum* growth remain to be developed, constituting an obstacle to the study of host-parasite interactions and standardization of *in vitro* drug screening assays. The present study evaluated the growth of *C. parvum* in polarized human intestinal epithelial cell lines as a physiologically-relevant model of the

intestinal lumen. Human colorectal adenocarcinoma Hct-8 cells and small intestinal epithelial FHs 74 cells were grown on polycarbonate membrane supports. Cell cultures were inoculated with *C. parvum* Iowa and infection was assessed qualitatively and quantitatively by immunofluorescence microscopy assay (IFA) and qRT-PCR, respectively. IFA experiments showed that trophozoite and meront I stages of *C. parvum* were predominant between 24 h and 72 h of infection in both cell lines. The larger meront II stages were more evident at 72 h in Hct-8 compared to FHs 74 cells. This difference was also apparent at 7 and 10 days of infection. Quantitative analysis of parasite growth by qRT-PCR showed peak levels of *C. parvum* 18S rRNA expression between 48-72 h in both cell lines and progressive decreases after 7-10 days of infection. Of note, different expression patterns between the cell lines were observed among genes coding for oocyst wall proteins, sporozoite surface antigens, metabolic proteins, and proteins with roles in sexual replication. In summary, we have established a framework to monitor the growth of *C. parvum* in different polarized cell lines in a qualitative and quantitative manner. Polarized FHs 74 cells offer a better model to visualize the intracellular stages of *C. parvum* and to study the asexual life cycle of the parasite. Optimization of *in vitro* models that better resemble the *in vivo* state as closely as possible should provide improved tools to study the pathogenesis and drug susceptibility properties of *C. parvum*.

## 1876

### COMPARATIVE TRANSCRIPTOMICS ANALYSIS OF ZOOTIC PROTOZOAN PARASITE, *BABESIA MICROTI* FROM MOUSE MODEL

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We have sequenced the transcriptome of the emerging human pathogen *Babesia microti* from mouse model and compared it with that of other protozoa. Our sequencing generated 731,487 reads with an average read length of 416 bp and GC content of 39%. The transcriptome was constructed using Newbler 2.6. Our de novo assembly yielded a database with 6,573 contigs (N50 of 1,317 bp) and 18,530 singlets, and generated 11,893 unigenes with CD-hit. In those unigenes, approximately 30% of them could be annotated to public database (swiss-prot and NR), and 1,683 unigenes were confirmed as orthology with *Babesia bovis*. Parasite ligands and proteases play a role in the merozoite invasion process, the moving junction complex protein apical membrane antigen 1 and its receptor, the rhoptry neck protein 2 (AMA1-RON2), a key factor of merozoite invasion mechanism was identified for *B. microti*. Furthermore, a new group of invasion related proteins containing the conserved TSP-1 motif has been identified from *B. microti*, namely merozoite specific thrombospondin-related anonymous protein (MTRAP) and thrombospondin related apical merozoite protein (TRAMP). However, the family of *Plasmodium falciparum* erythrocyte binding antigens (PfEBAs) and reticulocyte binding-like homologous proteins (PfRHs) as important invasion related proteins haven't been identified in *B. microti*, as well as SUB1, a member of subtilisin-like serine protease and serine-rich antigens (SERAs). Knowledge gained from these comparisons can shed light on the development of diagnosis methods, improved vaccines and new chemotherapy targets and provide a better understanding of the underlying biology and evolution.

## 1877

### WHOLE GENOME DNA SEQUENCE CAPTURE APPROACH REVEALS TREMENDOUS GENETIC DIVERSITY IN INTRACELLULAR PATHOGEN *THEILERIA PARVA*

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*Theileria parva* is a tick-transmitted apicomplexan parasite that causes East Coast fever (ECF), an acute fatal disease of cattle in sub-Saharan Africa. A vaccine for this disease currently exists, which is effective against cattle-transmissible strains but less effective against those originating from the African Cape buffalo, the natural reservoir of *T. parva*. Knowledge of genetic variation in both cattle- and buffalo-derived *T. parva* strains is critical for the design of next-generation vaccines against the parasite. The biology of *T. parva* has so far proven a powerful obstacle to the acquisition of DNA in sufficient quantity and quality for whole genome sequencing. DNA extracted early in the infection cycle is heavily contaminated with host DNA and parasite DNA from late-stage infections cannot be obtained sustainably on a large scale. We have adapted a DNA sequence capture approach to select *T. parva* from a mix of parasite and bovine DNA obtained from *T. parva*-infected bovine lymphocytes. In order to gain access to variable genomic regions that cannot be characterized through read mapping approaches, we assembled the captured reads *de novo* and analyzed the assemblies for completeness, including the presence of highly variable genes. From starting material of <1%-4% parasite DNA in a mixed sample from host and parasite, >98% of sequence reads post-capture mapped to the parasite genome, reflecting the method's high specificity. *De novo* assemblies generated from these data correspond to >97% of the reference genome, with all but two protein-coding genes present. This approach is successful even when applied to highly divergent *T. parva* isolates from buffalo, for which we present the first publicly available assembly. The ability to characterize genome-wide polymorphism based on *de novo* genome assemblies, an approach with higher resolution than read-mapping approaches, allows us to identify regions of greater diversity in the *T. parva* genome. We are able to identify the most divergent genes across isolates, the significance of their genomic location and use these results to inform the potential function of hypothetical proteins.

## 1878

### BLASTOCYSTIS AS A MARKER OF FECAL-ORAL OR WATER CONTAMINATION IS ASSOCIATED WITH AN INCREASED RISK FOR GASTROINTESTINAL PARASITIC INFECTION

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Polyparasitism is widespread in developing communities and may persist even after antiparasitic drug therapy. Risk factors for persistent infection may be attributed to resistance or reinfection. For proper intervention, the predominant risk factors must be determined. *Blastocystis* spp. are common intestinal protists whose role as human pathogens is disputed. To investigate whether the presence of *Blastocystis* is associated with gastrointestinal (GI) parasite infection in a rural location in Honduras regularly treated with Albendazole, stool samples from 99 children (mean age = 6.1 yrs) were analyzed using multi-parallel quantitative real-time PCR (qPCR) for *Ascaris lumbricoides*, *Ancylostoma duodenale*, *Necator americanus*, *Trichuris trichiura*, *Strongyloides stercoralis*, *Cryptosporidium*, *Entamoeba histolytica*, *Giardia lamblia*, and *Blastocystis*. Overall, 71.6%

of children tested positive for *Blastocystis*, 11.1% *Ascaris*, 2.0% *Necator*, 1.0% *Strongyloides*, 46.5% *Trichuris*, and 41.4% *Giardia*. Study participants infected with *Blastocystis* were more likely to be infected by one or more other GI parasites compared to participants not infected with *Blastocystis* (relative risk 1.946, 1.24 to 3.40; odds ratio 4.384, 1.62 to 10.4; sensitivity 83.1%, specificity 47.0%;  $p = 0.002$ ). Helminth infection was correlated to *Blastocystis* infection ( $p < 0.001$ ) while protozoan infection was not ( $p = 0.248$ ). Interestingly, our studies revealed a strong correlation ( $p = 0.0002$ ) between *Blastocystis* positive participants and *Trichuris* positive participants. Our data suggests that *Blastocystis* is a useful indicator of GI parasite exposure rates. Because *Blastocystis* is reported to be transmitted by both fecal-oral contamination and cyst-infected water sources, further studies will investigate the transmission modes and subtypes of *Blastocystis* and connections to the transmission of helminth infections.

## 1879

### SINGLE MOLECULE, REAL-TIME SEQUENCING OF PCR PRODUCTS REVEALS *THEILERIA* PARASITE SPECIES AND ANTIGEN DIVERSITY

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Infection of cattle with the apicomplexan parasite *Theileria parva* causes an acute and often deadly disease that imposes a significant economic burden on several countries in sub-Saharan Africa. Host CD8+ cytotoxic T cells are believed to play an important role in protection, which can be achieved by immunization via an infection-and-treatment method. However, this immunity is often strain-specific, and antigenic variation can play an important role in the effectiveness of the immunization regimen. Therefore, an understanding of the diversity of *Theileria* species that infect cattle in endemic regions, and the diversity of their antigens, is important for improving vaccine design and implementation. Here, we report a new molecular assay to characterize *Theileria* infections in cattle and buffalo, which will identify both multiple species as well as multiple strains of a single species. This assay is based on the PCR amplification of three *Theileria loci*, followed by deep sequencing of the amplicons, multiplexed across *loci* and samples with a Pacific Biosciences (PacBio) sequencing platform. One amplicon represents partial sequence of the 18S ribosomal DNA gene while the other two produce full length sequences of two highly variable *Theileria* antigens, Tp2 and Tp9, known to be recognized by CD8+ T cells activated in response to *T. parva* infection. Consequently, this assay also allows the investigation of genetic diversity in two critical parasite antigens. The assay was used in 23 cattle biopsies from a farm in Naivasha, Kenya, where cattle and buffalo co-mingle. Using bioinformatics approaches, we were able to uncover new, potentially biologically relevant, sequence variation in known parasite antigens and reveal patterns of co-infection with *T. parva* and a recently discovered parasite, *Theileria* sp. (buffalo). We propose that this approach could be used as an economical epidemiological tool to study the etiology of *Theileria* infection of bovinds in sub-Saharan Africa, as well as parasite antigenic diversity.

## 1880

### EVIDENCE OF RNA EDITING IN *BABESIA MICROTI*

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RNA editing is a molecular process by which isolated alterations can be made to specific nucleotide sequences within an RNA molecule after it has been transcribed by RNA polymerase, but before it is translated into protein. These changes may in turn lead to amino acid sequence differences in the resulting polypeptide relative to what is encoded in the DNA, with potential consequences to parasite metabolism. *Babesia microti* is the primary cause of human babesiosis, an emerging infectious

disease in the United States. In order to better understand the genetic and genomic diversity among *B. microti* isolates and thus improve diagnosis, treatment and prevention, we recently generated genomic and immunological data for seven *B. microti* strains. In addition, we generated strand-specific RNA-seq data for six of these isolates. Preliminary results from short read alignment of both genomic DNA (gDNA) and RNA-seq data of the same strain against the reference R1 genome suggest the presence of several unambiguous single nucleotide polymorphisms (SNPs) differences between the two data types generated from the same strain. These differences were confirmed by manual inspection of the read alignments. We are currently using systematic bioinformatics approaches to characterize, for each strain, the differences observed in the gDNA and RNA-seq data, including the nucleotides involved, directionality and genomic context of such changes. We are also investigating whether these differences are similar among strains, and whether this editing, if true, has potential public health implications.

## 1881

### KILLING OF *CRYPTOSPORIDIUM* SPOOROZITES BY LACTOFERRIN

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*Cryptosporidium* species are major causes of diarrhea morbidity and mortality in children from resource-limited countries specially children below 24 months of age. A large number of therapeutic agents have been evaluated for their anticryptosporidial activity, but only nitazoxanide has been approved by the FDA. Lactoferrin is a multifunctional glycoprotein present in breast milk with bacteriostatic, antimicrobial and anti-inflammatory activity. We conducted a study to determine the effect of lactoferrin during *Cryptosporidium* infection and on its sporozoites. An experimental study was conducted. We tested the effect of physiological concentrations of lactoferrin (0, 1, 2.5, 5, and 10 mg/mL) on *Cryptosporidium parvum* oocysts determined by the excystation rates via microscopy. The effect on sporozoite infectivity was evaluated by exposing excystated sporozoites to physiological concentrations of lactoferrin and the infection was evaluated in the infection model using HCT-8 cells and a RT-PCR. We observed that biological concentrations of lactoferrin did not affect excystation and had no effect on intracellular stages of the parasite. However sporozoites treated with  $\geq 5$  mg/mL lactoferrin (for 2 hours during the time of infection) resulted in significantly reduced numbers of parasites present after 24 hours of infection and parasites treated with 10 mg/mL lactoferrin didn't show fluorescence during microscopic analysis indicating that all parasites were killed after treatment, opposite to untreated parasites. Since the sporozoites are important during the infection process, then our data point to the potential of lactoferrin as a novel therapeutic agent for cryptosporidiosis and that continuous presence of breast milk in an infant's diet may decrease or inhibit *Cryptosporidium* infection. Further clinical studies are needed to confirm these findings in cases of cryptosporidiosis in children and other at-risk patients. If successful, lactoferrin may be a much-needed treatment option for cryptosporidiosis.

## 1882

### REAL-TIME PCR STRATEGY FOR DETECTION OF *TOXOPLASMA GONDII* FROM PERIPHERAL BLOOD CLOT

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Toxoplasmosis is a zoonotic disease caused by *Toxoplasma gondii*, an obligate intracellular coccidian parasite that infects humans as well as virtually all warm-blooded organisms. In most cases toxoplasmosis is asymptomatic; however, it can cause serious and life-threatening conditions in immunocompromised subjects. Diagnosis of toxoplasmosis includes serological tests and clinical and radiological response to treatment. However, a serological approach has low detection power since reactivation of the infection is not always followed by changes in antibody production, and correlation with other assays is needed for accurate diagnosis. For this reason, direct demonstration of the presence of the parasite in body fluids would mean a breakthrough for the diagnosis of this disease. The quantitative polymerase chain reaction (qPCR), directed to B1 and REP529 multicopy genes, has shown variable sensitivity which might be affected by the amount of parasite DNA present in different blood sample specimens. There is no consensus on which one yields the best performance. In this study we developed a qPCR method for accurate quantification of *T. gondii* loads using clot samples along with an adequate DNA extraction protocol. Additionally, we compared the sensitivity of the clot-based qPCR reaction with two other common types of blood specimens: whole blood in EDTA and guanidine EDTA blood (GEB). Before DNA extraction, clot specimens were transferred with a lysing matrix tube followed of an agitation cycle in a tissue homogenizer machine to ensure the clot disaggregation. The clot standard curve detected the lowest parasite load when compared to the ones from whole blood and GEB. The detection limit for clot samples was ten parasites with B1 as the target and one parasite using REP529. The high sensitivity of clot qPCR suggests widespread use of this specimen as a powerful tool for toxoplasmosis diagnosis. The future direction is to validate this technique using different blood samples from patients with neurological symptoms compatible with a *Toxoplasma gondii* infection.

## 1883

### URBANORUM SPP. EMERGING MICROORGANISM IN FECAL SAMPLES OF CHILDREN AT THE NATIONAL INSTITUTE OF CHILD HEALTH, AND OF ANIMALS: PIG AND CATTLE FROM LIMA, PERU FROM JANUARY TO MARCH, 2017

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*Urbanorum* spp. is described as "protozoan" emerging in Colombia, Ecuador, Venezuela and Mexico, and there is a first reported case in Peru in 2016; it is described as a protozoan similar to amoebae, with a round shaped form, 80-100µm of size, that releases "pseudopodia" and has been found in parasitic exams of adult and child patients, with a prevalence from 0-16%. The objective of this work is to investigate *Urbanorum* spp. in faecal samples obtained from children aged 0-16 years. A total of 305 samples from routine parasitological diagnostic in the National Institute of Child Health's Microbiology Service were processed and additional exams for *Urbanorum* spp. were realized at the Daniel A. Carrión Institute of Tropical Medicine of the National University of San Marcos, Lima, Peru, between January and March, 2017. Samples were processed with saline serum and Lugol, as well as other stains such as Ziehl Neelsen, Methylene Blue, Malachite Green, Safranin and Giemsa. *Urbanorum* spp. was found in 50 of the 305 (16.4%) samples from faecal samples of children. *Urbanorum* spp. was also studied in 200 faecal colon samples from pigs and recently slaughtered cattle from the Frigorífico La Colonial Sac, Lima, Peru, showing no presence of *Urbanorum* spp. Conclusion: *Urbanorum* spp. has been found in 16.4% of faecal samples of children and none in

the faecal samples of animals; it is necessary to perform microbiological studies including molecular biology, clinical epidemiology, pathogenesis, treatment and control of this emerging microorganism.

## 1884

### EVALUATION OF THE IMMUNOSUPPRESSIVE EFFECT OF DEXAMETHASONE IN SWISS MICE INFECTED WITH TOXOPLASMA GONDII ME49 STRAIN

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*Toxoplasma gondii* is a protozoan that can cause disease, especially in immunocompromised patients. The main form of dissemination of the parasite is by blood. In this study we evaluated leukocyte levels and the variation of their populations during experimental infection with *T. gondii* under immunosuppression. Three groups of mice were studied: group A: infected and immunosuppressed (n = 15), group B: uninfected and immunosuppressed (n = 10), group C: uninfected, non-immunosuppressed (n = 5). Group A were orally infected with 10 to 20 cysts of the Me49 strain, mice were immunosuppressed after 60 days of infection. The immunosuppression was intraperitoneally with 100 mg / kg of dexamethasone. We collected peripheral blood from the mouse tail at the beginning and end of the experiment at 0, 7, 21, 50 and 80 days post infection and 30 days after immunosuppression. Leukocyte quantification was performed using Turk method. The proportions of leukocytes were evaluate in a blood smear stained with Giemsa. The total leukocyte count showed : Group A, 17,220 cells / mm<sup>3</sup>, group B of 19,400 cells / mm<sup>3</sup> and group C 19,983 cells / mm<sup>3</sup>. After immunosuppression, group A: 3,166 cells / mm<sup>3</sup>, group B: 12,865 cells / mm<sup>3</sup> and group C: 27,350 cells / mm<sup>3</sup>. The percentage of leukocytes and neutrophils for mice in group A and B before immunosuppression was 90% and 25%, respectively. This percentages for these groups changed markedly after 60 days of immunosuppression: for group A it was 40% and 80% of lymphocytes (L) and neutrophils (N), respectively. For group B, it was 60% and 70%, of L and N, respectively. For the control group this percentage was 90% of L and 20% of N. In normal Swiss mice lymphocytes predominate more than neutrophils. Experimentally mice infected with *T. gondii* and subsequently treated with dexamethasone show a low lymphocyte population and increased neutrophils. The conclusion was the immunosuppression method was successful, confirmed by the cell count and the presence of a large number of cysts in the brains in group A. We suggest to develop this type of methodology in order to get biological samples for the diagnosis and to give better treatment of this parasitosis.

## 1885

### IMPACT OF IMPLEMENTING A STATE CERTIFIED IMPROVED COOKSTOVE ON CONCENTRATION LEVELS OF PARTICULATE MATTER (PM) AND CARBON MONOXIDE (CO) IN RURAL ANDEAN HOUSEHOLD IN PERU

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Worldwide, rural populations are exposed to numerous contaminants inside their homes by cooking or heating using traditional stoves or open fires burning biomass fuels (wood, coal, dung). Peru is among the six Latin American countries with the highest percentage of biomass fuel use. In this study we determined if a state-certified improved cookstoves reduce household air pollution levels in rural high-altitude homes of San Marcos, Cajamarca. This evaluation was embedded within a community-randomised controlled trial with two integrated health intervention packages (IHIP), a) the environmental health intervention comprised improved cookstoves (ICS), kitchen sinks and hygiene education (IHIP) and

b) the before-mentioned IHIP plus an early child development intervention (ECD). We determined 24hr indoor air concentration levels of CO and PM<sub>2.5</sub> in 33 kitchen environments, before and after (3-months after installment) the OPTIMA-improved stoves had been installed. Geometric means were calculated to describe PM and CO concentrations within households, due to the biased distribution to the right. To evaluate the impact of the intervention, the data were fitted to a mixed effects model; previously the data of PM and CO concentrations were transformed through a natural logarithm function. The geometric mean at baseline for PM levels was 46.60 µg/m<sup>3</sup>. After installing the OPTIMA-improved stove we observe a statistically significant (p-value <0.01) reduction (30.85 µg/m<sup>3</sup>) Regarding the CO levels, the geometric mean was also reduced when compared to baseline, (1.32 ppm for baseline to 1.19 ppm after installation). The difference is statistically significant (p-value <0.01). The OPTIMA-improved stove three months after installation shows a significant reduction in PM and CO.

## 1886

### IMPACT OF HOUSEHOLD WATER SOURCE ON SCHOOL ABSENCE AMONG CHILDREN LIVING OUTSIDE OF PORT-AU-PRINCE, HAITI

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As one of the poorest populations in the world, 56% of Haitians live in urban areas, less than 70% have access to improved drinking water sources and less than 30% have improved sanitation facilities. Although vaccination coverage has been increasing, less than 50% of children under the age of one year receive all recommended vaccines. Acute respiratory disease was the major cause of death (20%) among under-5 children in 2013. Respiratory diseases also affect older children and can impact school absence. Understanding the impact of household water source on children's health and its relationship with school absence is critical to design and enhance future interventions to improve health among Haitian families. Data were collected through surveys between June and July, 2016 from 39 different schools located outside Port-au-Prince region in Haiti. In total, 387 mothers described their socioeconomic status, educational level, children's health status, access to healthcare, among other characteristics. Chi-square tests, using SPSS (version 24), demonstrated associations. Mothers had a mean age of 36.9 years (SD 9.65) and 41.8% reported having no educational level, while only 14.1% completed secondary school. Only 18.6% of participants reported not having a source of income, compared to 81.4% employed. Father/partner contributed to household income in 84% of answers, resulting in a monthly income ranging between \$30.00-74.96 dollars for 45% of surveyed participants. Findings indicated more than 94% of participants had no water, electricity or latrine in the household; chi-square tests also indicated an association between illness-related school absences and water source. Cough/cold and/or fever accounted for more than 17% of cases. However, other built environmental factors, like access to latrines, were not significantly associated with illness related school absences. This study provides unique insight to the household dynamics of Haitian families and demonstrates the need for further qualitative investigations examining behavioral risk factors associated with respiratory disease spreading.

## 1887

### INFLUENCE OF ENVIRONMENTAL CONDITIONS ON NUTRITIONAL STATUS AMONG SCHOOL-AGE CHILDREN IN HAITI

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Understanding the influence of living conditions on indicators of malnutrition among Haitians school-children, is vital for guiding future interventions. This study investigates the potential influence of built environment on nutritional status, as measured by body mass index (BMI). Haitians aged 3-20 years were surveyed from 41 schools across Archaie Commune of Haiti, from June to July 2016. Data recorded includes age, sex, height, weight, BMI, and environmental factors including housing material, access to latrine, waste collection, and drinking water source. Logistic regression and Chi-square were performed to determine the association between malnutrition data and built environmental factors. A total of 4,964 schoolchildren were included in this study, of which 52% (2,584) were male and 48% (2,380) were female, of which 68.5% of the population had a low BMI (<18.5 BMI). Malnutrition was significantly associated with housing materials-roofing (OR: 1.37 [95% CI: 1.07-1.76]), flooring (OR: 1.91 [95% CI: 1.08-1.31]), and waste collection (OR: 0.67 [95% CI: 0.59-0.76]). Built environmental conditions like roofing, flooring, and waste collection are likely proxies for socio-economic status, however this survey was implemented in an impoverished rural mountain region. These findings add to the body of literature on malnutrition in Haiti, while investigating the associations between household environmental conditions and malnutrition measurements. Furthermore, this research suggests a potential link between household environmental conditions and nutritional outcomes among Haitians, which could potentially be used to identify at risk population.

## 1888

### THE INFLUENCE OF SANITATION ON CHILDHOOD DIARRHEA IN 2016 AND ITS IMPLICATIONS ON INTEGRATED COMMUNITY CASE MANAGEMENT OF ENDEMIC CHILDHOOD DISEASES IN ABIA STATE, NIGERIA

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Every second, a child in Nigeria dies as a result of poor sanitation. Childhood diarrhoeal disease is of public health significance in under fives being the second leading cause of death as well as the leading cause of malnutrition in this age bracket. Consequently Abia State of Nigeria has maintained high prevalence of diarrhoea and frequent outbreaks despite government- and non-government oriented interventions. This is a cross-sectional study carried out in Abia State from January 2016 to December 2016 among 403 caregivers with children under five in 27 randomly selected (multistage randomization) communities. Data were collected using a well structured interviewer-administered questionnaire. Ethical Approval was obtained from Abia State University Teaching Hospital Aba, and informed consents were obtained from all caregivers of children assessed. Data analysis was done with Statistical Package for Social Sciences version 22 (International Business Machine, New York). The results showed major gaps in sanitation as 79.4% of households do not treat their drinking water; 55.8% of respondents do not have private toilet facility; 15.4% practise open defecation; 75.7% of young children's faeces are not dropped into a toilet facility; 51.6% did not have hand washing device; and only 50.4% of respondents have ever received any health information about water hygiene. The prevalence of diarrhoea (over the previous 6 months) was high at 44.7%. Inferential analysis showed that many components of sanitation statistically significantly predicted

the prevalence of diarrhoea among children 0 to 59 months,  $f(18, 384) = 5.496$ ,  $p = 0.000$ ,  $R = 0.453$ . This study thus elucidates that sanitation statistically significantly predicted prevalence of diarrhoea among children, and the inadequate health education on sanitation compounds the problem. Thus, we recommend that sanitation components be integrated into efforts aimed at curbing endemic childhood diarrhoea, in order to guarantee their success and for greater impact on the scourge of childhood diarrhoea.

## 1889

### ENVIRONMENTAL IMPACT ON HELMINTH AND PROTOZOA INTESTINAL INFECTIONS IN URBAN SLUMS VERSUS RURAL COLOMBIA

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Intestinal parasites are globally widespread infectious agents disproportionately affecting children in resource-deprived areas. Environmental surroundings influence exposure to these parasites between different community settings (urban, rural). Stool samples from 132 children in an urban slum ( $n = 72$ , mean age = 2.5 yrs), and rural ( $n = 72$ , mean age = 2 yrs) areas were analyzed using multi-parallel quantitative real-time PCR (qPCR) for *Ascaris lumbricoides*, *Ancylostoma duodenale*, *Necator americanus*, *Trichuris trichiura*, *Strongyloides stercoralis*, *Blastocystis* spp, *Cryptosporidium* spp, *Entamoeba histolytica*, and *Giardia lamblia*. Prevalence for positive samples was 19%, 50% *Ascaris* ( $p = 0.002$ ); 0%, 2% *Strongyloides*; 6%, 46% *Trichuris* ( $p = 0.0001$ ); 86%, 76% *Blastocystis*; 19%, 0% *Cryptosporidium*; 0%, 20% *Entamoeba*; 63%, 68% *Giardia* for urban and rural respectively. Higher *Giardia* cyst per gram (CPG) of stool (calculate from qPCR standard curves) correlated to living in urban slums versus rural areas (4719 versus 702 CPG,  $p = 0.008$ ). *Blastocystis* DNA burden was higher in urban pre-school children (2 to 4 yrs) compared to infants (0 to 1 yrs) and school age (5 to 8 yrs) (3.4 versus 0.04, 0.025 fg/μl respectively,  $p = 0.020$ ). *Blastocystis* was associated with having any helminth infection in both urban and rural settings ( $p = 0.0491$ ). Helminth burden was correlated to qPCR derived eggs per gram (EPG) with higher *Trichuris* burden found in rural areas compared to urban slum (9953 versus 325 EPG,  $p = 0.0023$ ). Children infected with polyparasitism (2 or more parasites) correlated to living in rural areas compared to urban (59%, 19%,  $p = 0.001$ ). Our data shows the differences in infections between an urban slum and rural areas for intestinal parasites. Rural areas had significantly higher helminth infections likely due to soil exposure. Urban slum areas had surprisingly higher protozoa burdens, potentially due to crowding and sharing contaminated water. This study will guide public health initiatives on mass drug administration and water, sanitation projects.

## 1890

### HOUSEHOLD CONTAMINATION OF BABY BOTTLES USED FOR FORMULA FEEDING IN PERI-URBAN LIMA, PERU

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Feeding of infant formula or cow's milk using contaminated bottles may be an important transmission pathway of pathogens leading to enteric infections during the first two years of a child's life. This research aims to (1) estimate the levels of fecal contamination of baby bottles and (2) assess the behavioral determinants of such contamination in a peri-urban district outside of Lima, Peru. Samples are taken from bottles that are either in use or classified by the caregiver as "ready to use" at the sampling event. Caregivers' hands are also sampled to allow for the comparison

of bottle contamination with another widely recognized source of disease transmission within the home. Bottles and hands are sampled aseptically in the participant's home by rinsing with 50 mL of sterile water for 60 seconds. Samples are processed using membrane filtration and cultured for outgrowth of indicator organisms, total coliform and *E. coli*. A structured questionnaire is administered to the caregiver at each sampling event in order to evaluate practices associated with bottle use and hygiene. Variables to be derived from the questionnaire data include bottle characteristics (material, size, age of bottle, number of bottles in home), bottle preparation (time elapsed since bottle prepared, if bottle is stored in refrigerator when not in use), and bottle cleaning (use of detergent, sponge, or brush during washing, frequency of bottle cleaning and/or boiling). Preliminary results of bottle sampling and testing to date have demonstrated that 43.8% (21/48) of bottles are contaminated with *E. coli*. Of these, 42.9% (9/21) have concentrations of more than  $1 \times 10^2$  bacteria/mL of sample. Fecal contamination is more common and found in higher quantities in bottles as compared to caregivers' hands. Future analyses of the bacteriological and questionnaire data will help to identify factors associated with higher levels of contamination, and will shed light on practices that might be appropriate targets for a behavior change campaign.

## 1891

### A SCALABLE HOSPITAL-BASED HANDWASHING WITH SOAP AND WATER TREATMENT INTERVENTION FOR HOUSEHOLD MEMBERS OF DIARRHEA PATIENTS IN BANGLADESH (CHOBI7 TRIAL): INTERVENTION DEVELOPMENT AND USERS' EXPERIENCES

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Diarrhea is a leading cause of child death and a major contributor to morbidity globally. Household contacts of diarrhea patients are at a much higher risk of diarrheal disease than the general population during the one-week period after the patient presents in a health facility. This higher risk is likely due to shared contaminated environmental sources, such as household drinking water, and secondary transmission from poor hygiene practices in the home. The Cholera-Hospital-Based Intervention for 7 days (CHoBI7) is a one-week water treatment and handwashing with soap intervention, targeting diarrhea patients and their household contacts in Dhaka, Bangladesh. The intervention package, delivered at the patient's bedside, includes a pictorial module on diarrhea transmission, a handwashing station, soapy water bottle, sealed drinking water vessel, and chlorine tablets for water treatment. In collaboration with the Bangladesh Ministry of Health and Family Welfare, we conducted formative research to develop a scalable approach for integration of CHoBI7 into services provided to diarrhea patients in government and private health facilities in Bangladesh. Formative research included a total of 52 in-depth interviews with diarrhea patients and their household contacts, health facility staff, and government officials; hospital and household structured observations; and a pilot study of the developed CHoBI7 intervention. Interviews were also conducted with pilot household beneficiaries to explore experiences with each component of the intervention package, including 1) use and perceptions of chlorinated drinking water, 2) benefits and challenges of handwashing and water storage hardware, and 3) communication of key program messages among household members. Findings from interviews and observations, and feedback from pilot study participants, allowed us to identify key challenges for program implementation and

develop strategies to overcome these potential barriers to successful implementation. We are currently conducting a randomized controlled trial of our scalable approaches for delivery of the CHoB17 intervention.

## 1892

### VARIABILITY IN STRENGTH OF ASSOCIATION WITH DIARRHEA OF PATHOGENIC *E. COLI* ALONG AN URBAN-RURAL GRADIENT IN ECUADOR

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Pathogenic *E. coli* is a major cause of diarrhea in developing countries, yet it is often isolated from controls without diarrhea. Understanding why some infections are symptomatic and some are asymptomatic is a major outstanding question. We report on the results of a case-control study of diarrhea along a rural-urban gradient in Ecuador. We sampled ~100 subjects with diarrhea and ~100 age-matched controls at each of four sites: a large clinic in Quito, Ecuador's capital, a hospital in the capital of Esmeraldas Province, a hospital in the town of Borbón, and clinics in outlying rural communities. The urban-rural gradient also represents a gradient of access to clean and safe water. We identified pathogenic *E. coli* from fecal samples of study subjects through PCR and carried out whole genome sequencing on these isolates. In the two urban sites, diffuse adherent *E. coli* (DAEC) was the dominant *E. coli* pathotype, whereas in the more rural sites atypical enteropathogenic *E. coli* (EPEC) was the most prevalent pathotype. Infection with DAEC was associated with diarrhea symptoms in the urban sites [Quito: OR=2.8 (95% CI: 1.11, 7.06); Esmeraldas: OR=2.5 (95% CI: 1.19, 5.24)] but not in the rural sites. DAEC isolates causing diarrhea were found to be dispersed along the phylogenetic tree, suggesting that the diarrhea cases represent multiple lineages of DAEC rather than a large outbreak of a clonal genotype. Reported travel in the past year to Guayaquil, another major urban center, was associated with increased risk of DAEC infection, further suggesting that DAEC is associated with urban areas. Travel in the past year both into and out of Quito was a predictor for pathogenic *E. coli* infection. We found differential distribution of *E. coli* pathotypes in urban versus rural areas, and in urban areas *E. coli* pathotypes had stronger associations with diarrheal illness. This study highlights the potential for within-country "travelers' diarrhea" in a developing country context, and provides insights into countrywide transmission of pathogens. This information can help guide decisions on where to focus intervention strategies between urban versus rural regions.

## 1893

### DETERMINANTS OF DIARRHEAL DISEASE IN CHILDREN UNDER FIVE YEARS IN THE DEMOCRATIC REPUBLIC OF THE CONGO

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Two alternative approaches to community-led total sanitation (CLTS) are being carried out in the Democratic Republic of the Congo (DRC) as part of a health systems strengthening project (ASSP). This study examines data from a cross-sectional household survey conducted from April to May 2014 designed to capture information on factors related to water, sanitation, and hygiene (WASH) in the context of ASSP. A two stage – cluster sampling design was employed to select households from project supported health zones in three areas: 1) Tshopo and Maniema, 2) Kasai, Kasai Central, and Kasai-Oriental and 3) Nord Ubangi and Sud Ubangi, as well as matched clusters from non-project (control) areas. The survey measured two-week diarrheal disease prevalence among children under

five years of age as reported by the primary caregiver. Determinants of diarrheal disease were evaluated using bivariate and random effects logistic regression analysis. The analysis was limited to households with under-five children. 4,362 under-five children were identified in 2,223 households, however, only 3,942 observations were included in the final model due to missing values. The prevalence of diarrhea among provinces ranged from 8.92% to 16.56%. In multivariate analysis, significant risk factors for under-five diarrheal disease included primary maternal education [ORadj: 1.53, 95% CI (1.17, 2.00), p = 0.002], sharing a toilet facility with another family [ORadj: 1.63, 95% CI (1.27, 2.10), p < 0.001] and the presence of flies around the toilet facility [ORadj: 1.50 95% CI (1.09, 2.09) p = 0.014]. Understanding the determinants of diarrhea in children is crucial to the successful development and implementation of targeted WASH interventions. Although there is substantial evidence in the literature linking poor WASH to diarrheal disease in children under five, there is almost no evidence from the DRC that explores this association. The results of this analysis will help contextualize the outcomes of the CLTS approaches and comparing the effectiveness of these interventions will be critical in guiding the scale up of future WASH interventions in the DRC.

## 1894

### ASSOCIATION OF WATER SUPPLY WITH BRETEAU INDEX IN TWO RURAL CARIBBEAN POPULATION

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Vectorborne diseases represent an important problem that affect several regions worldwide. Features such as poor infrastructure, climatic conditions and water scarcity constitute some of the risk factors that increase this issue. Populations on tropical regions often exhibit features that worsen these conditions and consequently increase the presence of vectors. In this case, it was carried out a study in two rural populations of the coast Caribbean Coast, whose main difference is that one of them (Ararca) has a water supply system, while the other (Barú) lacks it. This particular difference allowed us to observe the influence that water supply system has in the increase of vector that transmit these diseases. It was examined 200 dwellings, and a total of 2012 containers between the two communities. Container index was higher in Barú (31.5%) than in Ararca (22.5) (p = 0.0002). Equally Breteau index, which was of 4.2 and 1.3 in Barú and Ararca respectively (p = 1.12x10<sup>-11</sup>). Aedes index was superior in Ararca (70.2%) than Barú (68.1%), but in these cases was not significant difference (p = 0.86). Due to the absence of water service supply, the Barú community has the necessity to acquire water through other ways which entails the storage of the resource for long periods, and this situation increases the container and Breteau index. Although these two indices were highest in Barú than in Ararca, did not happen the same with the Aedes index. This is due perhaps that Ararca also presents other risk factors that make it vulnerable (e.g. absence of sanitation facilities). Although also this information indicates that having a water supply system is not sufficient for improving some parameters as the Aedes index.

## 1895

### ASSESSMENT OF ABATTOIRS AND MARKETS SANITATION IN THE FEDERAL CAPITAL TERRITORY (ABUJA) AND ENUGU STATE, NIGERIA

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This study was done between June 2015 and November, 2016 on the assessment of abattoirs and markets sanitation in the Federal Capital Territory (Abuja) and Enugu state. The objective was to assess sanitary

conditions of the selected Markets and Abattoirs and to identify basic sanitary facilities present, and assess adequacy; determine levels of sanitation awareness and assess personal hygiene practices among workers in FCT and Enugu state. A cross sectional study design was employed. One thousand structured questionnaires were administered to the randomly selected traders, butchers, market and abattoir workers. Data collected were analyzed using descriptive and inferential statistics. Result showed that 969 (97.7%) respondents have heard about market and abattoir sanitation and hygiene. 60% of the respondents were not aware of Hazard Analysis and Critical Control Points (HACCP) principle in food and meat hygiene. 73.39% of respondents showed knowledge of waste management in markets and abattoirs. 675 (68%) of the respondents confirmed the non-availability of sanitary facilities in their market and abattoirs, vs 272 (27%) who affirmed availability of sanitary facilities. 45 (4.5%) had no idea, when compared with the statutory requirements for such premises. Traders, market and abattoir workers do not effectively use the toilet facilities. Refuse management and water supply facilities were inadequate in all study locations. We recommend investment during the developmental phase of market and abattoirs, enforcement of sanitary best practices and the deployment of Environmental Health Officers for risk assessment of markets and abattoirs.

## 1896

### EVERYBODY POOPS: SOCIAL AND CULTURAL NORMS AS PROXY MEASUREMENTS OF INDIVIDUAL-LEVEL DEFECATION PRACTICES

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There is growing evidence that latrine access does equate to uptake of latrines. To better implement sanitation interventions, it is necessary to first understand individual-level latrine use behavior. Using an ethnography that described defecation practices among individuals living in rural communities in coastal Ecuador and incorporating survey questions asked in other parts of the world, we designed a survey instrument to capture attitudes, norms, and beliefs related to latrine use and open defecation. We interviewed 89 adults with this tool. We applied Adaptive Elastic Nets (ENET) and Supervised Principal Component Analysis (SPCA) to reduce the data to a set of variables that predicted latrine use, as measured by self-report. The SPCA model reduced the dataset to 10 questions of importance, whereas Adaptive ENET identified 15 questions as having an association with reported latrine use. Demographic variables were not selected by either analytical approach and seven questions were selected by both models as having an association with the outcome. These models do not present statistically different results (chi-squared = 2.78, 1 degree of freedom, p-value = 0.10). Using machine learning techniques, we identified a list of social and cultural norms that predict individual-level latrine use. Indicators of cultural and social norms provide insight into behavior, and may also be subject to less misclassification than self-report of a sensitive behavior. These variables can be used in future work as a proxy measurement of latrine use, which would provide the WASH sector with a more robust variable for program evaluation than simply household access to a latrine.

## 1897

### COMPARATIVE PREVALENCE OF *PLASMODIUM FALCIPARUM* RESISTANCE-ASSOCIATED GENETIC POLYMORPHISMS IN PARASITES INFECTING HUMANS AND MOSQUITOES IN UGANDA

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Controlling malaria in high transmission areas, such as much of sub-Saharan Africa, will require concerted efforts to slow the spread of drug resistance and to develop strategies to impede transmission. Understanding the fitness costs associated with drug resistance, particularly within the context of transmission, can help guide policy decisions to accomplish these goals. Fitness constraints might lead to decreased transmission of drug-resistant strains. To determine if *Plasmodium falciparum* resistance-mediating polymorphisms impact on development at different parasite stages, we compared the genotypes of parasites infecting humans and mosquitoes from households in Tororo, Uganda. We genotyped 154 *P. falciparum* infected mosquitoes and 446 human samples at 14 polymorphic *loci* in genes encoding putative transporters (*pfprt* and *pfmdr1*) and folate pathway enzymes (*pfdhfr* and *dhrs*) using ligase detection reaction-fluorescent microsphere assays. Human and mosquito samples were from the same or nearby households within 40 days of each other. Of the 446 human samples that yielded data for our analysis, 122 were from the same household from which mosquitoes were collected and 324 were from a different household within 4 km (median distance 803 meters) of mosquito collection; matched human and mosquito samples were collected with 40 days of each other. Prevalences of key polymorphisms were similar between human and *Anopheles gambiae* s.l. infections, but pure mutant alleles were less common in human infections at the *pfmdr1* 86 (3% human vs. 19% mosquito,  $p < 0.001$ ) and *pfdhfr* 164 (0% in humans vs. 5% in mosquitoes,  $p = 0.001$ ) *loci*, and more common in human infections at *pfdhfr* 51 (99% humans vs. 93% mosquito,  $p = 0.003$ ). Mixed genotypes were more common in human infections, potentially explaining differences in allele prevalences. Overall, our results suggest modest directional selection resulting from varied fitness costs during the *P. falciparum* life cycle. Better appreciation of the fitness implications of different drug resistance mediating mutations can inform optimal malaria treatment and prevention strategies.

## 1898

### MOLECULAR SURVEILLANCE OF *PLASMODIUM FALCIPARUM* ANTIMALARIAL RESISTANCE IN SENTINEL SITES FROM MOZAMBIQUE

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The use of antimalarial drugs is one of the fundamental steps towards malaria control and elimination. The success of antimalarial treatment can be affected by drug resistant *Plasmodium falciparum* (Pf) populations, warranting the need to incorporate adequate molecular surveillance of known parasite factors associated with drug resistance for the early detection of resistant parasites. Pf positive dried blood spots from individuals (N=352) in four sentinel sites (Montepuez, Dondo, Moatize and Chokwe) from Mozambique were investigated for the presence of *k13* (dihydroartemisinin), *pfmdr1* (chloroquine), *pfcr1* (chloroquine, amodiaquine, artemether-lumefantrine) and *pfdhps* (sulfadoxine-pyrimethamine) polymorphisms using Sanger sequencing and restriction fragment length polymorphism. Plasmepsin (*pfpm*) 2 (piperazine) and *pfmdr1* (mefloquine and lumefantrine) copy numbers (CNs) were assessed by qPCR. The polymorphism analyses of *k13*, *pfmdr1*, *pfcr1* and *pfdhps* genes were successful in 98.3% to 100% isolates. Novel polymorphisms in *k13* (Leu619Leu, Phe656Ile, Val666Val and Gly690Gly) and *pfmdr1* (L1030L, D1061D, D1127D, S1137S, L1174L, D1179D, N1189N, T1192A, F1194S and Y1197N) were observed in the present study. The prevalence of *pfmdr1* 86Y and 184F mutant alleles was 3.1% and 46.7% respectively. The *pfcr1* mutant alleles (74I, 75E and 76T) were only present in Chokwe isolates (9.2%). The prevalence of *pfdhps* gene 436F, 437A and 540E mutant alleles were 9.3%, 26.7% and 82.2% respectively. Frequencies of *pfdhps* polymorphisms were significantly different between four sites. The prevalence of isolates with multiple copies (CN>1.5) of *pfpm2* and *pfmdr1* genes were 4/351 (1.1%) and 5/351 (1.4%) respectively. Markers of resistance to dihydroartemisinin were not found in the studied Pf isolates. At present the prevalence of Pf isolates with multiple copies of *pfpm2* gene is low. However, with adequate piperazine drug pressure resistant isolates may spread in Mozambique. This study also supports previous evidence for the return of chloroquine sensitive Pf isolates in Mozambique based on the low prevalence of *pfcr1* markers of resistance.

## 1899

### PATTERN OF K13 POLYMORPHISMS AMONG PLASMODIUM FALCIPARUM ISOLATES FROM BORDER AREAS IN THE MEKONG SUBREGION

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Emergence of artemisinin resistance of *Plasmodium falciparum* in Southeast Asia and the possibility of further geographical spread pose a serious threat to global elimination efforts, emphasizing the need to conduct surveillance of resistant strains. Mutations in the propeller domain of K13 gene of *P. falciparum* have been shown to correlate with delayed parasite clearance times and enhanced survival and are a prerequisite to meet the WHO definition of artemisinin resistance. In this study, we determined the prevalence of K13 polymorphisms by Sanger sequencing in samples collected from Western Cambodia, Eastern Thailand (along the Thai-Cambodian border), and along the Thailand-Myanmar border. From a total of 351 samples from Western Cambodia, the C580Y mutation was present in a majority of isolates (327; 93.16%) followed by R539T mutation (16; 4.56%) and wild type (8; 2.28%). Not surprisingly, this pattern was similar to that found in Thailand near the Cambodian border with C580Y mutation (74%, 49/66) also present in most of the isolates followed by R539T mutation (24.24%, 16/66) and R539I (1.52%, 1/66). In contrast, along the Thailand-Myanmar border, wild type was most common (72.92%, 35/48) followed by N537I (12.5%, 6/48), R561H (6.25%, 3/48), C580Y (4.17%, 2/48), G449A (2.08%, 1/48), and

P553L (2.08%, 1.48). Our study suggests a bimodal population of K13 polymorphisms that should be continuously monitored, especially in border areas in Thailand, where overlap of K13 resistant strains may accelerate fixation of delayed parasite clearance phenotypes and clinically significant artemisinin resistance. To identify minor K13 allelic variants circulating in individual patients that may not be picked up by Sanger sequencing alone, we are also performing amplicon deep sequencing on an Illumina MiSeq platform. These results, plus the prevalence of other molecular resistance markers such as *plasmepsin*, *pfmdr1* and *pfcr1* will be presented. Taken together, we hope to be able to identify major and minor resistance variant frequencies and develop a more accurate picture regarding the distribution of infection haplotypes in border regions.

## 1900

### IMPACT OF DIHYDROARTEMISININ-PIPERAZINE FOR INTERMITTENT PREVENTIVE TREATMENT OF MALARIA DURING PREGNANCY ON MALARIA INCIDENCE IN EARLY CHILDHOOD

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Intermittent preventive treatment of malaria in pregnancy (IPTp) with dihydroartemisinin piperazine (DP) is an effective and promising strategy in settings with widespread resistance to sulfadoxine-pyrimethamine (SP), but its impact on the risk of malaria during early childhood is unknown. We evaluated the impact of IPTp-DP on malaria incidence in early childhood in a birth cohort of children born to HIV-uninfected pregnant women living in Tororo, Uganda, who took part in a double blinded randomized controlled trial (NCT02163447). Pregnant women were enrolled at 12-20 weeks gestation and randomized to either SP given every 8 weeks or DP every 8 weeks or DP every 4 weeks. Children born to these mothers were given chemoprevention with DP every 12 weeks starting at 8 weeks of age, and followed from birth to 2 years of age. Women and children received all their medical care at a study clinic open 7 days a week. Incident episodes of malaria were defined as all treatments for malaria (fever and presence of malaria parasites by blood smear (BS)) not preceded by another treatment in the prior 14 days. Parasite prevalence (assessed by BS and loop mediated isothermal amplification (LAMP), and prevalence of anemia (hemoglobin <10g/dL), were assessed monthly and every 4 months respectively. The primary outcome was incidence of malaria from birth to 2 years of age; secondary outcomes included parasite prevalence and prevalence of anemia. The primary exposure was maternal IPTp randomization assignment. Through March 31, 2017, there were 192 live births, and 146 children have reached 2 years of age. During 355.2 person years of follow-up, there were 113 incident malaria episodes (overall malaria incidence of 0.32 episodes per person year). Overall prevalence of malaria parasites during monthly assessments by BS and LAMP was 50/4566 (1.1%) and 113/3652 (3.0%), respectively. Prevalence of anemia (hemoglobin <10 g/dL) was 155/1201 (3.0%). The last child will reach 2 years of age on May 18, 2017. Final unblinded results by maternal randomization will be presented at the time of the conference.

## 1901

### EFFICACY OF ARTEMISININ-BASED AND QUININE-BASED TREATMENTS FOR UNCOMPLICATED FALCIPARUM MALARIA IN PREGNANCY IN ASIA: A SYSTEMATIC REVIEW AND INDIVIDUAL PATIENT DATA META-ANALYSIS

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Pregnant women are more vulnerable to falciparum malaria and even asymptomatic infection can affect both mother and fetus. In non-African settings, early diagnosis and prompt treatment with effective drugs is the strategy recommended by WHO. However, the evidence to support current recommendations is sparse, as pregnant women have been systematically excluded from standard drug efficacy trials. In addition, there is no agreed standard method to assess antimalarial efficacy in pregnancy. We aim to summarise the current evidence of antimalarial efficacy in pregnancy and explore a standard methodology of assessment. We conducted a systematic review of antimalarial efficacy studies against uncomplicated falciparum malaria in pregnancy in Asia. Individual patient data (IPD) were sought and IPD meta-analysis was conducted using a random effects model to explore the clinical determinants associated with recrudescence failures. We identified 20 relevant studies, including two unpublished randomised control trials. Ten published studies used polymerase chain reaction (PCR) for differentiating recrudescence from reinfection, and all were conducted on the Thailand-Myanmar border. From all ten published studies with documented PCR, IPD of 1,466 malaria episodes from 1,396 women were analysed, of which 634 (43%) and 832 (57%) episodes were treated with quinine-based treatment (QBT) and artemisinin-based treatment (ABT) respectively. A total of 259 episodes occurred during the first trimester, 47 (18%) of them with ABT. Patients were followed up every 1–2 weeks until delivery (or for 42–63 days, whichever came later). PCR-adjusted adequate clinical and parasitological responses (ACPR) proportion of ABT were generally >90%, and this proportion decreased with longer follow-up duration. Risk of treatment failure was higher in patients treated with QBT compared to ABT. PCR-corrected ACPR of each ABT and QBT, and risk factors associated with treatment failure will be presented. This review highlighted a substantial methodological heterogeneity in clinical practices in MIP, calling for an urgent need to adopt a standardised methodology.

## 1902

### RELATIONSHIP BETWEEN LUMEFANTRINE PHARMACOKINETICS AND THE SELECTION OF DRUG RESISTANCE MUTATIONS FOLLOWING ARTEMETHER-LUMEFANTRINE IN HIV-UNINFECTED AND HIV-INFECTED CHILDREN ON ANTIRETROVIRAL THERAPY

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*In vitro* and *in vivo* studies have demonstrated that mutations in certain *Plasmodium falciparum* putative transporters, particularly *pfmdr1* and *pfcr1* are key mediators in resistance to many of the long-acting partner drugs used in artemisinin-based combination therapies (ACTs). In addition, recent *in vitro* data suggests that changes in *pfmdr1* may mediate resistance to the antiretroviral lopinavir/ritonavir (LPV/r). The impact of HIV infection, antiretroviral regimen, as well as the impact of long-acting partner drug

pharmacokinetics on the selection of drug resistant mutations in recurrent malaria has not been clearly elucidated. We conducted a prospective PK/PD study of artemether-lumefantrine in HIV-uninfected and HIV-infected children on three 1st-line antiretroviral regimens in a high endemic region of eastern Uganda. Drug levels were quantified at multiple time points, key molecular markers of drug resistance (*pfcr1* K76T, *pfmdr1* N86Y, *pfmdr1* Y184F) were assessed using a ligase detection reaction-fluorescent microsphere assay, *pfmdr1* copy number using real time PCR, and parasites were genotyped using six polymorphic markers to determine multiplicity of infection. Recurrent malaria (either clinical or parasitological failure) by day 42 was observed in 187 of 355 children. Drug levels in those with recurrent malaria varied dramatically between treatment groups (median day 7 lumefantrine in HIV-uninfected children, and HIV-infected children on efavirenz-, nevirapine-, and lopinavir/ritonavir-based ART were 345, 107, 515, and 620 ng/mL, respectively). Thus far, paired genotyping of baseline and recurrent infections for *pfmdr1* N86Y, *pfmdr1* Y184F, and *pfcr1* K76T has been completed in 75, 90, and 65 children, respectively. The prevalence of mutant alleles at baseline and the time of recurrent malaria was 18% and 4%, 67% and 68%, and 77% and 69%, for *pfmdr1* 86Y, *pfmdr1* 184F, and *pfcr1* 76T, respectively. Genotyping will be completed in all children, and the association of lumefantrine drug exposure and the selection of drug resistant markers at all putative drug resistance markers will be presented.

## 1903

### ELECTROCARDIOGRAPHIC EFFECTS OF THE ANTIMALARIAL DRUG DIHYDROARTEMISININ-PIPERAQUINE

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Concerns have been raised regarding the potential for the antimalarial drug dihydroartemisinin-piperazine to cause cardiotoxicity. Pharmacokinetic samples and electrocardiogram (ECG) measurements were obtained from a total of 1,000 patients, enrolled in a multi-centre trial in Burkina Faso, Mozambique, Ghana, and Tanzania [INDEPTH study]. All patients had uncomplicated *P. falciparum* malaria and received a standard 3-day treatment of dihydroartemisinin-piperazine. Nonlinear mixed-effects modelling was used to describe the relationship between piperazine exposure and QT-prolongation in order to evaluate the cardiovascular safety in patients with uncomplicated malaria. Both QTc-prolongation ( $\Delta$ QTc) and absolute QTc-intervals (QTc) were evaluated with separate modelling approaches. Linear and non-linear effects models, the impact of a circadian rhythm, and clinical covariates were evaluated with a stepwise modelling approach. A three-compartment disposition model with three transit absorption compartments described the piperazine concentration-time profile well. Body weight was included as an allometric function on all clearance and volume parameters, resulting in lower drug exposure in small children. The effect of piperazine on  $\Delta$ QTc was described by a linear relationship, resulting in a 6.14 msec QTc-prolongation per 100 ng/mL increase in population piperazine concentration. The effect of piperazine on the absolute QTc-interval was best described by a nonlinear model resulting in a maximum population QT-prolongation of 48.1 msec. The developed pharmacokinetic-pharmacodynamic model described the relationship between piperazine concentrations and  $\Delta$ QTc/QTc intervals effectively. The model demonstrated that an increased piperazine concentration was directly related to a prolongation of the QTc interval. However, the prolongation estimated from the model was considered to have an acceptable safety profile in the clinical setting of life-saving malaria treatment.

## 1904

**SURINAME ON THE ROAD TO ZERO MALARIA; AN EPIDEMIOLOGIC DESCRIPTIVE STUDY**

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Until 2004 malaria in Suriname showed a high disease burden with high *Plasmodium falciparum* (Pf) proportions. In 2004, Coartem® was introduced as first line treatment for Pf infections. Since then malaria control activities were scaled up and included improved access to diagnosis and treatment and mass distribution of Long Lasting Insecticide treated Nets. The population at risk is about 80,000 people and can be stratified as stable Maroon and Amerindian tribal communities and mobile migrant gold miner populations (MMP). This study describes the malaria epidemiology in Suriname between 2000 and 2015. Surveillance data were obtained from national healthcare providers, analyzed and described. Epidemiologic parameters were calculated. The trend of confirmed malaria cases shows peaks of cases until 2004. The highest peak, 15,967 cases, was observed in 2001. From 2004 onwards a steep decline is observed, reaching a nadir of 308 cases in 2014 (decrease of 98.1%). The Pf to non-Pf ratio has declined by 89.6% from 4.8 in 2000 to 0.5 in 2015. With both Pf and *Plasmodium vivax* (Pv) cases declining. Since 2011 cases among children below 14 years of age were very few. Hospital admissions for malaria have decreased enormously by 97.1% from 377 in 2003 to 11 in 2015. The proportion of admissions due to Pv infections increased from 13.4% in 2001 to 45.5% in 2015. Also, a shift in at risk populations was observed from stable communities to MMP populations. Deaths due to malaria have dropped from 24 in 2000 to 0 in 2015. In the current near elimination setting, management of imported cases has become increasingly important. In 2015 the proportion of imported cases was 73.5% of the total number of cases diagnosed. The majority originated from French Guiana as a consequence of cross-border moving miners. Suriname is on the brink of elimination. Artemisinin Combination Therapy as first line treatment for Pf infections and improved access to diagnosis and treatment have contributed to the malaria reduction. However, the high proportion of imported malaria poses the risk of re-introduction in areas which are currently malaria free. A regional approach is required to deal with this.

## 1905

**MALARIA ELIMINATION: ENGAGING COMMUNITIES THROUGH NATIONWIDE CAMPAIGNS**

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Malaria elimination requires political commitment, sustained funding, and a package of technical tools. Elimination will be impossible without engaging the direct beneficiaries: affected communities. These communities should not be considered merely as recipients of priority funding, but rather, as critical partners. PATH-MACEPA has worked with national malaria programs in Senegal and Zambia to create community demand for malaria elimination strategies, tailoring approaches as needed. In Senegal, the “Zéro Palu, je m’engage” campaign was launched in 2014 to bring together citizens at all levels of the country in the fight against malaria. One of the campaign’s initiatives is the deployment of “community champions” to facilitate malaria elimination interventions at the community level. These community champions are sponsored by private-sector companies, showcasing how public-private partnerships

can collaborate to eliminate malaria one community at a time. In Zambia, the “Malaria Ends with Me” slogan was first used in 2015 during a mass drug administration (MDA) trial. This trial presented challenges around the acceptability of a new approach, including giving a new drug to people who “felt fine”. Community engagement activities likely contributed to a low refusal rate of less than 1% by the end of the study. When MDA results demonstrated the issue of not finding people at home, community engagement was expanded to target mobile populations, school children, and religious leaders. As malaria decreases, identifying, treating, and following up each case is essential. Case investigation relies on neighbors of an “index case” accepting testing and treatment. In Senegal last year, close to 2,500 index cases led to investigations; out of over 51,600 people, only 143 refused to be tested. In Zambia, villages select individuals to serve as community health workers who test, treat, and track local and imported infections, while in Senegal, volunteers in malaria-free communities motivate travelers to get tested to avoid local spread of infection. These examples show the power of communities taking malaria elimination into their own hands.

## 1906

**USE OF ROUTINE HEALTH INFORMATION SYSTEM DATA TO EVALUATE IMPACT OF MALARIA INTERVENTIONS IN ZANZIBAR DURING THE PERIOD 2000-2015**

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Zanzibar aims to eliminate malaria locally-acquired malaria by 2018, following sharp declines in malaria morbidity and mortality since 2000. Interventions introduced and maintained in Zanzibar included universal diagnostic testing and artemisinin-based combination therapy (ACT) for case management of uncomplicated malaria from late 2003, and insecticide-treated nets and indoor residual spraying from 2006. These interventions were scaled up rapidly across both islands of Zanzibar: Pemba and Unguja. Evaluating the impact of these interventions on malaria burden using probabilistic methods is not possible due to the lack of contemporaneous control groups that did not receive interventions. We present analysis of routine health information management system (HMIS) data using a plausibility approach to estimate the impact of interventions applied during 2000–2015. An interrupted time series model was used to analyze changes in confirmed malaria incidence from 129 public outpatient facilities in Zanzibar. In addition, modeled counterfactuals were generated to estimate the number of malaria cases averted due to introduction of interventions. After accounting for climate variability, seasonality, testing rates, and outpatient attendance, the average monthly incidence rate declined following introduction of ACTs in 2003 compared to the pre-ACT period (incidence rate ratio, IRR 0.976). After vector control was introduced in 2006, the trend increased compared to the pre-vector control period (IRR 1.0184), but maintained an overall decline (IRR 0.9918). The combination of case management with ACTs and vector control is

estimated to have averted, on average, 8394 malaria cases per year during 2006–2015 across Zanzibar, while ACTs alone averted, on average, 1999 malaria cases per year during 2004–2005. This study presents a relatively simple yet rigorous approach to the use of routine HMIS data in evaluating the impact of malaria interventions.

## 1907

### EVIDENCE OF TRANSMISSION DECLINE DURING MASS DRUG ADMINISTRATION TRIALS IN SOUTHERN ZAMBIA THROUGH PARASITE GENOMICS: AN EXAMPLE OF BARCODING AND ITS UTILITY FOR MALARIA PROGRAMS

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Evaluation of changing genomic diversity of malaria parasite infections could provide critically sensitive measures of changing transmission dynamics. The Zambian Ministry of Health is exploring mass drug administration (MDA) with dihydroartemisinin-piperazine in combination with vector control to accelerate malaria elimination efforts. As part of a monthly sampled cohort of individuals followed over 18 months (December 2014–May 2016) from a community randomized controlled MDA trial in 60 health facility catchment areas in Southern province, parasites were assessed for patterns in genetic relatedness. Molecular barcode analysis was performed on *Plasmodium falciparum* genetic material extracted from discarded rapid diagnostic tests. Across 231 RDT positive samples with molecular barcodes over the study period, the proportion of monogenomic samples at baseline was 36%, and this increased substantially in the first six months of the study (final results will be available for presentation). Multiple instances of identical molecular barcodes were identified and fell into four types: those collected at the same month/time interval, those collected following the same MDA round, those from samples collected between MDA treatment rounds, and those collected from different clusters. Assessment of samples from individuals who were slide positive two months after treatment revealed that all of these infections were new parasite infections, since multiple positions of the 24-SNP molecular barcode were distinct between the sample pairs. These results suggest that genetic characterization of infections allows measures of increasing monogenomic infections and documentation of incident infections and possibly their source; these changes may correlate with both control efforts and with transmission decline. These findings support the use of parasite genomics to track changes in the parasite populations in response to the deployment of elimination programs; as transmission is reduced to very low levels, these metrics may be more sensitive to change than traditional tools.

## 1908

### PCR INCIDENCE OF *PLASMODIUM FALCIPARUM* INFECTIONS IN COHORT SAMPLES OVER TIME DURING A MALARIA MDA RANDOMIZED CONTROL TRIAL IN SOUTHERN PROVINCE ZAMBIA

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As part of a comprehensive package of interventions for achieving malaria elimination in Southern Zambia, a community randomized control trial was carried out in 60 health facility catchment areas (HFCAs) to assess the effectiveness of mass drug administration (MDA) on malaria prevalence and incidence. HFCAs were randomized to receive MDA (treatment given to all individuals), focal MDA (treatment given to all individuals living in a house where anyone tested positive), or standard malaria control activities that included high vector control coverage and community case management (control). A subset of individuals (all ages) in each HFCA was enrolled into a longitudinal study involving monthly visits where individuals were tested for malaria using the SD Bioline *P.f* RDT (and treated with artemether-lumefantrine if positive) as well as the collection of a dried blood spot (DBS) and (in some cases) a blood slide. A total of approximately 34,000 individual cohort visits were performed over the 18-month lifespan of the trial. To better understand malaria incidence in the different arms of the study, all available DBS are being tested by PET-PCR—a real-time fluorogenic primer-based PCR assay that detects the presence of *Plasmodium* at the genus and species level with a sensitivity many fold higher than that of conventional point-of-care diagnostics. From these data, we determined the level of (i) RDT subpatent parasite carriage, (ii) PCR microscopy and RDT concordance, and (iii) non-*Plasmodium falciparum* species prevalence. To date, 10,218 RDT-negative and 69 RDT-positive DBS were assayed by PET-PCR, representing the first six months of the samples collected, of which 2% were PCR-positive. The negative predictive value (NPV) of the RDT tests was found to be 99.7% while the positive predictive value (PPV) was 56.5%. The high NPV and the low PPV respectively showed that the RDT is highly specific but has moderate sensitivity. The implication of these findings on elimination strategies will be discussed.

## 1909

### PREVENTING REESTABLISHMENT OF MALARIA IN RECENTLY-ELIMINATED AREAS: A MODELING STUDY OF REACTIVE CASE DETECTION AND ADAPTIVE RESPONSE

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After elimination, areas with substantial vectorial capacity remain susceptible to reestablishment of malaria transmission through importation of infections. What types of operations are effective at preventing resurgence? What are the features of an effective surveillance system? How do programmatic needs differ based on local conditions such as pre-elimination transmission intensity, population density, and importation rate? To address these questions, we simulated malaria transmission

at the household level with an agent-based mathematical model and investigated the effects of various case management rates, maintenance of vector control, features of reactive case detection programs, and larger-scale drug- and insecticide-based responses on outbreak management and prevention of resurgence in three representative communities in southern Zambia. We find that excellent surveillance is a necessary condition for preventing reestablishment, and we predict that many areas of sub-Saharan Africa will require continued refreshing of vector control until elimination is achieved on a broader regional scale and importation becomes unlikely. The intensity of reactive activities needed to maintain elimination varies with both local receptivity and local configuration of households, with densely populated areas requiring larger radius of follow-up activities. Since asymptomatic infections remain likely when infections are imported into areas with substantial lingering immunity, adaptive responses where mass drug campaigns are triggered when clinical case counts surpass a pre-determined threshold can be a powerful tool for managing outbreaks. These results suggest a general set of guidelines for programs seeking to maintain elimination in areas that remain vulnerable to importation pressure.

## 1910

### DEVELOPING A NATIONAL MALARIA ELIMINATION INVESTMENT CASE: A FRAMEWORK AND APPLICATION

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The World Health Organization's *Global Technical Strategy for Malaria 2016-2030* calls for malaria elimination in at least 35 countries by 2030. To achieve this ambitious target, national malaria programs will require adequate financial resources to interrupt local transmission and prevent reintroduction. Donor funding for malaria elimination, however, has decreased in recent years, and competing priorities, coupled with a low priority given to malaria as a result of reduced transmission rates, often lead governments to withdraw funding for malaria at a critical juncture. Historical evidence suggests that ill-timed downsizing of malaria programs can lead to deadly and costly resurgences. To sustain political and financial commitment, policymakers responsible for resource allocation must be convinced of the economic returns of eliminating malaria. An investment case can present the rationale for investing in clear and concise terms. Building on existing literature, we developed a framework for national malaria elimination investment cases. The framework has four sections, namely (1) proposed investment, (2) rationale for investing, (3) financial landscape, and (4) issues to consider. The framework, when properly adapted, can help malaria programs gather and generate evidence on the costs, benefits, risks, and financial viability of national malaria elimination. We applied this framework to Papua New Guinea, a malaria endemic country that has aligned itself with the regional goal of making Asia Pacific malaria-free by 2030. Using the outputs of a dynamic transmission model, we estimated the total cost of eliminating malaria to be US\$454 million (range: US\$282-701 million) in 2016-2030, or \$30 million on average per year. This is roughly US\$14 million more than what was spent on malaria control in 2015. Elimination by 2030 can save over 14,500 lives and avert over 7.8 million cases. The net economic benefits of elimination compared to a business-as-usual scenario is roughly US\$5 billion (range: US\$4-7 billion). The median return on investment is 17, indicating that the incremental costs of elimination greatly outweigh its costs.

## 1911

### HIGH LEVEL EFFICACY IN HUMANS OF A NEXT-GENERATION *PLASMODIUM FALCIPARUM* ANTI-SPOROZOITE VACCINE: R21 IN MATRIX-M™ ADJUVANT

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It remains a global health priority to develop a durable and highly efficacious malaria vaccine. The most advanced malaria vaccine candidate, RTS,S/AS01 has completed Phase III testing in a multicentre study across several African sites and demonstrates low-level (~30%) efficacy against clinical malaria in children aged 5-17 months after a three dose schedule. Efficacy wanes rapidly over time and there remain some safety concerns that require further assessment in the planned pilot deployment trials due to commence in Africa in 2018. There is a need to improve on RTS,S/AS01 to achieve WHO development goals of a durable vaccine with >75% efficacy. RTS,S comprises recombinant particles expressing the central repeat and the C-terminus of the circumsporozoite protein (CSP) fused to Hepatitis B surface antigen (HBsAg). R21 has been developed at the Jenner Institute, University of Oxford. This is an improved HBsAg-CSP-based construct, but without any unfused HBsAg protein which is present at a four-fold molar excess in RTS,S particles. This excess of HBsAg was required for RTS,S to form a particle; however, R21 forms particles without excess HBsAg by utilizing the better expressing yeast, *Pichia pastoris*. Matrix-M (MM) is a promising, novel saponin-based adjuvant that has been tested safely with vaccines for a number of diseases in over 1000 subjects. We previously showed that R21 administered with MM is safe and has comparable immunogenicity at much lower doses compared to RTS,S/AS01: 10µg doses of R21/MM induced the same antibody titres to the CSP repeat as 50µg of RTS,S/AS01B. Durable humoral responses at 6 months were higher for the 10µg than the 50µg dose of R21/MM. We report a Phase IIa efficacy trial using controlled human malaria infection in healthy UK adult volunteers. Three doses of 10µg R21 adjuvanted with MM given 4 weeks apart demonstrated high level sterile efficacy [(81.8%; n=11); p=0.0009]. Furthermore, we observed significantly reduced reactogenicity compared to reported data on the standard RTS,S/AS01 regimen. These data provide strong support for this R21/MM vaccine to be evaluated further in African adults, children and infants.

## 1912

### SAFETY AND IMMUNOGENICITY OF THE NOVEL *PLASMODIUM FALCIPARUM* BLOOD-STAGE VACCINE RH5.1/AS01B IN A PHASE I/IIA CLINICAL TRIAL

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The development of an effective blood-stage malaria vaccine holds significant promise, not only for reducing the morbidity and mortality associated with malaria, but also for reducing transmission, either alone or as a crucial component of a multi-stage vaccine. The reticulocyte-binding protein homologue 5 (RH5) is the most promising blood-stage *P. falciparum* candidate antigen to date. It is essential for parasite survival and erythrocyte invasion, has limited polymorphism, and antibodies induced by RH5 in preclinical *in vitro* studies overcome two of the long-standing difficulties associated with other merozoite antigens: first, they can block erythrocyte invasion to high efficiency (requiring lower antibody concentrations); and second, cross-inhibit all *P. falciparum* lines and field isolates tested to-date. Efficacy has been shown against a stringent heterologous strain blood-stage challenge in an *in vivo* Aotus monkey-*P. falciparum* challenge model, following vaccination with RH5 protein. Protection was strongly correlated with anti-RH5 serum IgG antibody concentration and *in vitro* functional growth inhibition activity (GIA). Here we report on a first-in-human Phase Ia clinical trial (NCT02927145) of the recombinant protein RH5.1 delivered with GSK's adjuvant AS01B to 48 healthy, malaria-naïve UK adult volunteers (four groups of n=12). Group 1 receive a lead-in dose (2 µg) of RH5.1 (given 3 times 4 weeks apart) before dose escalation to 10 µg in Group 2, and then 50 µg in Group 4 (same schedule). Group 3 receive 50 µg twice 4 weeks apart followed by a delayed, fractional dose (10 µg) boost at 6 months. All groups receive the same dose (0.5 mL) of AS01B. This is the first RH5 protein/adjuvant vaccine to be tested in humans. The vaccine is well tolerated at all doses. There have been no safety concerns and no SAEs to-date. T cell, B cell and serum antibody responses have been assessed by ELISpot, flow cytometry and quantitative ELISA and purified IgG is tested for anti-parasitic function using the *in vitro* GIA assay. These promising data will be presented and RH5.1/AS01B will be tested for efficacy in a Phase IIa blood-stage CHMI trial in late 2017.

## 1913

### INTEGRATED ANALYSIS OF ANTIBODY, CYTOKINE AND T CELL RESPONSES INDUCED BY RTS,S/AS01E VACCINATION WITHIN THE AFRICAN PEDIATRIC PHASE 3 TRIAL: SEARCHING FOR CORRELATES OF PROTECTION

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The RTS,S/AS01E malaria vaccine is the most clinically advanced, however there is still a need to better characterize and integrate antibody and cellular immune responses elicited upon vaccination to understand its moderate and short-lasting protection. We assessed the antibody and cellular responses to the RTS,S/AS01E antigens circumsporozoite protein (CSP) and Hepatitis B surface antigen (HBsAg) at baseline and one month post primary vaccination by luminex and intracellular cytokine

staining and flow cytometry in RTS,S-vaccinated and control-vaccinated children recruited in two of the African sites of the phase III trial: Manhiça (Mozambique) and Bagamoyo (Tanzania). We aimed to explore the association between antibody and cellular responses induced by the vaccine, focusing on specific cell types and cytokines of the T helper phenotype. RTS,S vaccination induced polyfunctional CSP- and HBsAg-specific CD4+ T cells and antibodies of different isotypes/subclasses and degrees of avidity to multiple CSP B cell epitopes (NANP, C-terminal and full length). RTS,S-vaccinated children had significantly higher frequencies of CSP- and HBsAg-specific CD4+ T cells producing IL-2, TNF-α and CD40L and HBsAg-specific CD4+ T producing IFN-γ and IL-17 than controls or baseline frequencies; these responses were confined in central and effector memory compartments. Additionally, effector memory CD4+ T cells producing IL-4 and IL-21 were detected for both vaccine antigens. Furthermore, we identified distinct profiles of antigen-specific cytokine responses associated with RTS,S vaccination and protection/risk of malaria. We will present data on the correlation between such cytokine profiles and antibody responses induced by the vaccine in African children. Data thus far show that RTS,S/AS01E vaccine induces antibody and T cell responses of higher quality and complexity than previously thought. The joint analysis of responses detected in memory CD4+ T and B cell compartments may provide better correlates of RTS,S/AS01-induced immunity and duration of protection that may guide development of second generation improved candidates.

## 1914

### IGG PROTEOMICS AND BCR SEQUENCING OF SPECIFIC B CELLS FOR ANTIBODY REPERTOIRE ASSESSMENT AFTER MALARIA TRANSMISSION BLOCKING VACCINATION IN MALIAN ADULTS

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Transmission blocking vaccines induce antibodies that target sexual-stage antigens expressed by the parasite in the mosquito. Antibodies against Pfs25 and Pfs230, molecules present on the surface of the sexual stages of *Plasmodium falciparum*, can reduce parasite burden in the mosquito. Antibody repertoire analyses, performed by BCR sequencing and/or proteomics of serum IgG, have been employed to evaluate vaccine responses. Here, we used a Pfs25 tetramer to sort Pfs25-specific single B cells (Pfs25+, CD3-, CD19+, CD20+) generated after a fourth dose of Pfs25-EPA immunization in Malian adults, and sequenced light and heavy chain of antibody from these cells. In a second approach, Pfs25-specific antibodies were affinity-purified from plasma of vaccinees and analysed by mass spectrometry. For peptide analysis, we used an *in-house* database, generated by immunosequencing a 130-nt fragment of the CDR3 region of the IgH from PBMCs from the same donors. We compared two groups of samples: those with antibodies able to reduce parasite burden in the midgut of a mosquito; and those without such antibody activity. For both groups, we compared IgG sequence alignment/coverage using the PBMC immunosequencing database, CDR3 length, and post-translational modifications. Our results showed that all Pfs25-IgG samples from the high antibody activity group yielded peptides matching CDR3 region sequences generated from study PBMC. Coverages higher than 75% were found only in the high antibody activity group. Hexose was found as post-translational modification only in peptide sequences from the high antibody activity group, suggesting a glycosylation process. CDR3 length did not differ between high and low activity groups. These preliminary analyses demonstrate that proteomics techniques can detect specific IgGs from the vaccine trial. For the BCR sequencing of antigen-specific

single cell, we were able to find information about heavy and light chain for dozens of cells per sample. We will combine all datasets (CDR3 sequencing; BCR sequencing of single cell; MS/MS analysis) to define BCR repertoire, expansion, and hypermutation after vaccination.

## 1915

### DIFFERENTIAL IMMUNE-RESPONSIVENESS TO PFSPZ VACCINE IN MALARIA-NAÏVE, SEMI-IMMUNE AND IMMUNE POPULATIONS FROM INFANCY TO ADULTHOOD

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Sanaria is developing whole *Plasmodium falciparum* (Pf) sporozoite (SPZ) vaccines spurred by an international scientific alliance of ~200 investigators from ~40 organizations in ~20 countries. Unprecedented clinical outcomes have resulted from 14 PfSPZ-based Vaccine trials conducted to date; >90% protection against controlled human malaria infection has been achieved in 6 clinical trials in the USA, Germany, Mali, and Tanzania. The high level of observed efficacy must necessarily rely on and be defined by wide-ranging and robust induction of immune responses. T cell responses directed against infected hepatocytes are considered to be the primary mediators of protection, but antibodies against PfSPZ have also been implicated, and may also be a marker for T cell responses. We have systematically assessed antibody levels against, 1) the most-abundant protein on PfSPZ, the circumsporozoite protein (CSP), by ELISA; 2) whole PfSPZ surface antigens by an automated immunofluorescence assay (aIFA); and live PfSPZ by inhibition of sporozoite invasion (ISI) of hepatocytes, on a high throughput platform, for all trials. In semi-immune Tanzanian adults the median antibody responses to PfCSP by ELISA, were 6.5-10 times lower than in malaria naïve Americans, but higher than in immune Malians who received the same immunization regimen. T cell responses implicated in durable protection in the face of waning antibodies, followed the same trend. Overall, the lower vaccine specific immune responses correlated with poor protective efficacy in adult Tanzanians. Most notably though, antibody responses increased in magnitude in younger age groups in Tanzania with 6-12 month olds attaining highest levels, similar to those in naïve adults in the USA. Future systems-based analyses, are expected to highlight the immunological underpinnings driving differential responsiveness to PfSPZ Vaccines across age-groups and exposure levels, and how to overcome immune dysregulation in adults due to long term exposure to malaria.

## 1916

### HUMAN TO MOSQUITO TRANSMISSION OF *PLASMODIUM VIVAX* GAMETOCYTES DURING CONTROLLED HUMAN MALARIA INFECTION AND DEVELOPMENT OF *VIVAX* SPOROZOITES

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*Plasmodium vivax* is a major cause of morbidity and the most globally widespread malaria parasite. However, the development of drugs and vaccines to control *P. vivax* infection has been limited in comparison to progress with *P. falciparum*. This is largely due to the inability to continuously culture the parasites *in vitro*, thus hindering the evaluation of interventions against asexual parasite cultures, preventing the *in vitro* generation of gametocytes for transmission blocking studies, and limiting the production of sporozoites that could be used to infect hepatocytes for the evaluation of hypnozoites. Here we describe the iterative development of a safe and reproducible clinical trial system using *P. vivax*-infected erythrocytes to initiate a blood stage infection in malaria-naïve volunteers (n=32), that could potentially be exploited to study all stages of *P. vivax* parasite biology. We demonstrate the ability to monitor the development and clearance of asexual parasitemia and gametocytemia with qPCR and RT-qPCR, respectively. *P. vivax* gametocytes were detected in all volunteers and were successfully transmitted to *Anopheles* mosquitoes using direct skin feeding assays (DFA) and direct membrane feeding assays (DMFA). Transmission success was related to the level of gametocytemia and the prevalence of infection was up to up 63% after DFA and up 12% after DMFA. Membrane feeding assays were also performed following the enrichment of gametocytes and resulted in 93% of the mosquitoes infected with up to 10 oocysts/midgut and ~4,300 sporozoites/infected mosquito. This work demonstrates the potential utility of *P. vivax* controlled human malaria infection as a model to evaluate asexual stage and transmission blocking interventions. Furthermore, this system could facilitate the study of multiple *P. vivax* parasite stages by providing a source of gametocytes for standard membrane feeding assays and generating sporozoites to enable the evaluation of hypnozoites. This system is amenable to further optimisation and thus may provide an opportunity to support the development of drugs and vaccines against all stages of *P. vivax*.

## 1917

### A NOVEL HIGHLY PROTECTIVE *PLASMODIUM* ANTIGEN - A *FALCIPARUM* VACCINE CANDIDATE

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The development of an effective malaria vaccine depends upon inducing an immune response capable of preventing infection, disease, and transmission. A malaria vaccine is feasible as radiation-attenuated sporozoites (RAS) elicit sterile protection in mice and humans. While both RTS,S and whole sporozoite vaccines show potential, these vaccines may not achieve the long-term efficacy and cross-strain protection necessary. Alternative pre-erythrocytic (PE) antigens must therefore be identified, characterized and evaluated as single or multi-component subunit

vaccines. Recently, we screened a panel of new *Plasmodium yoelii* (Py) PE antigens for their ability to recall T cell responses from RAS-immunized mice. Vaccines expressing antigens that were positive in this screen were then systematically evaluated for the ability to induce sterilizing protection in mice against a sporozoite challenge, identifying viable candidates for the development of a vaccine. We adopted a matrix strategy to test a large number of antigens in combinations of three, with and without PyCSP in mice. The immunization regimen consisted of priming with DNA and boosting with adenovirus 5 (Ad5) constructs. As a result of these studies, we have identified a novel PE antigen (E140) capable of consistently inducing 100% sterilizing protection in inbred and outbred mice (alone or in combination with other PE antigens), against a sporozoite challenge. PyE140 immunization of mice induced high titer antibodies to sporozoites, liver, and schizont stages, which correlate with protection. We have further examined the duration of the PyE140 protection, routes of immunization, blood stage challenge, and antigen localization on these parasite stages. In addition to these results, preliminary *P. falciparum* E140 immunogenicity data will be presented. These data support the advancement of the *P. falciparum* E140 ortholog in a subunit malaria vaccine.

## 1918

### HELMINTH INDUCED ALTERATIONS IN T CELL, B CELL, DENDRITIC CELL AND MONOCYTE SUBSETS AND THEIR REVERSAL FOLLOWING TREATMENT

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Although helminth infections are known perturb innate and adaptive immune responses, whether these alterations are related to expansion of particular cell populations or to alterations of specific cell function is unknown. Thus, we enumerated the numbers of a variety of T cell, B cell, monocyte and dendritic cell (DC) subsets in a group of individuals with asymptomatic, *Strongyloides stercoralis* (Ss) infection (INF) and a group of uninfected controls (UN). These cell frequencies were also repeated in the INF individuals 6 months following anthelmintic treatment. Our data show that Ss infection is characterized by significantly increased number of naïve (CD45RA+ CCR7+ p=0.0135), effector (CD45RA-CCR7- p=0.0088) and regulatory (CD4+ CD25+ Foxp3+ CD127dim p=0.0404) CD4+ T cells in comparison to UN individuals. In contrast, there were no significant differences in the number of cells in the CD8+ T cell compartments between the groups. Ss infection was also characterized by significantly increased numbers of naïve B cells (CD45+ CD19+ CD21+ CD27- p=0.0188) and significantly decreased numbers of immature (CD45+ CD19+ CD21+ CD10+ p<0.0001) and activated memory (CD45+ CD19+ CD21- CD27+ p<0.0001) B cells. INF was also associated with increased numbers of myeloid (Lin- HLA-DR+ CD11c+ p<0.0001) and plasmacytoid (Lin- HLA-DR+ CD123+ p<0.0001) DCs as well as increased numbers of non-classical (CD45+ HLA- DR+CD14dimCD16hi p=0.030) but decreased numbers of intermediate monocytes (CD45+ HLA-DR+ CD14hi CD16dim p=0.0041). Anthelmintic treatment resulted in a significant reversal of the cell frequencies to those seen in uninfected populations. Thus, Ss infection leads to alterations in the numbers of T cell, B cell, DC and monocyte subsets, alterations that revert toward "normal" and after anthelmintic treatment.

## 1919

### IMMUNOLOGIC IMPACT OF ASCARIASIS ON THE HOST LUNG

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Ascariasis (roundworm) is the most common intestinal helminth infection globally and accounts for significant morbidity including allergic airway disease. This study aims to evaluate the lung immunologic changes that occur during *Ascaris* infection in a murine model. We hypothesize that *Ascaris* infected mice will demonstrate a type 2 immune response consistent with allergic airway disease. Balb/c mice were administered *Ascaris suum* embryonated eggs by oral gavage. Plethysmography was completed and respiratory system resistance (RRS cm H<sub>2</sub>O·s·ml<sup>-1</sup>) was measured on day 12 post-infection. Bronchoalveolar lavage (BAL) was collected and total cell counts were determined using cytospin and microscopy. BAL fluid and lung tissue were collected for cytokine analysis using Luminex MAGPIX®. Additional lung tissue was used for histopathologic evaluation (H&E, PAS, and PAFS stains). *A. suum* infected mice had severe airflow resistance compared to controls (p= 0.0087). Total immune cell counts in BAL were significantly elevated in *Ascaris* infected compared to controls (2,637,714 vs 167,500; p< 0.001) including macrophages (p = 0.0047), lymphocytes (p = 0.0159), neutrophils (p < 0.0001) and eosinophils (p= 0.0012). *Ascaris* infected mice had a robust type 2 cytokine inflammatory response in both the BAL fluid and lung tissue including Interleukin-4 (IL-4) (p < 0.01; p< 0.01), IL-5 (p< 0.001; p< 0.001), IL-13 (p< 0.001; p< 0.01) relative to controls. On histopathology, *Ascaris* infected mice demonstrated severe neutrophilic and eosinophilic inflammatory infiltrate, goblet cell metaplasia, mucin production and smooth muscle hypertrophy consistent with a type 2 mediated immune response. We found that ascariasis causes profound airway hyper-responsiveness and type 2 inflammatory lung pathology consistent with severe allergic airway disease. Further evaluation of the immunologic molecular mechanism by which *Ascaris* induces allergic airway disease will allow for recognition of novel therapeutic targets. Interventions to reduce *Ascaris* infection have the potential to prevent significant morbidity in millions of children around the world.

## 1920

### ALLERGIC SENSITIZATION COINCIDENT WITH HELMINTH INFECTION DRIVES A TH2-DOMINATED IMMUNE RESPONSE THAT LIMITS PARASITE BURDEN

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More than three billion people worldwide are infected with helminth parasites and/or suffer from allergic diseases. A common feature of atopic disorders and helminth infections is their association with type 2 immune responses; however, the impact of helminth infections on allergic diseases is based largely on epidemiological studies. Previously, we have demonstrated that parasite- and allergen-specific CD4+ T cell responses in subjects with filarial infections and coincident allergic sensitization (filarial [Fil]+ allergy [A]+) have hyperreactive parasite Ag-specific Th2-associated immune responses, when compared to 3 appropriate control groups (Fil-A-, Fil-A+, Fil+A-), which correlated with serum IgE levels and levels of circulating activated eosinophils. To better understand the mechanisms underlying the augmented TH2-associated immune responses seen at the helminth/allergy interface and how it might influence the parasitologic outcome of the helminth infection, we used a murine model of house dust mite (HDM)-induced allergic asthma and coincident helminth infection (*Ascaris spp*). Our data show that HDM sensitization prior to infection with *Ascaris* led to a strong Th2 response (IL-4, IL-13, IL-10) in the lung with

markedly increased BAL cellularity (primarily eosinophils and macrophages) compared with non-HDM-sensitized mice. Moreover HDM-sensitization prior to *Ascaris* infection led to a 66% reduction in the number of lung-stage *Ascaris* larvae. Moreover, when airway reactivity and pulmonary mechanics were evaluated, the HDM-allergic and *Ascaris*-infected mice had a marked increase in the Forced Vital Capacity and a significant reduction in the lung resistance compared with the non-HDM sensitized but *Ascaris*-infected mice. Our data, therefore, suggest that allergic sensitization coincident with helminth infection leads to a TH2- dominated immune response that not only limits parasite burdens but may also be involved in tissue remodeling allow more quick resolution of the local inflammation-associated morbidity.

## 1921

### TAXONOMIC AND FUNCTIONAL MICRO BIOME ASSEMBLAGES IN INDIVIDUALS INFECTED WITH STHS

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To understand the dysbiosis associate with soil transmitted helminth (STH) infections in the human gut ecosystem we compared microbial community taxonomic and functional assemblages among infected and uninfected individuals and before and after anthelmintic treatment. We focused on identifying and validating biologically meaningful associations by: a) comparing the microbiome of moderate/heavy infected individuals to uninfected individuals, b) validating the findings using subjects from different geographical cohort, c) identifying STH-discriminatory taxa (including those conserved among subjects from the different cohorts), d) delineating microbiome assemblages after de-worming, and, e) identifying microbiome-encoded biological functions associated with infection. While answering these questions, technical aspects were also considered to understand how study design, diagnostic accuracy, precise STH burden estimation and depth of coverage affect optimal profiling of the microbiome. We identified twelve bacterial taxa associated with infections in these two geographically very distinct cohorts, including taxa previously related to reducing gut inflammation (*Olsenella*), being increased in other disease states (*Flavonifactor*) or nematode infections (*Enterococcus*). We also identified taxa that responded to treatment, including *Clostridium XVIII*, which was also lower in infected individuals but recovered abundance after treatment, and *Butyricimonas*, which is a butyrate-producing bacteria (butyrate is inhibitor of gut inflammation). Among functional categories identified as associated with hookworm infection was mucin-type O-glycan biosynthesis, indicating that hookworm infection results in changes to the mucosal layer, which is needed for the parasite to penetrate in order to attach on the lining of the intestine and feed on blood. These findings have important implications for designing mechanistic studies to fully understand the STH-microbiome interactions that could facilitate development of affordable microbiome driven intervention for sustainable STH control.

## 1922

### COMPARISON OF WHOLE GENOME TO 16S SEQUENCING ANALYSIS OF INTESTINAL MICROBIOME IN ARGENTINIAN CHILDREN WITH HELMINTH AND PROTOZOA INFECTIONS

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Next generation sequencing for microbiome analysis is commonly performed using 16S rRNA gene sequencing or whole genome shotgun sequencing (WGS) method. We carried out both WGS and 16S sequencing on human fecal samples from a 122 Argentinian cohort study focusing on two groups- helminth infected (*Ascaris*, *Ancylostoma*, *Necator*, *Strongyloides*, and *Trichuris*) versus no-parasite groups verified by multi-parallel real-time quantitative PCR. WGS had higher resolution allowing the reads to classify to the bacterial strain level and in some cases even sub-strain level. 16S sequencing could not resolve further than genus level. Shannon alpha diversity metric comparing only bacterial reads within groups showed no statistically significant difference for helminth infected group ( $p = 0.999$ ) and for no-parasite group ( $p = 0.400$ ) indicating that either method has similar sensitivity to detect alpha diversity differences. However, WGS has a significant increase in "difference of means" (DOM) as compared to 16S rRNA gene sequencing, with a delta gap increase of  $1.49 \times 10^{-3}$  proportion of reads (medians of  $5.3 \times 10^{-5}$  versus  $-0.001437$  respectively,  $p = 0.0001$ ). DOM is a measure of the change in proportion of specific bacterial sequences for helminth and no-parasite groups. It provides information on the capacity of an assay to discriminate between 2 experimental groups and small effect size. The WGS method provides rich metagenomic information compared to 16S rRNA sequencing. Metagenomic information for 16S rRNA reads can be inferred using PICRUST software through taxonomic information, but it lacks the direct evidence of genes found in WGS. As such, 16S sequencing is computationally inexpensive, while WGS data is challenging to manage relying on software using complex algorithms to analyze data. Our data shows that WGS offers higher taxonomic resolution and discrimination along with metagenomic information while 16S provides a reasonable option if the taxonomy and diversity are the primary focus of a study. This study provides important information for potential tropical medicine research in selecting the optimal assay based on function and price.

## 1923

### WHOLE GENOME SEQUENCING OF ASCARIS LUMBRICOIDES WORMS REVEALS CHANGES IN SPACE AND TIME

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As deworming becomes more common, more health departments and global health organizations are looking for "end game" strategies. A

greater understanding of who infects whom in settings where helminth prevalence is low but persistent could make deworming results more sustainable. Of 530 *Ascaris lumbricoides* adult worms collected at two time-points (baseline and 3 months after treatment) in five villages in rural western Kenya, the whole genomes of 77 were sequenced using Illumina paired-end sequencing. Reads were processed and mapped onto a new reference *A. lumbricoides* genome assembled from the reads from the germline DNA from one of the 77 worms. SNPs were called relative to the reference genome, and nearly one million variants were identified. Neighbour Joining clustering was done repeatedly based on sampling 200,000 single-nucleotide polymorphisms (SNPs) at a time. Significant clustering was found within villages and from the same time-point, but not within houses or at the individual level. Furthermore, genetic distance between worms was significantly associated with geographical distances between host residences ( $r=0.22$ ,  $p=0.001$ ) suggesting that worms in different villages may be partially reproductively isolated from each other. The genomic data indicated that worm populations changed post-treatment, which is corroborated by independently collected morphological data. Nevertheless, many members of the original population(s) were still present 3 months post-treatment. In environments such as this one, where worm transmission appears to cluster within villages, the village may be the appropriate unit for deworming programs to target. These data also stress the importance of monitoring genomic changes in worm populations following treatment.

## 1924

### THE EFFECT OF SOIL-TRANSMITTED HELMINTHS ON CHILD DEVELOPMENT: IS IT MEDIATED BY ANEMIA?

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It has been hypothesized that soil-transmitted helminths (STHs) adversely affect child development and that this effect is mediated by anemia (i.e. STH infections cause anemia and anemia affects development). However, no formal mediation analysis has yet been performed to investigate this hypothesis. The current objective, therefore, was to investigate the extent to which the effect of STH infection on child development is due to mediation by anemia. A longitudinal cohort study was conducted in Iquitos, Peru between September 2011 and July 2016. Children were recruited at one year of age and followed-up yearly to five years of age. Every year, STH infection was measured using the Kato-Katz technique and child development was measured with the Wechsler Preschool and Primary Scale of Intelligence (WPPSI) in a random sample of 880 children. Anemia was measured at 3, 4 and 5 years of age. STH infection and anemia were categorized into the number of times each child was detected STH infected/anemic throughout the study. A total of 781 children completed the WPPSI at five years. In multivariable linear regression analyses, children found STH-infected more times had statistically significantly lower WPPSI scores at five years of age compared to children who were never found infected (adjusted  $\beta$  values (95% CI) for the total net effect of 1, 2, 3, 4-5 detected infections compared to never infected, respectively: -1.7 (-3.9, 0.6); -3.9 (-6.3, -1.5); -4.4 (-6.9, -2.0), -2.9 (-5.5, -0.3)). Decomposition of the total net effect of STH infection on child development revealed that, although anemia was found to have an independent effect on child development (data presented elsewhere), the observed STH effects were not notably due to mediation by the number of times found anemic. The percent of the total net effect of STH infection on child development that was due to mediation by anemia was: 12%, 3%, 5% and 7% for 1, 2, 3 and 4-5 STH infections, respectively. These results suggest that other mechanisms, such as malnutrition and inflammatory and immunological responses, should be investigated as possible explanations for the effect of STH infection on child development.

## 1925

### A CLOUD-BASED EPIDEMIOLOGICAL SURVEILLANCE PLATFORM WITH APPLICATION TO CHAGAS DISEASE VECTOR CONTROL

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Epidemiological surveillance is a critical mission in public health, providing advanced warning and prevention at the earliest stages of an epidemic. In the case of vector-borne diseases, surveillance often requires surveying a large number of sites over an expansive area for disease vectors. Ideally, surveillance site selection would be informed by historical and epidemiological data integrated in some way to generate prediction of local risk. To this end, we developed an epidemiological app that we call EpiReportR. EpiReportR is a versatile and user-friendly platform that addresses a variety of spatial epidemiological problems. Implemented in the open source language R, EpiReportR provides neighborhood maps for use by health inspectors during door to door surveillance for domesticated *Triatoma infestans*, the vector of Chagas disease. In the maps, each house appears as a point that is colored according to its relative risk of infestation with the insect and the maps are updated in real time based on field results. EpiReportR also includes tools for electronic data collection, eliminating the laborious digitalization of paper forms following data collection. EpiReportR also provides a number of other capabilities for spatial epidemiology work, including access to predictive spatial models, analytic capabilities, and visualization. The cost and expertise required for EpiReportR are minimal, and modification of the app requires only knowledge of R and SQL. The app is fully-functional across computers and mobile devices, open source, and can be easily adapted to a variety of epidemiological scenarios. EpiReportR is currently being used for Chagas disease entomological surveillance by health inspectors in nine districts of Arequipa, Peru, and data is currently being collected on its use.

## 1926

### MOLECULAR EPIDEMIOLOGY OF CUTANEOUS LEISHMANIASIS AMONG REFUGEES IN NORTH LEBANON

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Cutaneous leishmaniasis (CL) is the most prevalent neglected tropical disease in the conflict zone in the Middle East, which currently affects more than hundred thousands of refugees annually. In Lebanon, whose population has increased ca. 25% because of mass migration from Syrian conflict zones has historically had a very low annual incidence of CL; however, recent outbreaks of CL are being reported by hospitals among Syrian refugees. Here our study is designed to investigate the current situation of CL among refugees in North Lebanon. The pilot study was carried out at Al-Bachaer medical center situated in North Lebanon from 1st December till 1st April 2017. 36 patients infected by CL have been diagnosed by dermatologist according to clinical symptoms. The majority of patients (78%) are younger than 18 years. Needle aspirations were taken for direct smear to seen under microscopy. In addition, Isohelix swab has been taken for each patient then all swabs have been processed for molecular diagnosis. *Leishmania* species were identified by PCR-RFLP analysis of the ribosomal Internal Transcribed Spacer 1 (ITS1). The refugees had displaced from endemic and non-endemic areas of North Syria. Most of refugees had migrated from Edleb (52.7%; 19/36), followed by Hama

(22.2%; 8/36), Rakkah (8.3%; 3/36), Aleppo (11.1%; 4/36), Homos (5.5%; 1/36) and Tartous (2.7%; 1/36). All these patients are living in the poorest region of North Lebanon where environmental factors could drive infectious disease particularly CL as it would be favorite ecology for sand flies to breed and transmit. As clinical manifestation, the lesions were mainly located in the facial area. Nodular and papular lesions, with or without ulceration, were the most predominant lesion types (44.4% 16/36). On molecular level, 15 samples (41.6%; 15/36) were positive for *Leishmania* sp. and patients were mainly coming from Edleb, Hama, Rakka and Aleppo. Possibility of local transmission is observed through female patient who settled in Lebanon for 4 years and then noticed the appearance of ulcerative lesion after more than 42 months of settling in Lebanon. CL infection was confirmed by microscopy and PCR.

## 1927

### FIELD TRIAL TO ASSESS LEISHMANIASIS VACCINE EFFECTIVENESS AS A POTENTIAL IMMUNOTHERAPY IN ASYMPTOMATIC DOGS

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Visceral Leishmaniasis is targeted for elimination on the Indian subcontinent. One of the greatest challenges to date for achieving this goal is how to stem transmission from asymptomatic-infected people. Studies indicate that vaccination could prevent transmission from asymptomatic-infected individuals by boosting nascent immunity. Due to inherent risks of vaccinating already exposed populations, no study has evaluated the ability of a vaccine to decrease VL in asymptomatic-infected individuals. Dogs are the predominant reservoir host for *L. infantum*, with similar immunopathogenesis of disease to people. A vaccine trial in dogs would show proof of principle to decrease parasite load and progression to VL. We report efficacy results from a double blind, randomized, vaccine field trial to prevent VL progression. 557 *Leishmania*-negative or asymptomatic dogs were enrolled. *Leishmania* infection status was determined via qPCR, serology, and clinical data. Non-clinical dogs had less than two clinical signs of VL and were diagnostic positive. Dogs were vaccinated 3 times at 14 day intervals and followed every 3 months for 12 months. To provide a standard that will allow comparison to human clinical trials, a data safety-monitoring board evaluated the statistical plan and nine-month interim data. Analyses include comparison between treatment groups for overall clinical score to evaluate disease progression, seroconversion, *L. infantum* qPCR positivity, time to event analyses for clinical disease and death, and vertical transmission. Xenodiagnosis was performed on a subset of dogs to evaluate vector transmissibility. This trial, the first to vaccinate asymptomatic dogs, demonstrated that vaccinating asymptomatic infected animals was safe. Preliminary results indicate that vaccination decreased all-cause mortality. Results demonstrate ability of this novel vaccine/immunotherapy to alter seropositivity and parasitemia, which correlates directly with likelihood of transmission. This study underscores the need for vaccines that target asymptomatic leishmaniasis as part of the toolbox to promote eradication goals globally.

## 1928

### LOCAL DYNAMICS, SPATIAL INTERACTIONS AND DISPERSAL ROUTES OF VISCERAL LEISHMANIASIS IN 45 MUNICIPALITIES OF SÃO PAULO STATE, BRAZIL

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Visceral leishmaniasis (VLeish) has a worldwide distribution with Brazil harboring 90% of cases in Latin America. The appearance of endemic areas depends on the adaptation of sandflies to naïve ecological niches. The disease has been a major health concern in São Paulo state since 1998 and its continuing expansion is not so far clearly comprehended. We hypothesize that Tobler's first law of geography can be applied to justify the spatial interactions of many transmission sites of the vector and reported cases in São Paulo. The overall goal is to analyze dispersion routes by spatial modeling and spatial interactions of VLeish, and to demonstrate that VLeish risk can be measured effectively using spatial interactions theory, elucidating active cores and residual borders of transmission. 45 municipalities in western São Paulo were selected due the consistency of the databases, and because they belong to an important hub in the state. The databases consisted of: (i) *entomological data and notification* – Superintendence of Control of Endemics (SUCEN) and The Center of Epidemiological Surveillance (VL and CL); (ii) *environmental data – Moderate-Resolution Imaging Spectroradiometer (MODIS) – Land Surface Temperature (LST), Normalized Difference Vegetation Index (NDVI)*; (iii) *socioeconomic variables* – Human Development Index (HDI) and the Index of Social Vulnerability (ISV). Using multi-criteria analysis was possible to group the results in: I – Core of VLeish or presence of vector *Lu. longipalpis*, especially in the west, and presence of vegetation remnants. II – The active border of high transmission, presence of *Lu. longipalpis*, hot and humid climate, medium social vulnerability, presence of native remaining refuges of vegetation. III – VLeish residual border, absence of vectors *Lu. longipalpis*, wet weather, native vegetation. IV – Low incidence of VLeish, absence of *Lu. longipalpis*. The potential for transmission of leishmaniasis does not imply the occurrence in these areas, bearing in mind that evidence was assessed for a number of other factors that may enhance the transmission cycle.

## 1929

### USING DYNAMIC MODE DECOMPOSITION TO PRIORITIZE REGIONAL SCREENING FOR HAT IN THE DEMOCRATIC REPUBLIC OF CONGO

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The WHO has set a goal to eliminate Human African Trypanosomiasis (HAT) as a public health problem by 2020. With limited means available, a major challenge is to prioritize the distribution of resources to the areas that are most at risk of continued transmission. Overall, reported HAT cases in the Democratic Republic of Congo (DRC) have steadily declined in recent years. Dynamic Mode Decomposition (DMD) is an equation free method with the ability to find coherent spatial-temporal patterns in data arising from nonlinear systems. We applied DMD to ten years of active and passive screening data from DRC as a fast, data-driven method for identifying areas which have deviated from the national trend. Not only do the results select specific *foci* of interest for increased future screening or surveillance, but they may also highlight features of potentially problematic regions where little screening has been performed in the past. In particular, DMD pinpointed *foci* along riverine transportation routes, which supports further analysis of the role of migration in maintaining transmission. We

discuss some of the limitations to this method, what efforts can be made to improve its use in the future, and how it might be complemented by epidemiological modeling.

## 1930

### USE OF SALIVA FOR LARGE SCALE *TRYPANOSOMA CRUZI* SCREENING

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Vector control for Chagas disease was aggressively addressed during 1990s, and in 2006, Brazil was certified as free of vector transmission caused by the main vector *Triatoma infestans*. Nevertheless, residual infestation and recolonization can occur, threatening the long-term success of the vector control. Large scale serological surveys using blood collection are complex to perform and have been used only focally to monitor areas where sporadic acute cases were detected. The development of an antibody detection method based on saliva would facilitate large scale and systematic screening of children in endemic regions. The aim of this study was to evaluate if plasma could be replaced by saliva in commercial available T cruzi EIA. We have collected saliva from 100 T.cruzi infected patients and 50 healthy individuals using Sallivete®, Sarsted. Five assays were evaluated (ARCHITECT Chagas – Abbott; Chagas REC – INVITRO; GOLD ELISA Chagas – REM; ELISA recombinant v.4.0 – Wiener and ELISA Chagas III – Grupo Bios S.A – Diasorin). Test parameters such as sample and conjugate dilutions, incubation time and conjugate manufacturer were modified. A better discrimination between Chagas patients and controls were achieved using the ELISA recombinant v.4.0 – Wiener kit under the following conditions: no sample dilution, no conjugate dilution, sample and conjugate incubation period of 60 minutes and a cut-off of two standard deviation above the mean of the controls. Under these conditions the assay sensitivity and specificity were 97% and 100% respectively, showing that saliva could replace plasma for large screening surveys in endemic areas.

## 1931

### POLICY RECOMMENDATIONS FOR REACHING ELIMINATION OF VISCERAL LEISHMANIASIS ON THE INDIAN SUBCONTINENT: A COMPARISON OF MULTIPLE TRANSMISSION MODELS

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Introduction: On the Indian subcontinent (ISC), visceral leishmaniasis (VL) is targeted for elimination as a public health problem by 2020. The elimination target is defined as an annual VL incidence of <1 per 10,000 capita at (sub-)district level. Interventions focus on vector control, surveillance and on diagnosing and treating VL cases. Aim: To explore whether and within which timeframe current interventions may lead to reaching the VL elimination target. Methods: We present multiple mathematical models of VL transmission on the ISC with structural differences regarding the main reservoir of infection, including those with a prominent role of asymptomatic infection. We compare their predictions for achieving the WHO VL elimination targets with ongoing and alternative treatment and vector control strategies. Results: All the transmission models suggest that the WHO elimination target will be met in Bihar, India, before or close to 2020 in sub-districts with a pre-control

incidence of 10 VL cases per 10,000 people per year or less, when current interventions (60% coverage of indoor residual spraying (IRS) of insecticide and an average delay of about 40 days from onset of symptoms to treatment) are maintained. Conclusion: Increasing the IRS coverage and to a lesser extent reducing the time from onset of symptoms to treatment will both decrease the time to elimination. However, in all cases the models suggest there is likely to be ongoing transmission after 2020 and so control measures will have to be kept in place for several years to achieve the longer-term aim of breaking transmission.

## 1932

### A LONGITUDINAL ASSESSMENT OF GAMETOCYTE PRODUCTION AND INFECTIVITY IN CHRONIC AND ACUTE *PLASMODIUM FALCIPARUM* INFECTIONS

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Gametocytes are essential for onward transmission to mosquitoes. In *Plasmodium falciparum*, gametocytes appear 10-12 days after asexual parasites and often circulate at very low densities. It is currently unclear whether individuals are infectious to mosquitoes before symptoms arise or before malaria infections are detectable by microscopy. We assessed gametocyte production and infectivity in two cohorts in Burkina Faso. In the first cohort, children (5-10 years) were cleared of pre-existing infections and were subsequently weekly monitored by molecular methods for incident infections. Upon first detection of infection, sampling was performed daily and monitoring continued for 42 days. Mosquito feeding assays were performed at day 0 (day of detection of infection), d14 and d35. The second cohort was identical in design but malaria infections were not cleared. Intensive follow-up commenced once an asymptomatic infection was detected for 2 consecutive months (a chronic infection). 51 acute and 39 chronic *P. falciparum* infections were monitored for asexual parasite and gametocyte dynamics and infectivity to mosquitoes. Only 13% (7/51) of children with acute infections remained fever free after the first detection of malaria infection by nPCR, whilst 72% (28/39) of all children with chronic malaria infections remained asymptomatic during 42 days of intensive monitoring. Although mature gametocytes were detected at low densities, none of children with acute malaria infections infected mosquitoes compared to 69% (27/39) of children with chronic infections. Of the latter, some were infectious at 2 or 3 occasions during the 35-days of intensive follow-up and infected up to 98% of mosquitoes. Taken together, our results suggest that intensive monitoring allows the detection of symptomatic malaria before infected individuals become infectious to mosquitoes. Asymptomatic malaria-infected children, on the other hand, are often infectious for several weeks; their identification will be key to the success of malaria elimination strategies.

## 1933

### MALARIA BURDEN THROUGH ROUTINE REPORTING: RELATIONSHIPS BETWEEN INCIDENCE ESTIMATES

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Malaria burden remains high in Uganda, ranked 4th by number of cases and 11th by number of deaths worldwide and accounting for 30-50% of outpatient visits. The burden is estimated using routine surveillance for planning, implementation, and evaluation of public health practice. Standard routine measures of burden include total suspected or cases, and test positivity rate (TPR) as incidence proxies. Additional estimates of incidence commonly used are evaluated in this study. We aim to explore relationships between incidence estimates from routine data to improve interpretation of burden reported through routine reporting. Three high-level facilities located at varied malaria endemicities in Uganda (Nagongera, Walukuba & Kihhi) were included. Study participants were children under 11 years suspected to have malaria, and seen between Oct-2011 and Jun-2016. Estimates of incidence (TPR, malaria positive fraction - MPF, mean incidence (reported case) rate - MIR, and standardized incidence ratio - SIR) were derived and pairwise relationships between them explored. Nagongera, Walukuba & Kihhi HCIV saw 46,049 (79%), 31,861 (52%) and 32,675 (78%) study participants of which 33.3%, 34.0% and 60.7% respectively, had home village recorded as located within the facility's sub-county. Strong nonlinear relationships were observed between MPF and MIR over time (monthly). And, positive linear relationships observed between MPF and SIR over time (Coef=0.140,  $p < 0.001$ ; Coef=0.215,  $p = 0.002$ ; Coef=0.198,  $p < 0.001$ ). Similarly, weaker linear relationships were observed across space (Coef=-0.012,  $p = 0.785$ ; Coef=-0.013,  $p = 0.589$ ; Coef=-0.026,  $p = 0.133$ ). Burden over time is not implied across space. Improved interpretation of burden requires an account for both time and space/location. Full results will be presented at the conference.

## 1934

### LONGITUDINAL CLINICAL AND MOLECULAR ANALYSIS OF ASYMPTOMATIC MALARIA INFECTION IN MALAWI

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In Malawi, asymptomatic *Plasmodium falciparum* infections are common and may drive transmission. The frequency, persistence, and clinical outcome of asymptomatic infections is unknown. Although school aged children (SAC) carry the majority of prevalent infections, this may be due to increased exposure to infection or a prolonged duration of asymptomatic infections. Whether and how many asymptomatic infections progress to clinical disease and eventually prompt treatment is unknown. We characterize the age-specific dynamics of asymptomatic infections in a high-transmission setting and examine the association between asymptomatic infection and clinical disease. In total, 120 participants, aged 1-50 years, with uncomplicated malaria (treated with artemether-lumefantrine) were enrolled and followed monthly for up to two years. Samples from all visits were tested for parasites using both microscopy and qPCR. Genotyping with *m*sp1 and *m*sp2 was used to characterize the complexity of each infection. Molecular force of infection was defined as the number of unique infecting genotypes/person/year. Analysis has been completed for 1702 person months of follow up time. Asymptomatic infections were detected in 23% of visits. Asymptomatic infection, the longest of which persisted for 16 months, was associated with increased time to next clinical malaria (HR 0.45,  $p < 0.001$ ) in all ages. The mean duration of persistence of individual infections will be calculated by age.

Overall, 785 incident infections were detected; 35% at a visit when no symptoms were reported. We found SAC are a distinct risk group, and have a significantly higher molecular force of infection (IRR 2.4,  $p < 0.001$ ) than other age groups. In our setting, clinical malaria was more likely to be due to newly acquired infection (OR 4.6, 95%CI 2.5-8.5) than to a persistent infection. Asymptomatic infections constitute a significant reservoir of *P. falciparum* in Malawi and may be protective against clinical malaria.

## 1935

### TRACKING MALARIA: PREGNANT WOMEN AS A SENTINEL POPULATION FOR MALARIA SURVEILLANCE

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With increasing spatial heterogeneity of malaria transmission and an age shift of the disease burden towards older children and adults, pregnant women attending antenatal care (ANC) have been proposed as a pragmatic sentinel population for malaria surveillance. However, the representativeness of routine ANC prevalence data and how it is related to the prevalence in other population subgroups has yet to be investigated. For this study, we obtained monthly ANC malaria prevalence data from all Tanzanian health facilities reporting to the national data warehouse DHIS2 between January 2014 and May 2016. Among 4,354,911 pregnant women attending ANC, 49.6% were tested for malaria. The average malaria infection prevalence in pregnant women was 7.6% (95% CI 7.2-7.9) with little monthly variation. In 2015, malaria prevalence was generally higher in school children and in children aged 6-59 months than in pregnant women, with substantial variability between regions and districts. A high correlation was found between regionally aggregated ANC prevalence and prevalence in children aged 6-59 months (Spearman rho:  $p = 0.90$ ;  $p < 0.0001$ ) as well as school children (regional:  $p = 0.84$ ;  $p < 0.0001$ ; district:  $p = 0.80$ ;  $p < 0.0001$ ). On the other hand, correlation was low with district aggregated prevalence predicted by the Malaria Atlas Project ( $p = 0.56$ ;  $p < 0.0001$ ). In all comparisons, the correlation was substantially better in districts or regions with one rainy season compared to areas with biannual rain. The results of this study provide strong support for using pregnant women attending ANC as a sensible and pragmatic sentinel population to assess malaria trends in Tanzania. However, ANC malaria prevalence cannot be used to directly predict the prevalence in other population subgroups. Further work is required to identify covariates that influence the relationship between ANC and other population subgroup prevalences particularly in areas of more intense seasonality.

## 1936

### COMBINING LONG-LASTING INSECTICIDAL NETS AND INDOOR RESIDUAL SPRAYING FOR MALARIA PREVENTION IN ETHIOPIA: A CLUSTER RANDOMIZED CONTROLLED TRIAL

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Long lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) are effective tools to prevent malaria, but the effectiveness of combining the two are not yet fully understood. This study compared the separate versus combined effect of LLINs and IRS on malaria incidence and anemia. This cluster randomized controlled trial was done in the Adami Tullu

district in south-central Ethiopia for 119 weeks, from September 2014 to January 2017. Prior to the trial, we did a pilot study to estimate the sample size, and registered 8 malaria episodes per 10,000 person-weeks of observation (PWO). 6072 households (HHs) were randomly assigned to four intervention arms: LLINs + IRS, LLINs, IRS, and no intervention implemented by the project (control). The primary outcome was malaria incidence. Analysis was based on an intention-to-treat principle. Each arm had 44 clusters with an average of 35 HHs per cluster; the total population was 34,548. PermaNet 2.0 LLINs were distributed in September 2014, and IRS (with propoxur) was carried out yearly. Active and passive case detection were done weekly. Malaria was diagnosed using a rapid diagnostic test (CareStart™). Anemia was assessed through yearly surveys on children. The yearly rainfall was 60% of expected in 2015 (El Niño effect), entomological studies were simultaneously conducted. Results The overall malaria incidence was 2.91 per 10,000 PWO (37% of pre-intervention incidence), and similar in the four arms with 2.99 (LLINs + IRS), 2.92 (LLINs), 3.01 (IRS) and 2.72 (control). Generalized estimation equation showed that LLINs did not provide individual protection. Entomological findings showed greater outdoor mosquito human-biting activities than indoor. The yearly mean hemoglobin concentrations were similar among the trial arms. Conclusions The malaria incidence was low and similar in the intervention arms. The severe drought might have contributed in too few malaria cases to evaluate the interventions. However, residual transmission could be an important cause of malaria transmission in the area. Our results could be relevant for situations with low malaria incidence.

## 1937

### DEVELOPMENT OF A NOVEL GENOTYPING AND MATHEMATICAL ALGORITHM FOR ESTIMATION OF MULTIPLICITY OF INFECTION OF MALARIA PARASITES

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Multiple strain infections with malaria parasites in humans are common in endemic areas, and quantifying the multiplicity of infection can be used as a surrogate marker for transmission intensity. Here we present a novel multiplex polymerase chain reaction and next-generation sequencing approach for differentiating *Plasmodium falciparum* malaria strains within a host. This novel method allows recovery of information for all 24 SNP sites (identified by Daniels et al 2008, Malaria Journal) using 3 multiplex PCRs. The disambiguation algorithm relies on consistent amplicon ratios across SNP sites to search through all possible amplicon ratios and barcodes to identify up to 4 strains and their frequencies within a sample. Both the laboratory and analytic pipelines were developed using a collection of spiked laboratory isolates consisting of whole blood and cultured ring-stage parasites with known SNP barcodes and frequencies. We assessed multi-strain infections in available smear-positive samples from four population surveys in western Kenya from 1996 (n=64) and after scale up of malaria interventions in 2001 (n=64), 2007 (n=47) and 2012 (n=67). Over this time period, prevalence of infection in young children (<5 years old) dropped from approximately 80% at baseline to 26% in 2008 and plateaued at 40% in 2009 through 2012. Over time, the proportion of lower-diversity infections with only one or two strains in a sample among smear-positive samples increased (p<0.001, Wald test for regression coefficient) (Over time: 1 strain: 7.8%, 9.3%, 27.7%, 26.9%; ≤2 strains: 23.4%, 37.5%, 36.2%, 50.7%; ≤3 strains: 51.6%, 67.2%, 59.6%, 76.1%). Proportion of samples with four or more strains decreased over time (48.4%, 32.8%, 40.4%, 23.9%) (p=0.013 via Wald test). These trends indicate that even during the plateau in prevalence from 2009 and 2012, the total infection complexity was decreasing as reflected by within-host strain diversity. This approach provides a tool to

measure dynamics of transmission intensity and to evaluate the impact of targeting interventions over time, information that is critical in planning malaria control interventions.

## 1938

### UNDERSTANDING THE HIGHLY DYNAMIC NATURE OF DECLINING MALARIA TRANSMISSION IN PAPUA NEW GUINEA

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A renewed emphasis on malaria control in Papua New Guinea (PNG) has resulted in a significant overall reduction in the prevalence and incidence of malaria. However these reductions have not been uniform in all areas and recent data suggests a trend toward increased incidence of malaria in some locations. A suite of health facility surveillance and community-based surveys, undertaken throughout the past decade, combine sensitive serological and molecular diagnosis of infections with operational, demographic, human and vector behavioural and spatial data. Across all sites and surveys, the reductions had been very pronounced for *P. falciparum* (Pf), with qPCR prevalence declining from >50% to <10% in community surveys. In children 1-5 years, the molecular force of blood-stage infection (molFOB) declined from 5.9/child/year (2006) to 1.6 /child/year (2013) and the incidence of clinical episodes from 2.0 (2006) to 0.1 (2013) episodes/child/year. For *P. vivax* (Pv), qPCR prevalence declined from 42% (2006) to 13% (2010) before returning to 20% (2014) in communities on the north coast of Madang Province. In 1-5 year old East Sepik children, PCR prevalence ranged from 60% and 65% in 2006 to 2008 respectively, to 18% in 2013, whilst molFOB dropped from 14.0/child/year (2006) to 2.4/child/year (2013) and the incidence of clinical episodes declined from 2.0 to 1.1 to 0.1/child/year. Parasite densities decreased 5-fold, with 72% of Pf and 87% of Pv infections submicroscopic in 2013/14. Very recent surveys on north coast Madang indicate that whilst Pv prevalence remained unchanged at 20%, Pf has resurged to a prevalence of 30%. The relationship between low-density asymptomatic infections, parasite genotypes and population level immunity will be presented, as well as a detailed analysis of the relationship between host and vector behavior and infection status. Understanding the highly dynamic nature of malaria transmission in PNG and the driving factors is critical to inform appropriate surveillance and intervention strategies to sustain the gains made with a decade of malaria control in PNG and advance towards elimination.

### GENETIC RELATEDNESS OF *VIBRIO CHOLERAE* ISOLATES WITHIN AND BETWEEN HOUSEHOLDS DURING OUTBREAKS IN DHAKA, BANGLADESH

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Household contacts of cholera patients have a 100 times higher risk of developing a cholera infection than the general population. To compare the genetic relatedness of *Vibrio cholerae* isolates from infected household contacts and water sources within patient households, we performed whole-genome-sequencing (WGS) and multilocus-variable-number tandem-repeat analysis (MLVA) on isolates across three cholera outbreaks in Dhaka, Bangladesh. The WGS analyses revealed that 80% of households had water isolates that were more closely related to clinical isolates from the same household than to any other isolates. While in another 20% of households an isolate from a person was more closely related to clinical isolates from another household than to water isolates from their own household. The mean pairwise differences in single nucleotide variant (SNV) counts for isolates from the same household were significantly lower than those for different households (2.4 vs. 7.7  $p < 0.0001$ ), and isolates from the same outbreak had significantly fewer mean pairwise differences compared to isolates from different outbreaks (mean: 6.2 vs. 8.0,  $p < 0.0001$ ). Based on MLVA in outbreak 1 we observed that the majority of households had clinical isolates with MLVA genotypes unrelated to water source isolates from the same household. While in outbreak 3 the majority of households had clinical and water source isolates with the same MLVA genotypes. The beginning of outbreak 2 resembled outbreak 1 and the latter part resemble outbreak 3. We validated our use of MLVA by comparing it to WGS. Consistent with WGS results, the number of pairwise differences in the five MLVA *loci* for isolates within the same household was significantly lower than isolates from different households (mean 1.6 vs. 3.0, ( $p < 0.0001$ )), and isolates from the same outbreak had significantly fewer *loci* differences compared to isolates from different outbreaks (mean 2.5 vs. 3.2,  $p < 0.0001$ ). These results suggest that transmission patterns for cholera are a combination of person to person and water to person cholera transmission with the proportions of the two modes varying within and between outbreaks.

### 1940

#### IL-23 EXPRESSION DISTINGUISHES MUCOSAL IMMUNE RESPONSES TO LIVE COMPARED TO KILLED *VIBRIO CHOLERAE*

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Natural *Vibrio cholerae* infection provides durable protective immunity, while vaccines composed of killed whole cell *V. cholerae* may result in more limited protection. To better understand the difference in the innate immune response to live toxigenic *V. cholerae* O1 versus killed organisms, we used human macrophage cell culture to identify pathways that discriminate between responses to stimulation. To screen for differences in gene expression, we used RNAseq to examine the complete transcriptome, and found that expression of IL-23 was 28-fold higher in cells exposed to

live *V. cholerae* compared to killed organisms. Next, we found that IL-23 release from human macrophages was also higher in cells exposed to live versus killed *V. cholerae*, measured by enzyme-linked immunosorbent assay (mean difference 4.3 ng/ml, CI 3.5-6.2,  $p < 0.001$ ). In addition to IL-23, IL-1 $\beta$  and IL-6 expression were also increased, comprising a group of cytokines known to act together to promote Th17 CD4+ responses. The IL-23 response was dependent on stimulation by live *V. cholerae* with enzymatically active toxin, because the stimulation effect was markedly reduced in both an isogenic cholera toxin mutant and in cells stimulated with killed organism and exogenous holotoxin, but could be restored with exogenous holotoxin plus the cholera toxin mutant strain of *V. cholerae*. Prior studies demonstrate that Th17 immune responses are prominent in natural *V. cholerae* infection and lacking in persons vaccinated with whole-cell killed *V. cholerae*. B cell memory responses are promoted by Th17 CD4+ cells, and through this mechanism, IL-23 may contribute to long-term immunity to *V. cholerae*.

### 1941

#### DEVELOPMENT OF A NEW DIPSTICK FOR RAPID DETECTION OF *VIBRIO CHOLERAE* O1 IN ACUTE WATERY DIARRHEAL STOOLS

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Cholera is a severely dehydrating diarrheal disease that results in approximately 3-5 million cases and over 100,000 deaths per year globally. Cholera is caused mainly by *Vibrio cholerae* O1, and less commonly by *V. cholerae* O139. Recognizing cholera cases early, especially in an initial phase of an epidemic, and in areas where cholera has not previously circulated is a high public health priority. Laboratory capacity in such settings is usually limited. To address this, we have recently developed an immunochromatographic lateral flow device (dipstick), Cholkit, for rapid diagnosis of cholera cases. The dipstick contains a monoclonal antibody to the O-specific polysaccharide (OSP) component of *V. cholerae* O1 lipopolysaccharide that recognizes both Inaba and Ogawa serotypes. We tested the Cholkit dipstick using fresh stool specimens of 76 adults and children presenting with acute watery diarrhea to the International Centre for Diarrhoeal Disease Research in Dhaka, Bangladesh (icddr,b). We compared Cholkit's performance with those of microbial culture, PCR (rfb and ctxA genes) and Crystal VC, the latter a commercially available dipstick designed to detect both *V. cholerae* O1 and O139. We found that all stool specimens with a positive culture for *V. cholerae* O1 (n=19) were positive by Cholkit. We then used Bayesian latent class modeling to estimate sensitivity and specificity of each diagnostic assay. The sensitivity of Cholkit, microbiological culture, PCR, and Crystal VC was 98% (95% CI: 88-100), 71% (95% CI: 59-81), 74% (95% CI: 59-86) and 98% (95% CI: 88-100), respectively. The specificity for *V. cholerae* O1 was 97% (95% CI: 89-100), 100%, 97% (95% CI: 93-99) and 98% (95% CI: 92-100), respectively. Of note, two Crystal VC assays were positive for *V. cholerae* O139, although neither stool sample was confirmed by culture or PCR, and *V. cholerae* O139 is not recognized to be circulating in Dhaka at present. Conclusion: the Cholkit dipstick is simple to use, requires no dedicated laboratory capacity, and has a sensitivity and specificity of 98% and 97%, respectively. Cholkit warrants further development and evaluation.

## 1942

**BIVALENT ORAL CHOLERA VACCINE INDUCES MEMORY B CELL RESPONSES**

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Cholera has become endemic in Haiti since its introduction in 2010. A killed whole cell bivalent oral cholera vaccine (BivWC) has now been used in multiple countries; however, a complete understanding of the immune response to this vaccine is still lacking. To determine whether this vaccine generates detectable circulating memory B cell (MBC) responses, we followed a cohort of 73 Haitian adults who received two doses of BivWC (Shanchol) for 1 year following vaccination. We assessed immune responses at day 0 (baseline), day 7 (7 days after the first vaccination), day 21 (7 days after the second vaccination), and again on days 44, 90, 180, and 360 following the initial vaccination. We observed a significant increase in circulating IgA MBC responses targeting the O-specific polysaccharide (OSP; Ogawa and Inaba) of *Vibrio cholerae* O1, starting 21 days following vaccination. We also observed an increase in the level of circulating IgG memory B cells targeting V. cholerae O1 Ogawa OSP at day 44 following vaccination; the Ogawa serotype has been the predominant circulating strain of V. cholerae in Haiti since the introduction of cholera there in 2010. These results provide evidence that the bivalent oral cholera vaccine is capable of inducing and boosting systemic memory B cell responses targeting V. cholerae.

## 1943

**IMMUNE RESPONSES AGAINST O-SPECIFIC POLYSACCHARIDE (OSP) DEVELOP AFTER VACCINATION WITH ORAL CHOLERA VACCINE CVD 103-HGR (VAXCHORA) AND THESE RESPONSES ARE ASSOCIATED WITH PROTECTION AGAINST EXPERIMENTAL INFECTION WITH VIBRIO CHOLERAЕ O1 EL TOR INABA IN NORTH AMERICAN VOLUNTEERS**

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Cholera is a dehydrating diarrhea caused by *Vibrio cholerae* O1/O139. The mediator(s) of protective immunity against cholera are not well defined. A growing body of evidence suggests that protection may be afforded by antibodies targeting V. cholerae O-specific polysaccharide (OSP). A single-dose live attenuated oral cholera vaccine was recently FDA-approved in the U.S (CVD 103-HgR, Vaxchora; PaxVax). Oral vaccination with CVD 103-HgR was associated with protection against experimental wild type

challenge against cholera in North American volunteers. Protection was associated with vibriocidal seroconversion observed ten days after vaccination. The vibriocidal response is largely comprised of IgM responses against V. cholerae LPS. Protection against cholera is serogroup specific, and serogroup-specificity is defined by the OSP component of LPS. We were therefore interested in assessing whether OSP antibody responses occurred after vaccination with CVD 103-HgR, and whether such responses correlate with protection against disease. In brief, adult North American volunteers (n=46) were immunized with 5×10<sup>8</sup> colony-forming units (CFU) of CVD 103-HgR and then challenged with approximately 1×10<sup>5</sup> CFU of wild-type V. cholerae O1 El Tor Inaba strain N16961, either 10 or 90 days post vaccination. Vaccination was associated with induction of significant increases in IgM and IgA anti-OSP antibody levels by day 10 after vaccination that fell back to baseline by day 90 in the absence of challenge. There was significant boosting of anti-OSP IgM and IgA responses and development of IgG anti-OSP responses following day 90 challenge, but no boosting following day 10 challenge. IgM and IgA anti-OSP responses on day 10 following vaccination were associated with protection against moderate or severe diarrhea following wild type challenge (Spearman r=-0.44, p=0.002; r=-0.362, p=0.01). Conclusion: Oral vaccination with live attenuated cholera vaccine CVD 103-HgR (Vaxchora) induces antibodies that target V. cholerae OSP, and these anti-OSP responses correlate with protection against experimental challenge with V. cholerae O1.

## 1944

**EPIDEMIC CHOLERA AND MICRONUTRIENT DEFICIENCY — GRANDE SALINE, HAITI, 2011**

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Epidemic cholera typically affects vulnerable communities with unsafe water and poor nutrition. Most *Vibrio cholerae* infections are asymptomatic, and risk of symptomatic cholera is thought to increase with micronutrient deficiencies. Cholera was introduced to Haiti for the first time in October 2010. In March–April 2011, we conducted a cross-sectional seroepidemiologic survey in Grande Saline, Haiti, to assess risk factors for symptomatic infection, including micronutrient deficiencies. We tested sera of participants ≥2 years of age for ferritin, a marker of iron deficiency; retinol-binding protein (RBP), a marker of vitamin A deficiency; and C-reactive protein (CRP) and alpha-1-acid glycoprotein (AGP), markers of inflammation. We adjusted ferritin and RBP for CRP and AGP using a multiple linear regression model from a previous study to account for potential confounding effects of inflammation. We used multivariable logistic regressions to examine associations between self-reported cholera diagnosis or V. cholerae seropositivity and micronutrient deficiencies, adjusting for age, sex, and education. Of 772 participants tested (age range 2–90 years; 3% <5 years), 150 (21%) reported a cholera diagnosis during October 2010–April 2011; 466 (60%) were seropositive. Low ferritin (<12 µg/L for age <5 years, <15 µg/L for age ≥5 years) was found in 19%; 3% had low RBP (<0.7 µmol/L). Elevated CRP or AGP (>5 mg/L or >1 g/L, respectively) was found in 20%. No associations between self-reported cholera diagnosis and low micronutrient measures were observed. Participants with low ferritin had higher odds of seropositivity than those with normal ferritin levels (odds ratio 2.1; P <0.001). The association between seropositivity and iron deficiency suggests a higher risk of cholera infection among people with malnutrition, though symptomatic infection was not associated; however, causality cannot be inferred. Improved access to nutritious foods and safe water are critical in impoverished communities, and further studies to better characterize the relationship between symptomatic cholera and micronutrient deficiencies are warranted.

## 1945

**WHO IS AT RISK OF CHOLERA IN AFRICA? QUANTIFYING POTENTIAL VACCINE DEMAND AND IMPACT POLICY-RELEVANT SPATIAL LEVELS**Sean M. Moore<sup>1</sup>, Andrew S. Azman<sup>2</sup>, Heather S. McKay<sup>2</sup>, Justin Lessler<sup>2</sup><sup>1</sup>University of Notre Dame, Notre Dame, IN, United States, <sup>2</sup>Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

Africa is subject to high-mortality cholera epidemics and areas of persistent incidence. Oral cholera vaccine (OCV) is becoming an important tool for cholera control as increased production has led to a growing interest in proactively vaccinating populations at high risk for infection. However, despite increases in OCV availability, the number of OCV doses available (~10 million in 2017) is too low to conduct large, generalized campaigns in at-risk countries. To prioritize the use of current supplies and quantify future production needs it is important to identify populations living at high risk of cholera infection who would derive the most benefit from prophylactic OCV use. Using cholera incidence reports from 2010-2016 over multiple spatial scales, we map the incidence of cholera in 20x20 km grid scales throughout Africa. To provide risk estimates at a policy-relevant scale, we identify districts (2nd ISO administrative level) with a significant at-risk subpopulation that comprises either  $\geq 10\%$  of a district's population or is  $\geq 100,000$  people. Based on these criteria, 69.2 (95% CrI: 49.8-89.2) million people live in high-risk districts in Africa based on an annual incidence threshold of  $\geq 1$  per 1,000, and an additional 141.5 (95% CrI: 106.7-184.0) million people live in moderate-risk districts with incidence between 1 per 1,000 and 1 per 10,000. Assuming only direct vaccine protection at levels consistent with a recent meta-analysis, vaccinating everyone living in moderate- and high-risk areas could directly prevent more than 582,000 (95% CrI: 339,000-1,044,000) cases over a five-year period, but would require 421.5 (95% CrI: 313.0-546.4) million doses, more than 50x the global annual production of OCVs in 2016. However, if districts are sequentially prioritized by incidence rate, 50% of these cases could be prevented with only 12% of the OCV doses (50.7 million doses, 95% CrI: 49.0-54.2 million), and 95% could be prevented with only 51% of the required doses (214.5 million doses, 95% CrI: 205.1-232.6 million).

## 1946

**MALARIA TRANSMISSION AT THREE SENTINEL SITES IN WESTERN KENYA FROM 2002 TO 2016: THE RESURGENCE AND CAUSALITY ANALYSIS**Guofa Zhou<sup>1</sup>, Guiyun Yan<sup>1</sup>, Andrew K. Githeko<sup>2</sup>, Harrysone E. Atieli<sup>2</sup><sup>1</sup>University of California Irvine, Irvine, CA, United States, <sup>2</sup>Kenya Medical Research Institute, Kisumu, Kenya

The long-lasting insecticidal nets (LLINs), indoor residual spraying of insecticide (IRS) and artemisinin-based combination therapy (ACT) have been scaled up in Africa to control and eliminate malaria in the past decade. These intervention measures have led to overall significant declines in malaria burden. However, several studies have also documented little change in some sites in Africa. High-quality evidence for malaria burden changes and especially the mechanisms underlying these changes are often lacking. We conducted a long-term evaluation of malaria transmission dynamics in three sentinel sites with varying transmission intensity in western Kenya. The 15-year longitudinal monthly observational study found that in two highland sites, currently indoor resting vector density has resurged to the level of 2004-2005, the period before the LLIN mass distribution. Parasite prevalence remained unchanged at two sites since 2009 and bounced back to the level of 2005 at one site. We investigated the potential causes of the resurgence, including LLIN coverage rate, vector insecticide resistance, vector species composition shift, insecticidal decay effect of the LLINs, and climatic anomaly. Generalized additive model was used to determine the relative

contributions of different factors through a stepwise feeding procedure. The results indicated that indoor resting *Anopheles gambiae* s.l. density was mainly affected by long-term trend and annual variability of climate regardless of study sites, whereas density and proportion of indoor resting *An. funestus* were correlated with resistance level and climatic factors. Parasite prevalence were strongly correlated with insecticide resistance, *An. funestus* indoor resting density, LLIN killing ability and climatic factors. The results suggested that insecticide resistance and shift of vector species became key factors affecting malaria control in this highly endemic area of Africa.

## 1947

**HIGH PLASMODIUM FALCIPARUM OOCYST LOADS IN NATURALLY INFECTED MOSQUITOES IN AFRICA**Anais Bompard<sup>1</sup>, Dari F. Da<sup>2</sup>, Serge Yerbanga<sup>2</sup>, Isabelle Morlais<sup>1</sup>, Thierry Lefèvre<sup>1</sup>, Thomas S. Churcher<sup>3</sup>, Anna Cohuet<sup>1</sup><sup>1</sup>Institut de Recherche pour le Développement, Montpellier, France,<sup>2</sup>Institut de Recherche en Sciences de la Santé, Bobo-Dioulasso, Burkina Faso,<sup>3</sup>MRC Centre for Outbreak Analysis and Modelling, Infectious Disease Epidemiology, Imperial College, London, United Kingdom

The population dynamics of human-to-mosquito malaria transmission in the field has important implications for the genetics, epidemiology and control of malaria. The number of *Plasmodium falciparum* oocysts in naturally infected mosquitoes in the wild is poorly understood, though past work indicates that most mosquitoes have only one or two oocysts. The per bite mosquito force of infection (the mean number of oocysts gained from an infectious bite) is also unclear despite the force of infection influencing factors such as the efficacy of novel transmission blocking interventions currently under development. Here a yearlong analysis of malaria transmission in three sites in Burkina Faso and Cameroon is reported. Naturally fed mosquitoes were caught inside houses and dissected to assess the prevalence and intensity of oocysts and sporozoites 3 and 7 days after collection. Cross-sectional surveys of the resident human population were carried out to determine the prevalence and intensity of sexual and asexual parasites. Results show that oocysts intensity in naturally infected mosquitoes is substantially higher than previous estimates. In the rainy season infected mosquitoes had on average 9-18 oocysts per mosquitoes 3 days after collection, with one mosquito harboring 786 oocysts. Multivariate analysis indicated that village, season and bednet use in the local population to be associated with the prevalence and intensity of oocysts and the sporozoite rate. A dynamical mathematical model of transmission was used to estimate the per-bite transmission probability, the proportion of superinfections and average parasite exposure per bite for each location. The implications of high parasite exposure on biology of transmission and the development and use of transmission blocking interventions in the field are discussed.

## 1948

**IMPACT OF PYRETHROID EXPOSURE ON RESISTANT MOSQUITO FITNESS**Alida Kropf<sup>1</sup>, Behi Kouadio Fodjo<sup>2</sup>, Marius Zoh Gonze<sup>2</sup>, Bassirou Bonfoh<sup>2</sup>, Chouaibou Mouhamadou<sup>2</sup><sup>1</sup>MIE, Bouake, Côte D'Ivoire, <sup>2</sup>CSRS, Abidjan, Côte D'Ivoire

The use of insecticides is the current backbone of vector control. But, it is widely believed that the current rise and spread of insecticides resistance in mosquito population could compromise its efficacy as exposures to LLINs or IRS are no longer able to kill their target. However very little is known about the long-term effect of insecticide exposure on resistant mosquito. We here hypothesize that mosquitoes that have survived insecticide exposure will have a reduced fitness, which may be expressed in a shorter lifespan, reduced blood-feeding success or reduced egg output. We tested this hypothesis in an experimental design where we measured different life history traits on 2 groups of resistant mosquitoes. One group was exposed repeatedly to insecticides before being allowed to have a

blood meal while the other group acted as our control was not exposed. The experiment was conceived in order to recreate a situation as close to reality as possible, using a pure *An. gambiae* s.s resistant strain, Tiassale S molecular form and a bed net furnished and distributed to the populations by the National Malaria Control Program in Côte d'Ivoire. Each measured life trait after each exposure has subsequently been broken down into a gonotrophic cycle and the parameters were compared within and between the different gonotrophic cycles for each mosquito group. We were able to put in evidence a sub-lethal effect of pyrethroid exposure on resistant mosquitoes. Affected were life traits such as the blood feeding, the egg laying, immediate mortality and delayed mortality. As all of these traits play a major role in the transmission and thus the epidemiology of malaria. They were, in a second step, included into a vectorial capacity model (MacDonald Ross formula). These results help understanding why vector control remains somewhat effective in reducing malaria transmission and that finally the impact of resistance on vector control may be much less than previously assumed.

## 1949

### TARGETING CATTLE FOR MALARIA ELIMINATION: MARKED REDUCTION OF *ANOPHELES ARABIENSIS* SURVIVAL FOR OVER SIX MONTHS USING A SLOW-RELEASE IVERMECTIN FORMULATION

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Behavioural plasticity has allowed malaria vectors to avoid home-centered vector control strategies such as, Indoor residual spraying and insecticide-treated nets which are both remarkably effective and affordable. This unsuppressed large population is now responsible for residual malaria transmission, a major challenge in elimination efforts. Partial zoophily is the key factor in residual malaria transmission as evidenced by large proportion of mixed cattle and human blood meals in a number of vector species. Mosquitoes that feed on peridomestic livestock tend to avoid contact with insecticides, reproduce and survive to continue transmission once human blood is available again. Modelling shows that targeted use of veterinary endectocides (drugs that kill mosquitoes feeding on treated subjects) could result in incremental transmission reduction after roll-out of long-lasting insecticidal nets, indoor residual spraying and other core vector control tools. Veterinary endectocides hold potential to become a complementary strategy for malaria elimination. We conducted a trial of a long-lasting, implantable veterinary formulation of ivermectin that can sustain mosquito-killing levels of this drug for more than months. 3 calves were randomly assigned to be implanted with five silicon rods or nothing as control. 50 *Anopheles arabiensis* (triplicates) females were fed on their flanks every 2 weeks. Mosquito mortality was then assessed by counting and removing the dead for 10 days. Our results show a marked and significant reduction in *Anopheles arabiensis* survival that does not decay in magnitude even six months after implantation. Slow release ivermectin formulations could complement on the success achieved by home-centered measures and aid malaria elimination.

## 1950

### INVESTIGATING THE ACTIVITY OF THE MACROCYCLIC LACTONES IVERMECTIN AND MOXIDECTIN AGAINST MALARIA VECTORS

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Livestock that live in close proximity to human hosts are alternative blood sources for many malaria vector mosquito species. In such areas, treating peri-domestic animals with insecticides such as ivermectin or moxidectin may reduce the mosquito population, hence reduce transmission of malaria. Moxidectin has the theoretical advantage of increased lipophilic properties and longer half life. The aim of this study was to investigate the efficacy of these two macrocyclic lactones with known activity against arthropod ectoparasites. Activity was tested against the dominant malaria mosquito vector in Oceania, namely *Anopheles farauti*. Initially, activity of these drugs was tested against colony mosquitoes by membrane feeding assays where the drugs were prepared in the blood meals. Drug levels were measured in the plasma and red cell compartments in the blood meals and IC50 and IC90 levels determined. Subsequently, in-vivo efficacy was investigated by conducting a clinical trial where pigs were treated with ivermectin and moxidectin in doses appropriate for human administration. Then *An. farauti* mosquitoes were allowed to feed on the pigs. Drug levels in the skin and blood of the pigs and mosquito mortality were monitored. Results of both the *in vitro* and *in vivo* experiments will be presented. Results of this study will assist in evaluating the potential of an innovative malaria control strategy utilising domestic animals for mosquito vector control applicable in the field setting.

## 1951

### THE CONTRIBUTION OF SYMPTOMATIC AND ASYMPTOMATIC INFECTIONS TO THE INFECTIOUS RESERVOIR OF *PLASMODIUM FALCIPARUM* AND *P. VIVAX* IN ETHIOPIA

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The level of asymptomatic malaria infections is considerably higher than previously thought. Whether these infections need to be targeted to further strengthen control efforts/accelerate malaria elimination needs detailed investigation. In this study we performed detailed assessments of parasite carriage and transmissibility to mosquitoes to investigate the relative contribution to malaria transmission of clinical and asymptomatic malaria. We successfully performed mosquito feeding assays using locally reared *A. arabiensis* mosquitoes on clinical malaria episodes detected by the routine health system (n=41) and asymptomatic malaria infections detected by microscopy (n=41) and PCR (n=88) in Adama, central Ethiopia. Membrane feeding experiments yielded 5,000 mosquito observations. Mosquito infection was investigated using CSP based ELISA on 20-30 mosquitoes per experiment that were sacrificed 12 days post feeding; infection was confirmed with 18S gene based qPCR. Microscopy and PCR revealed 8.4%(41/487) and 26.5%(129/487) asymptomatic infections, respectively. Most of the infections were attributed to Pv 60.0%(98/164), while only 31.7%(52/164) were due to Pf. Among the feeding experiments analyzed so far, 2 out of 8 asymptomatic Pf carriers were infectious to mosquitoes (5 and 30% infected mosquitoes) but none of the clinical patients (0/8) were found infectious. Among 13 Pv carriers, 2/5 asymptomatic individuals were infectious to mosquitoes (5.5 and 25% infected mosquitoes) while 7/8 symptomatic individuals were infectious to mosquitoes and infected on average 57% of mosquitoes. Transmission data on an additional 4000 mosquitoes from 140 individuals will be completed in the coming months. Preliminary results from this study demonstrate that asymptomatic Pf infections play significant role in the maintenance of the infectious reservoir whereas clinical malaria cases are particularly relevant for Pv transmission. The findings fill an

important knowledge gap on the relative contribution of symptomatic and asymptomatic individuals in an area of moderate-low transmission of vivax and falciparum malaria.

## 1952

### OUTDOOR PRIMARY AND "SECONDARY" VECTORS CONTRIBUTING TO RESIDUAL TRANSMISSION IN ZAMBIA

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Historically data across much of sub-Saharan Africa showed high levels of exposure to malaria mosquitoes indoors in the middle of the night. The use of insecticide-treated nets and indoor spraying of insecticides targeting these behaviors have been attributed to 70% of over 600 million cases of malaria averted between 2000-2015 in this region. Despite the impressive gains and continued high coverage of interventions, malaria transmission persists in many areas. Contributing factors to this residual transmission may be the persistence of vectors with altered behaviors; those that evade contact with insecticides can maintain transmission. To assess the contribution of outdoor foraging of anopheline vectors to malaria transmission, light trap collections of mosquitoes were made in sites of low and high malaria transmission in Zambia. Collections targeted to areas within homesteads frequented by residents in the evening or night were compared in a Latin Square design. In Macha, the low transmission site, southern Zambia, light traps were set outdoors next to kitchens, animal shelters and exit points from homesteads. Here, hourly collections at kitchens indicated sizable exposure to anophelines prior to when residents entered houses, however larger catches were made from animal shelters; collections were 26-fold those next to kitchens. Of the mosquitoes caught near animals, 63% were of the species *Anopheles squamosus*, previously shown to be a potential malaria vector in the area. In the high transmission site, Nchelenge, northern Zambia, light traps were set outdoors where food is cooked at night, next to animal pens and at wash rooms. Highest catches were made where people gather at night with 2.4-fold the catch of traps set next to animals. 85% were identified as *An. funestus* s.l. Data from these divergent sites show potential exposure to a variety of vectors outdoors in both pre-elimination and holoendemic malaria sites in Zambia. These findings stress the necessity for combining malaria interventions targeting both indoor and outdoor foraging mosquitoes as the dominant vectors and their associated behaviors may be difficult to predict.

## 1953

### IMPACT OF PNEUMOCOCCAL CONJUGATE VACCINE ON INVASIVE PNEUMOCOCCAL DISEASE IN THE GAMBIA: POPULATION-BASED SURVEILLANCE OVER 9 YEARS

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*Streptococcus pneumoniae* is a major cause of child mortality. Pneumococcal conjugate vaccines (PCV) have reduced the incidence of invasive pneumococcal disease (IPD) in affluent countries. Many low-income countries have introduced PCV, yet data on vaccine impact from these settings are lacking. We measured the impact on IPD of Gambia's PCV program which introduced PCV7 in Aug 2009 without a catch-up program and PCV13 in May 2011. We conducted standardized population-based surveillance for suspected meningitis, sepsis or pneumonia among those aged  $\geq 2$  months in the Basse Health & Demographic Surveillance System (BHDSS) in rural Gambia. Sterile site samples were analysed by conventional microbiology and pneumococcal isolates were serotyped by latex agglutination. The rate of enrolment per unit population varied over time, so we adjusted annual counts of IPD to the mean rate of enrolment of patients with suspected meningitis, sepsis or pneumonia. Incidence was calculated using BHDSS mid-point population estimates. We published results to the end of 2014. Here we report updated results to the end of 2016. We investigated 24 179 patients, identifying 372 cases of IPD. The baseline incidence of vaccine-type IPD in the 2-23 month age group from May 12, 2008 to May 11, 2010 was 195 and fell to 14 per 100,000 person-years in 2015/16; incidence of non-vaccine type IPD increased from 49 to 74 per 100,000 person-years; all IPD decreased from 253 to 88 per 100,000 person-years. In the 2-4 year age group the incidence of vaccine-type IPD fell from 99 to 15 per 100,000 person-years; non-vaccine type IPD increased from 14 to 18 per 100,000 person-years; all IPD decreased from 113 to 33 per 100,000 person-years. In the 5-14 year age group the incidence of all IPD decreased from 12 to 1 per 100,000 person-years. In the  $\geq 15$  year age group the incidence of all IPD fell from 9 to 1 per 100,000 person-years. Of 25 cases of vaccine-failure, 11 occurred within 12 months of the 3rd dose of PCV and 14 occurred more than 12 months after the 3rd dose of PCV. Overall cases of IPD have fallen by around 65% in the under 5 year age group and herd protection effects are evident in older children and adults.

## 1954

### SAFETY, TOLERABILITY AND EFFICACY OF A THREE-DOSE REGIMEN OF RADIATION ATTENUATED *PLASMODIUM FALCIPARUM* NF54 SPOROZOITES (PFSPZ VACCINE) IN HEALTHY MALIAN ADULTS

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A double blind, randomized clinical trial was conducted in Mali, West Africa to assess a 3-dose regimen of Sanaria® PfSPZ Vaccine (1.8x10<sup>6</sup> PfSPZ) administered by direct venous inoculation against natural malaria exposure over 6 months of surveillance. An open label dose escalation pilot study was completed first and demonstrated the safety and tolerability of PfSPZ Vaccine (4.5x10<sup>5</sup>, 9x10<sup>5</sup>, and 1.8x10<sup>6</sup> PfSPZ). After establishing safety and tolerability, the targeted dose (1.8x10<sup>6</sup> PfSPZ) was then administered at 0, 8, 16 weeks to the main cohort (n=120) in a double blind, randomized, placebo-controlled trial starting in Mar 2016.

Participants received artesunate/amodiaquine to eliminate Pf before first and last vaccination. The incidence and severity of local and systemic AEs occurring within 7 days after each dose were solicited. During the malaria transmission season (Aug-Dec 2016), volunteers were examined and blood smears obtained every 2 weeks for 24 weeks in total; the primary efficacy endpoint was detection of first positive blood smear following third vaccination. 60 subjects received at least one dose of PfSPZ Vaccine and 60 subjects received placebo. 57 (95%) received all 3 doses of PfSPZ Vaccine and 55 (91.7%) subjects received normal saline placebo. We detected no significant differences in local or systemic AEs or laboratory abnormalities between PfSPZ Vaccine and placebo groups. The primary efficacy endpoint was the time-to-infection during the transmission season. 55 subjects in the PfSPZ Vaccine group and 54 subjects from the placebo group were evaluable for per protocol analysis. Of these participants, 42 (77.8%) from the placebo group and 32 (58.1%) from the vaccine group developed Pf infection. Per protocol, the vaccine efficacy was 51% ( $p=0.004$ , 95% CI 20-70) by time-to-infection analysis (ITT 39%,  $p=0.033$ ) and 24% ( $p=0.031$ , 95% CI 2-41) by proportional analysis (ITT 22%,  $p=0.041$ ). PfSPZ Vaccine was well tolerated and safe at higher doses. This 3-dose vaccine regimen of PfSPZ Vaccine showed similar protection against Pf infection as previously reported in African adults receiving a 5-dose regimen.

## 1955

### HEPATITIS C, SYPHILIS, AND G6PD DEFICIENCY IN CAMEROONIAN BLOOD DONORS

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Glucose-6-phosphate dehydrogenase (G6PD) deficiency, the most common genetic condition in the world, remains a well-known risk factor for hemolytic anemia and kernicterus in newborns. A wide range of prevalence has been reported in Africa, though little is known about the region of Cameroon. To identify prevalence of G6PD deficiency in Cameroon and correlate with demographic, hematologic, and infectious markers. Blood was collected at Mbingo Hospital, a large referral center in Northwest Cameroon, as part of a previously established hospital blood donor program. Demographic information, ABO, hemoglobin, and infectious markers [syphilis (RPR), hepatitis C virus (antigen), hepatitis B (HBsAg), HIV] were performed per hospital donor screening protocol. Samples were frozen, with freezer temperatures maintained between -20 and -80C. G6PD testing was then completed in batch with thawed samples. Deficiency was determined using the Beutler Fluorescent spot test. We compared prevalence rates of each infection among G6PD-deficient vs non-deficient males by chi-square. We screened 747 blood donors and identified 40 (5%) deficient donors. Out of 539 (72%) males, 38 (7%) had G6PD deficiency. The prevalence of HCV in G6PD-deficient males versus non-deficient males was 8.3% vs. 1.3% ( $p = 0.02$ ). The prevalence of RPR positivity was 13.5% vs. 5.4% in G6PD-deficient vs non-deficient males ( $p = 0.05$ ), respectively. We found no significant difference in rates of hepatitis B virus or HIV in G6PD-deficient versus sufficient males. ABO typing in G6PD-deficient males showed 16.2% A, 18.9% B, 66.2% O, and 2.7% AB, similar to that of the study cohort ( $p = 0.93$ ). This is the first study to correlate G6PD deficiency with infectious markers. This raises important questions regarding the relationship between G6PD deficiency, hepatitis C virus, and syphilis. Further investigation is required to evaluate whether G6PD deficiency represents an independent risk factor for the development of hepatitis C and syphilis.

## 1956

### OUTCOMES OF A PILOT HYDROCELE SURGERY CAMP IN ETHIOPIA

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Globally an estimated 19.5 million men suffer from hydrocele due to lymphatic filariasis. Two major challenges in addressing the burden of hydrocele are patient identification and access to safe hydrocele surgery. In Ethiopia, LF patient estimates for three regions suggest 1,245 suspected hydrocele patients (0.04% of population), likely an underestimate due to stigma. Trained health extension workers developed patient line listings going door-to-door with color photos depicting hydroceles to aid in identification. A hydrocele surgery camp was then piloted at one hospital in collaboration with local and federal authorities and with support from the USAID-funded MMDP Project. Clinical officers screened 252 patients and confirmed 198 hydrocele cases (79%). Eight surgeons or surgical officers participated in a five-day training in resection (excision) surgery using theory-based modules and the Filariace Anatomical Surgical Task Trainer (FASTT) simulator. Trainees practiced on the FASTT simulator before operating on live patients. Each trainee then performed at least five live surgeries under the supervision and support of a master trainer. Over the 11-day period of the training and camp, 175 hydrocele surgeries were performed. All patients were hospitalized for three days and received a nine-day course of antibiotics, starting one day before surgery. At day five post-surgery, when 154 (88%) patients were followed up by trained community clinical workers, no patients were found with hematoma or infection. However, by day 14, out of 167 patients followed up, one (0.6%) had a hematoma and 19 (11.4%) had infections. This may indicate a need for improved post-surgical care. All patients with complications were referred to local health facilities. Other studies have found rates of complications ranging between 3-7% at day five, and increasing at later follow up. Critical to the camp's success was the standardized training, appropriate use of antibiotics, and routine patient follow up. Based on the ease of identifying a cohort of patients and the small number of post-operative complications, this strategy may be applicable in other countries.

## 1957

### IDENTIFYING CLINICAL PREDICTORS FOR PROGRESSION TO CHRONIC KIDNEY DISEASE IN MESOAMERICAN NEPHROPATHY

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Mesoamerican nephropathy (MeN) is a mysterious kidney disease of unknown etiology. The epidemic, to which greater than 20,000 deaths are owed, is unrelenting across Central America. MeN primarily affects young agricultural workers who lack traditional risk factors for kidney disease. MeN has been characterized as a chronic kidney disease (CKD), but we recently identified an acute phase of MeN, involving acute kidney injury with interstitial nephritis. The goal of this analysis is to pinpoint clinical characteristics of acute MeN that predict progression to CKD. Using univariate analysis, we compared patients with acute MeN who developed CKD to those who did not to identify clinical risk factors for CKD. Physicians at a private hospital in Nicaragua completed case reports detailing acute clinical encounters on cases of MeN and provided follow-up data on subsequent CKD diagnoses. From Feb 2015-Jan

2017, 468 cases of acute MeN were reported, mostly male (91%) and young (median age 27 yrs). Most (92%) had acute kidney injury (AKI). Frequent symptoms were fever (62%), nausea/vomiting (72%), back pain (61%), and headache (52%). Leukocytosis (80%), neutrophilia (84%), lymphopenia (53%), elevated C-reactive protein (76%), and anemia (59%) were common, along with leukocytes (99%) and leukocyte casts (30.2%) in urine. 30 patients (6%) progressed rapidly (median 90 days) to CKD, with half (51%) progressing to  $\geq$ Stage 3 CKD. We found that age  $>$ 25 years (Prevalence Ratio [PR] 2.90 [1.03, 8.19],  $p=0.045$ ), hyperuricemia (2.56 [1.26, 5.21],  $p=0.010$ ), anemia (3.37 [1.31, 8.67],  $p=0.012$ ) and hyponatremia (PR 3.20 [1.46, 7.02],  $p=0.004$ ) were associated with CKD. Leukocytosis (PR 0.39 [95%CI 0.19, 0.81],  $p=0.012$ ) and leukocyturia (PR 0.36 [0.17, 0.75],  $p=0.006$ ) during the acute phase of MeN were found to be protective. Our data suggest that acute systemic inflammation during acute MeN may mediate progression to CKD. Our ongoing longitudinal analysis will enhance our understanding of clinical events during disease progression and shed light on the mechanism of injury. This is the first of clinical factors associated with CKD and with recovery in MeN.

## 1958

### A NOVEL, PORTABLE INFRARED 3D SCANNER QUICKLY PROVIDES ACCURATE LIMB VOLUME AND CIRCUMFERENCE MEASUREMENTS IN PATIENTS WITH FILARIAL LYMPHEDEMA

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Despite progress in reducing transmission of lymphatic filariasis (LF), millions remain affected by filarial lymphedema. Current tools for measuring lymphedema include water displacement (WD), limb circumference (LC), and ultrasound skin thickness (UST). Each of these methods has drawbacks: WD is difficult to perform in the field, LC measurements are cumbersome and variable, and UST measurement is difficult to standardize and requires expensive equipment. A portable infrared scanner has recently been developed that can quickly and accurately measure limb volumes. We compared the scanner with the above-mentioned modalities in patients with filarial lymphedema. Six examiners were trained in each modality over a 2-day period that was followed by a test period with 52 patients. Limbs were measured by 2 (WD) or 3 (each other modality) separate examiners. Limbs with differing grades of lymphedema were examined (N = 28, 19, 20, 21, 2, 4, and 10 for grades 0-6, respectively). Limb volumes ranged from 1.6L to 11.0L and circumference ranged from 17.8 to 71.1 cm. The portable scanner calculated limb volume and circumference measurements that correlated nearly perfectly with WD ( $r^2 = 0.997$ ) and LC values ( $r^2 = 0.992$ ) over the entire range measured. The scanner required an average of 2.2 min to acquire measurements for both legs, compared to 17.4, 7.5, and 31.7 min, respectively, for WD, LC, and UST. Coefficients of variation for limb volume measurements performed by 2 (WD) or 3 (scanner) examiners were 1.4% for WD and 2.2% for the 3D scanner. CVs for circumference measurements were 1.8% by tape measure and 1.6% by scanner. UST was the least precise measure tested; median inter-observer variability was 5.5% (IQR 3.2% - 9.1%; range 0.2% - 42.0%). These results show that accurate and precise lower extremity volume and circumference measurements can be obtained in approximately 2 minutes with a non-invasive, portable scanner. This technology has the potential to revolutionize assessment and monitoring of lymphedema in the clinic and in the field.

## 1959

### ABORTION RATE IS MUCH HIGHER THAN MICROCEPHALY RATE IN ZIKA VIRUS INFECTIONS OCCURRING IN THE FIRST TRIMESTER OF PREGNANCY

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Zika virus (ZIKV) infections in pregnant women have been associated to an increased number of severe malformations, such as, microcephaly and arthrogryposis. Much of this knowledge has been acquired through clinical studies but very few prospective studies have been done in cohorts of pregnant women, and this type of study is crucial to define ZIKV infections as the cause of congenital disease. In our prospective study, a cohort of pregnant women admitted to the study, independently of their stage of pregnancy, was followed up to the end of pregnancy. The primary goal of the study was to detect infections in these women and to compare the pregnancy outcomes of women infected or not with ZIKV. Pregnant women presenting with a zika-like disease, with duration of less than four days, were evaluated and followed by an ObGyn physician and zika diagnosis was performed by RT-PCR. STORCH syndrome was investigated in every case. From February 2016 to March 2017, 1,125 pregnant women were admitted to the study and 268 became sick during the follow-up (70, 102 and 96 on the first, second and third trimester, respectively). According to these trimesters, ZIKV-infections were detected in 42, 74, and 62 pregnant women and it was evident that the zika-related complications were only observed when infection occurred in the first and second trimester. Adverse pregnancy outcomes of ZIKV-positive mothers included 4 microcephaly, two newborns with normal cephalic perimeter but with cerebral calcifications, one arthrogryposis, and most important, 9 abortions, while outcomes of ZIKV-negative mothers included two microcephaly and two abortions. The newborn with arthrogryposis died a few hours after birth. One of the abortion cases associated to ZIKV-negative patients was from a 42-year-old patient and the other was from a patient infected with dengue-1 virus. Although the number of symptomatic pregnant women was not very large, it was clear that ZIKV infections were associated to a higher number of microcephaly and, most important, to a much higher rate of abortion, especially when the infection occurred in the first trimester of the pregnancy.

## 1960

### PROJECT PREMONITION PROJECT: FIELD TRIALS OF A ROBOTIC SMART TRAP FOR MOSQUITO IDENTIFICATION AND BIONOMICS

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Using traps for surveillance of vector and pathogen populations is an important component of many vector management programs, however this technology has remained largely unchanged over many years. It is inaccessible in developing countries and limited in developed countries, largely due to costs and personnel requirements. Project PREMONITION has developed an autonomous mosquito trap that captures live mosquitoes

individually for higher-throughput lower-cost mosquito surveillance and pathogen discovery. The PREMONITION trap uses infrared sensors and algorithms to identify flying insects by wing beat frequency, capable of capturing only target mosquito species and reducing non-targeted captures. In addition to recording putative species identification, abiotic data such as precise time of capture, temperature, humidity and ambient light, are recorded effectively providing foraging activity parameters throughout the collection period. Due to a unique design, each specimen is tagged with the data it produced, enabling new bioinformatics analyses. Field trials of the PREMONITION trap were conducted in Houston, Texas, in summer 2016 to evaluate trap performance under native environmental conditions. We performed 87 deployments across 20 sites in Houston. Over 20 GB of mosquito data paired with high-resolution environmental data were collected and analyzed. Over 22,000 mosquito events were detected and key species activity was captured at the sub-minute temporal scale. Data showed over 90% accuracy at identifying *Culex* and *Aedes* mosquitoes at the genus level. The resulting bionomic data provide unprecedented insight to the diurnal and nocturnal activity of these vectors.

## 1961

### PREVENTING MALARIA PARASITE TRANSMISSION WITH TRANSGENIC ENTOMOPATHOGENIC FUNGI

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We compared arthropod genes encoding insect-specific sodium, potassium and calcium channel blockers for their ability to improve the efficacy of *Metarhizium* against insecticide-resistant anophelines. Toxins increased lethality to mosquitoes at spore dosages as low as one conidium per mosquito. One of the most potent strains, expressing the EPA approved Hybrid (Ca<sup>++</sup>/K<sup>+</sup> channel blocker), was studied for efficacy in a MosquitoSphere in Burkina Faso. We found that suspending *Metarhizium* in locally produced sesame oil and spreading that on netting or black sheets achieves a long-term effect in the MosquitoSphere. Compared to the wild-type fungus, Met-Hybrid killed anopheline mosquitoes in half the time and at much lower spore doses in semi-field conditions, which increased the percent of lethality of infected mosquitoes and the effective persistence of the pathogen. The results surpass the WHO threshold for successful vector control agents. Met-Hybrid also has important pre-lethal effects that include sexual transmission and reduced blood feeding by infected mosquitoes. This NIH funded, international effort represents an important step in the progression of transgenic mosquito control technologies into field application.

## 1962

### MITIGATION OF PYRETHROID-RESISTANT *Aedes aegypti* USING PRE-SEASON, NON-PYRETHROID INDOOR RESIDUAL SPRAYING

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Prevention of *Aedes*-transmitted viruses (dengue, chikungunya, Zika) relies strongly on reactive vector control interventions (e.g., indoor space spraying, ultra-low volume spraying, thermal fogging) in response

to symptomatic cases, yet these methods are less effective against indoor-resting *Aedes aegypti* and of limited impact in mitigating rapidly propagating outbreaks. Additionally, the evolution of pyrethroid resistance further reduces the efficacy of vector control. Indoor residual spraying of insecticides (IRS) can effectively prevent transmission, although it is time consuming and difficult to deploy during outbreaks. Applying IRS using non-pyrethroid insecticides before the start of the transmission season may both reduce vector populations and mitigate the effect of pyrethroid resistance. We compared the entomological efficacy of IRS when applied two months before peak transmission within Merida, Mexico, an area with high prevalence of pyrethroid resistance. A randomized controlled trial was performed to compare the efficacy of IRS with Bendiocarb, to which *Ae. aegypti* is susceptible, to an untreated control. Houses were sampled for presence and abundance of *Ae. aegypti* 15 days before and 15 days, one, two, and three months after IRS. All indices of adult *Ae. aegypti* (e.g., proportion of houses positive, total abundance, and abundance of bloodfed females) were reduced immediately following application of IRS with Bendiocarb compared to the untreated control and reductions remained three months post application, well beyond the peak transmission period. Mean abundance of *Ae. aegypti* per house was reduced from 2.0 ± 0.3 (untreated) to 0.48 ± 0.1 (Bendiocarb) 15 days post IRS. Lower abundance per house with IRS was consistent 3 months post spraying, 0.59 ± 0.1 (untreated) to 0.26 ± 0.1 (Bendiocarb). These data indicate that early application of IRS with non-pyrethroid insecticides can effectively reduce populations of pyrethroid-resistant *Ae. aegypti* throughout the transmission season and merit more thorough explorations on the epidemiological impact of preventive vector control with residual insecticides.

## 1963

### EPI INFOTM FOR MOSQUITO SURVEILLANCE: A FREE MOBILE APPLICATION AND ANALYSIS DASHBOARD TO IMPROVE FIELD DATA COLLECTION AND PROVIDE AUTOMATED ANALYSIS THAT ENABLES DATA-DRIVEN DECISION MAKING FOR VECTOR CONTROL

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The Zika virus outbreak in the Americas highlighted capacity gaps of many vector surveillance programs in the region to rapidly conduct mosquito surveillance for supporting the vector control measures necessary to halt the outbreak. As a result, many countries employed generalized vector control, with patchy data on mosquito abundance, densities, or resistance to insecticides, which prevented them from more efficiently targeting their efforts. To improve the tools available for supporting decision making for outbreak control, the US Centers for Disease Control and Prevention (CDC) developed a tablet-based, mobile application for mosquito surveillance and a desktop data analysis dashboard, using existing free Epi Info™ software. The mobile app allows users to enter mosquito surveillance and control data directly in the field. In addition to a module collecting demographic information, the app consists of four modules: mosquito trapping (multi-day, for ovitraps and adult traps), mosquito surveys (single day, for immature surveys and aspiration), cone bioassays (for monitoring insecticide-treated surfaces), and vector control (for recording vector control actions, including efficacy studies). GPS coordinates, dates, and times are automatically collected, and sampling locations can be captured automatically using scanned barcodes. Field-collected data sync with the master database upon the tablet's detection of a wireless connection, and are uploaded immediately to the analysis dashboard. The dashboard automatically calculates relevant mosquito indices, places sampling locations on a map, and presents the user with graphical representations of trends over time, all of which can be filtered by dates and locations of interest. The dashboard is designed for use by mosquito surveillance and control personnel to make operational decisions based on data that is collected in near-real time. Using the Epi Info™ app and dashboard

eliminates the need for paper-based mosquito surveillance systems, enabling programs to more rapidly make data-driven vector control decisions. The app is freely available in multiple languages.

## 1964

### NEXT GENERATION MOSQUITO SURVEILLANCE TECHNIQUE: THE NEAR INFRA-RED SPECTROSCOPY

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Mosquito control programs need high throughput, rapid and cost effective surveillance tools to estimate the impact of interventions, determine exposure risk and direct interventions to disease hot spots. Traditional surveillance tools such as those that estimate mosquito survival or those that detect infections in mosquitoes are either laborious, technically demanding or costly. Our team is currently testing the applicability of the near infrared spectroscopy (NIRS) as an alternative surveillance technique for malaria, dengue and Zika control programs. NIRS can predict the age and species of the major malaria vector, *Anopheles gambiae* s.s. and *An. arabiensis* simultaneously with accuracies of up to 90% and can predict the age of laboratory reared *Anopheles stephensi* and *Ae. Aegypti* with accuracies of up to 95%. Prediction of the 1) presence or absence of Wolbachia in *Aedes aegypti* was up to 96% accurate and 2) presence of dengue in laboratory reared *Ae. aegypti* was 91% accurate. Other applications that NIRS has shown potential include detection of Plasmodium parasites in *Anopheles* and Zika virus in *Ae. Aegypti* mosquitoes. The team is exploring alternative analysis options such as artificial neural networks to improve the predictive accuracy of NIRS. NIRS has shown potential as an alternative surveillance tool for mosquito control programs. It is a non-destructive one stop shop surveillance technique that can be used to measure multiple characteristics of a mosquito sample such as infection and age with just a single, 3-second reading of the mosquito. The technique does not require reagents to operate and hundreds of samples can be scanned and analyzed immediately. However, for operational use, NIRS requires field validation.

## 1965

### CHARACTERIZING THE BEHAVIOR OF SUSCEPTIBLE AND RESISTANT STRAINS OF ANOPHELES GAMBIAE AT THE LLIN INTERFACE USING SIMPLE NEW LABORATORY VIDEO TESTS

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The central role of long-lasting insecticidal nets (LLINs) in malaria prevention in Africa is threatened by pyrethroid resistance. Investigating how mosquito populations interact with different LLIN types is fundamental to understanding the nature of resistance, evaluating new net treatments and managing future resistance emergences. We used two new simple bench top tests, the Cone Video test and the Thumb test, to explore detailed behavioral events associated with insecticide exposure, in two insecticide susceptible and two resistant strains of *Anopheles gambiae* at three LLIN types (PermaNet® 2.0, Olyset® and Duranet®). The Cone Video test employs scan sampling to quantify activity during a standard WHO cone test (resting on net, cone or in flight; 5 mosquitoes; 3min) using a cellphone camera. In all tests, susceptible and resistant mosquitoes rested preferentially on untreated and treated netting except for susceptible mosquitoes on Olyset®, where net contact was significantly reduced compared with other LLINs. Non-contact assays suggest this reduced contact was due to irritancy induced by contact with the net surface rather than repellency; interestingly this effect was not seen in

pyrethroid resistant populations. Reduction in net contact correlated with mortality post-exposure. The Thumb test video records detailed events of a single mosquito at a human-baited LLIN interface, with or without blood feeding. Here, susceptible and resistant mosquito strains readily fed through all LLIN types though the duration of the blood meal was reduced in both strain types when feeding through an LLIN compared to an untreated net. The results of both assays will be compared and considered in relation to behavioral data from other studies. The results demonstrate the potential of these simple assays for increasing understanding of the repellent or contact irritant properties of existing and new insecticides or LLINs, and the full characterization of their modes of action.

## 1966

### DELTA METHRIN RESISTANCE IN Aedes Aegypti RESULTS IN VECTOR CONTROL FAILURE IN MERIDA, MEXICO

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Insecticide resistance has emerged as a worrisome outlook for the implementation of insecticide-only approaches to vector control. Particularly for *Aedes aegypti*, it is generally argued that this rapid rise of insecticide resistance may compromise the effectiveness of control programs. Yet, empirical evidence of such negative operational impact is lacking. We present results from a randomized controlled trial performed in the city of Merida, Mexico, quantifying the efficacy of indoor residual spraying (IRS) against adult *Ae. aegypti* in houses treated with either deltamethrin (to which local *Ae. aegypti* expressed a degree of resistance) or bendiocarb (to which local *Ae. aegypti* were susceptible) as compared to untreated control houses. This trial was designed to quantify the operational impact of deltamethrin resistance on the efficacy of insecticide applications to control *Ae. aegypti*. Indoor residual spraying (an effective method to control *Ae. aegypti*) was performed from October 31 to November 22, 2015 and aimed at full coverage of entire city blocks and close entomological monitoring consisting of indoor adult collections using Prokopack aspirators at 2 weeks prior to spraying (baseline) and at 15 days, and 1, 2 and 3 months post-spraying. While no statistical differences between treatment and control houses were found for all adult *Ae. aegypti* infestation indices at baseline, post-spraying *Ae. aegypti* infestation and abundance were significantly lower in houses treated with bendiocarb compared to untreated houses (odds ratio <0.75, P<0.05; incidence rate ratio < 0.65, P<0.05) whereas no statistically significant difference was detected between the untreated and the deltamethrin-treated houses. On average, bendiocarb spraying reduced *Ae. aegypti* abundance by 60% during a 3-month period. Results demonstrate that vector control efficacy can be significantly compromised when the insecticide resistance status of *Ae. aegypti* populations is not taken into consideration.

## 1967

**TOXOCARA INFECTION IN AN ECUADORIAN BIRTH COHORT: FROM BIRTH TO 5 YEARS OF AGE**

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Human infection with toxocarasis is a systemic zoonotic helminth infection with worldwide distribution but which is most prevalent among populations living under conditions of poor hygiene in tropical regions. Infection is acquired through accidental exposure to embryonated eggs of *Toxocara canis* and *Toxocara cati*, excreted in the faeces of the definitive hosts, dogs and cats, respectively. Although *Toxocara* infections can be transmitted through the intrauterine route in natural and paratenic hosts, there are few data on possible *in utero* transmission in humans. Further, there is limited information from humans on the burden of disease morbidity attributable to these infections in endemic populations. To address these questions, we have been using data and stored samples from an ongoing birth cohort, the ECUAVIDA cohort, of 2404 newborns and their mothers recruited in a rural District of Esmeraldas Province in coastal Ecuador. For the present analysis, plasma samples collected from a sample of 290 mothers and their children at birth (cord blood), 7, 13, 24, 36, and 60 months were analyzed for the presence of IgG antibodies to *Toxocara canis* excretory-excretory substances (TES) by ELISA. TES-specific antibodies were detected in 80.3% of mothers and in 84.1%, 0%, 9.3%, 48.4%, 64.9%, and 80.9% of children at 7, 13, 24, 36, and 60 months, respectively. The incidence rate of infection was greatest during the second year of life (39.3% of seronegatives) and seroprevalence reached that of the children's mothers (i.e. >80%) by 5 years of age. Significant risk factors for infection varied by age but consistently included household dogs and or cats. Our data indicate lack of *in utero* transmission of *Toxocara* infection in a hyper-endemic region of Ecuador with rapid acquisition of infection between 7 months and 24 months of age. Future analyses will estimate the burden of disease that can be attributed to *Toxocara* infection in this cohort.

## 1968

**SEROPREVALENCE OF ANTIBODIES TO TOXOCARA SPECIES IN THE UNITED STATES AND ASSOCIATED RISK FACTORS, 2011-2014**

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Toxocarasis results from infection with larval stages of a dog and cat intestinal nematode and causes human morbidity. Current US estimates of *Toxocara* exposure are over 17 years old. We used a multiplex bead based assay (Tc-CTL-1MBA) with purified *Toxocara canis* antigen to estimate *Toxocara* antibody seroprevalence in serum of 13,509 persons six years and older from the National Health and Nutrition Examination Survey (NHANES), 2011-2014 and identified seropositivity risk factors. We tested a subset of 500 samples with the *T. canis* enzyme immunoassay test previously used in NHANES III (1988-1994) to estimate seroprevalence had the prior survey samples been tested by Tc-CTL-1MBA. The age standardized estimate of *Toxocara* seroprevalence was 5.0% (95% confidence interval [CI], 4.2%-5.8%), lower than previously reported in 1988-1994 even accounting for increased Tc-CTL-1MBA specificity. Risk factors for seropositivity from multiple logistic regression were older age (odds ratio [OR], 2.1; 95%CI, 1.1-3.9 in 50-59 year olds; OR, 1.7; 95%CI, 1.0-2.8 in 60-69 year olds; and OR, 2.6; 95%CI, 1.5-4.7 in ≥70 year olds versus 6-11 year olds), non-Hispanic Black race/Hispanic origin (OR, 1.4;

95%CI, 1.0-2.0 versus non-Hispanic White), male sex (OR, 1.9; 95%CI, 1.6-2.2), living below poverty level (OR, 1.9; 95%CI, 1.4-2.6), a household with >0.5 persons per room (OR, 1.3; 95%CI, 1.0-1.6), less than college education (OR, 1.9; 95%CI, 1.5-2.4), and birth outside the United States (OR, 3.6; 95%CI, 2.6-5.1). *Toxocara* seroprevalence estimates in 2011-14 were lower than in a study from NHANES III, 1988-94, but seropositivity risk factors remained the same. These findings could help frame prevention efforts.

## 1969

**CHARACTERIZING SOIL-TRANSMITTED HELMINTH SINGLE AND MULTIPLE INFECTIONS RESPONSE TO TREATMENT WITH BENZIMIDAZOLES AND OTHER DRUGS**

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The benzimidazoles albendazole and mebendazole are given as preventive chemotherapy to school-aged children or entire communities for soil-transmitted helminthiasis caused by *Ascaris lumbricoides* (Al) *Trichuris trichiura* (Tt) and hookworm (Hw). Efficacy varies with the species, location and time. A database made of 12 clinical trials of albendazole (alone and combinations), mebendazole (alone and combinations), other treatments, or placebo was analysed to characterise infections and study treatment effects on individual and multiple species. Baseline infection intensities were expressed as eggs per gram of faeces (EPG); treatment outcomes were expressed as egg reduction rates using arithmetic means (ERR) and as distribution of individual-participant ERR (where no change or an increase was expressed as 0%). 4,981 patients were diagnosed with 9,525 infections overall with one (35%) or several STH species (65%): Al 21%, Tt 45% and Hw 34%. Baseline infection intensity was significantly higher for each STH species in co-infections than mono-infections. The ERR met the WHO minimal efficacy criteria of >95% for Al, >50% for Tt and >90% or >70% for Hw in just 55% of studies of albendazole and mebendazole alone or in combination, independent of whether mono- or multiple-infections. Considering individual responses, placebo achieved 100% ERR (hence cure) in 19% of Al and 11% of Tt and Hw infections. Albendazole alone gave ERR=0 in 1%, 27% and 8% of Al, Tt and Hw infections, and ERR=100% in 79%, 52% and 45%, respectively. The equivalent figures for mebendazole are 2%, 22% and 26%; and 93%, 25% and 21%. All treatments were compared through network meta-analysis. Co-infections were frequent and had higher infection intensities, but did not influence treatment performance. Just over half of treatments meet the WHO minimal efficacy criteria. Individual ERR response distributions allow identifying the proportions of suboptimal responders and comparing treatment performances.

## 1970

**A SINGLE DOSE OF IVERMECTIN, DEC PLUS ALBENDAZOLE IS SUPERIOR TO DEC PLUS ALBENDAZOLE FOR TREATMENT OF TRICHURIS TRICHIURA IN INDONESIA**

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*Trichuris trichiura* is a prevalent soil transmitted helminth (STH) in Indonesia and known for its poor susceptibility to common anthelmintics including benzimidazoles. Therefore, lymphatic filariasis (LF) elimination programs using diethylcarbamazine (DEC) combined with albendazole (DA) may have suboptimal effects on STH. Recently, a new regimen combining ivermectin, DEC and albendazole (IDA) was studied in a large community clinical trial for LF. As part this study, we have compared the efficacy of a single dose of IDA and DA against STH infection. Residents in a village in Southwest Sumba District were treated either with IDA or DA. A total of 262 participants provided stool samples at two time points, before treatment and two to four weeks after treatment. The presence of STH and the fecal egg count per gram (EPG) were determined using the Kato-katz method. Baseline infection rates for *Ascaris lumbricoides*, hookworm, and *Trichuris trichiura* in the study area were 30.5%, 75.6%, and 78.2%, respectively. There were no significant differences of STH prevalence rates between the IDA and DA groups at baseline. Following treatment, prevalence of any STH infection differed significantly between the two groups (85.5% vs 51.5%, respectively), especially for *T. trichiura* (67.7% vs 12.0%). Reduction of infection intensity for each species was significant in both groups (all  $p < 0.0001$ ). The STH cure rate (CR) was significantly higher in the IDA group (48.1%) compared to the DA group (13.3%,  $p < 0.0001$ ). Analysis by species showed only for *T. trichiura* a significant difference in the CR (IDA 89.1% vs DA 26.5%) as well as in the incidence of new infections (IDA 15.9% vs DA 46.2%). The egg reduction rate (ERR) of *T. trichiura* was also higher in the IDA compared to the DA group (94.1% vs 40.1%). The triple drug therapy using IDA showed higher efficacy against *T. trichiura* compared to the current standard regimen for LF elimination using DA. This result supports the inclusion of ivermectin in LF and STH elimination programs in Indonesia.

## 1971

**SOIL-TRANSMITTED HELMINTH INFECTION AND MASS DRUG ADMINISTRATION IN MYANMAR: ARE ADULTS PERPETUATING TRANSMISSION?**

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Myanmar has had ongoing mass drug administration (MDA) programmes against soil-transmitted helminths (STH) and lymphatic filariasis (LF) for at least 14 years. Data reported to the WHO preventive chemotherapy (PCT) databank show national coverage of the STH MDA programme, annually treating school-aged children (SAC) with albendazole, has been above 75% since 2006. However, national coverage of the separate LF MDA programme, which annually treats the whole community with albendazole and DEC, has only been above 75% since 2014. Few epidemiological studies include adults, preferring to focus on SAC. However, MDA compliance is usually lower in the older age groups and it is possible that treatment is insufficient in these age groups to adequately reduce prevalence and intensity. Our epidemiology study, conducted in two villages in lower Myanmar that have received four years of MDA ( $n=659$ ), recorded an overall prevalence of 20.5% STH infection, with 16.9% STH infection in adults. Whilst STH infection remains higher in SAC (28.5%),

there is a substantial burden of infection in adults. Prevalence of infection with any STH is statistically different between pre-SAC, SAC and adults ( $p=0.002$ ). The most prevalent STH species overall, and in each age group, was *Trichuris trichiura*. Only 6.33% of infected adults were infected with more than one STH species. When analysing STH prevalence in adults alone ( $n=467$ ) we found no statistical difference between the study villages, sex of study participant, education level or whether the participant shared a household with children. However, there was a statistically significant difference between stratified age groups within the adult age class ( $p=0.002$ ), with prevalence decreasing as adult age group increased. In conclusion, despite adults in these Myanmar communities receiving annual MDA for four years, STH prevalence is surprisingly high. Further study is required to better understand STH infection in adults if country NTD control programmes plan on targeting elimination. Adults are likely acting as an important reservoir of infection, perpetuating transmission in the community.

## 1972

**ENZYME-LINKED IMMUNOSORBENT ASSAY AS AN ENDGAME DIAGNOSTIC METHOD FOR SOIL-TRANSMITTED HELMINTH INFECTIONS**

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With the success of mass deworming programmes against soil-transmitted helminths (STH) and the known limitations of the current techniques, improved diagnostic methods are necessary to ensure detection of low-intensity infections. Immunoassays measuring antibody titres against specific STH antigens can provide a new way of measuring programme impact and recording rates of reinfection. A study was conducted in Bungoma District, western Kenya, where blood and stool samples were collected from 2273 individuals during two cross-sectional surveys in Mar-Apr and Aug-Sep 2014. Stool samples were analysed using duplicate Kato-Katz and intensity of infection recorded as eggs-per-gram (EPG). Plasma was extracted from the blood samples and analysed by indirect enzyme-linked immunosorbent assay (ELISA) to measure antibody titres against two *Necator americanus* recombinant proteins (Na-ASP-2 and Na-SAA-2) and two *Ascaris* antigens, A. suum haemoglobin (AsHb) and A. lumbricoides extract. Prevalence and intensity of infection were analysed together with antibody seroprevalence against the four antigens, searching for correlations between EPG and antibody titres at individual and community level, as well as changes in seroprevalence post-community-wide deworming. Despite a positive correlation between the age-prevalence profile of *N. americanus* and seroprevalence of Na-ASP-2, no other correlations were found between infection status or EPG levels and antibody titres, and no significant changes in seroprevalence were found three months after deworming treatment. There was also some evidence of cross-reactivity between the four antigens tested. Immunoassays might be used as alternative diagnostic methods in populations targeted by deworming programmes, but cross-reactivity between species and difficulties in distinguishing between past, prepatent and current infections remain. Their use might be limited to an analysis of community-wide exposure rather than accurate individual diagnosis of present or past infection. In the endgame, seroconversion in young children could be a good marker of continued exposure to infection.

## 1973

**POOL THE STOOL: POOLING STOOL SAMPLES AS A STRATEGY FOR INCREASING THE EFFICIENCY AND EFFECTIVENESS OF REAL-TIME PCR FOR SOIL-TRANSMITTED HELMINTHS (STH)**

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Due to mass-drug administration (MDA) efforts the incidence of soil-transmitted helminth (STH) infection is declining in many countries. As such declines occur, the need for cost-effective and sensitive strategies capable of monitoring the changing rates of infection is becoming increasingly critical. While PCR-based molecular techniques have repeatedly demonstrated their improved diagnostic capacities, the costs of DNA-isolation and subsequent PCR screening can be prohibitively high. Accordingly, sample pooling strategies, capable of reducing the costs associated with such screening efforts, would provide one means of potentially overcoming these economic obstacles. Such strategies have previously been utilized for the molecular testing of urine, blood and vector insect samples, and the capacity to detect soil-transmitted helminths in pooled stool samples has been previously evaluated using microscopy-based diagnostic techniques. In this study, we aimed to demonstrate that a similar pooling approach would not substantially reduce the sensitivity of real-time PCR-based detection of soil-transmitted helminths, when compared with the testing of individual human stool samples. Accordingly, pools of 5, 10 and 20 samples with a series of known egg-per-gram (epg) concentrations, 0 epg (control), 20 epg, 50 epg, 100 epg and 200 epg were prepared for six human-infecting species of STH (*Necator americanus*, *Ancylostoma duodenale*, *Ancylostoma ceylanicum*, *Strongyloides stercoralis*, *Trichuris trichiura* and *Ascaris lumbricoides*). Samples underwent DNA isolation, and resulting extracts were tested using previously validated and published multi-parallel real-time PCR assays for the detection of each species of STH. Results of testing have enabled us to identify the largest pool sizes that can be tested while still maintaining the required sensitivity at low epg concentrations. These results will help to guide programmatic decision making efforts with respect to sample pooling strategies, as the need to reduce the cost and labor associated with STH control efforts becomes increasingly pressing.

## 1974

**IMAGE-BASED TECHNOLOGY IDENTIFIES CANDIDATES FOR THE IN VITRO SINGLE-TREATMENT OF *TRYPANOSOMA CRUZI* AND *TRYPANOSOMA BRUCEI* PARASITES FROM THE MMV PATHOGEN BOX COLLECTION OF COMPOUNDS**

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Chagas disease, endemic to 21 countries in the Americas, is caused by the protozoan parasite *Trypanosoma cruzi* and is recognized by the World Health Organisation as one of the world's 17 most neglected tropical diseases. The disease causes some 14 000 deaths per year and a further 8 million people are threatened with *T. cruzi* infection. Treatment of Chagas disease is limited to the drugs which have associated side effects and display lack of efficacy against the chronic phase of the disease. *Trypanosoma brucei* sub-species cause Human African Trypanosomiasis (HAT), endemic to 36 countries within sub-Saharan Africa. This disease moves through clinically distinct clinical phases and in the latter, parasites cross the blood brain barrier and infection is difficult to treat. In addition, treatment regimens are complicated and not effective against all species. To identify much-needed new compounds with activity against *T. cruzi*, a phenotypic, high-content, 384-well image-based assay was developed to identify *T. cruzi* amastigotes within host cells following compound

exposure. In the same well, the effect of compound activity on host cells is determined. This assay was used to profile the activity of the Medicines for Malaria (MMV) Pathogen Box collection, containing 400 compounds selected for chemical diversity and drug-like properties. Fifteen compounds exhibited selective and unpublished activity against the parasite and 12 of these compounds were also active against *T. brucei* parasites. The majority of the IC50 values of these compounds were sub- $\mu$ M and provide a resource of potential new targets shared by these kinetoplastid parasites. To establish the most effective molecules for prioritisation, compound time to kill and maximum efficacy were investigated. As a preliminary identification of the mode of action, the impact of these molecules on parasite mitochondrial health and replication were investigated utilising Mitotracker Red and nucleoside analogues, respectively. Novel to *T. cruzi* drug discovery, nucleoside analogues were coupled with click chemistry for the image-based assessment of parasite replication.

## 1975

**ANTI-LEISHMANIAL LUCIFERASE BASED IN VITRO HIGH THROUGHPUT SCREENING OF INTRACELLULAR AMASTIGOTES OF GEOGRAPHICALLY DIVERSE PARASITES**

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Leishmaniasis is a neglected vector-borne tropical disease caused by a protozoan of the genus *Leishmania*. The clinical spectrum of leishmaniasis ranges from self-healing cutaneous ulcers to an irreparable damage of the soft and cartilaginous tissues and even to fatal systemic illness. Most of the drugs available today are difficult to use, have unpleasant side effects, and have variable efficacy. Consequently, there is a continued search for safer, less expensive and more effective drugs. Experimental Therapeutics' (ET) mission is to develop drugs to treat cutaneous leishmaniasis for US service members. ET's testing paradigm focuses primarily on evaluating compounds' activity on *L. major*, starting with *in vitro* IC50s of intracellular amastigotes. Promising compounds from the *L. major* screen can now be evaluated further on a geographically diverse set of parasites. In this study, a pLEXY-hyg based vector was used to generate *Leishmania* parasites that constitutively express the firefly luciferase gene. These nine transgenic species (*L. major*, *L. guyanensis*, *L. infantum*, and *L. mexicana*, and *L. panamensis*, *L. donovani*, *L. tropica*, *L. amazonensis*, and *L. peruviana*) were evaluated in comparison to their wild type counterparts and characterized for their utility for use in a high throughput screening format to find novel, broad-spectrum anti-Leishmanial compounds with activity against geographically diverse parasites.

## 1976

**COORDINATE REGULATION OF CELLULAR PROCESSES BY INOSITOL PHOSPHATES DURING *TRYPANOSOMA BRUCEI* LIFE CYCLE DEVELOPMENT**

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*Trypanosoma brucei* changes surface protein, metabolism and morphology during its life-cycle. In the mammalian host bloodstream forms (BFs) differentiate into stumpy BFs, then to procyclic forms (PFs) in the fly midgut and to other stages in the salivary gland. We showed that the inositol phosphate pathway helps regulate these developmental steps. Three genes that affect PI(4,5)P2, function in regulating surface protein expression and antigenic variation. Knockdown of inositol polyphosphate 5-phosphatase (IP5Pase), which dephosphorylates Ins(1,4,5)P3, (a product of the above enzymes) results in a cell density dependent differentiation of slender to stumpy BFs. Knockdown of inositol polyphosphate multikinase (IPMK), which phosphorylates Ins(1,4,5)P3, results in differentiation of BF to PF. This differentiation changes surface protein (VSG loss and procyclin expression), cell morphology, and the transcriptome. RNAseq following IPMK knockdown in BFs shows upregulated stage-specific gene sets

including those for sugar and amino acid transporters, cAMP signaling, oxphos, and specific RNA-binding proteins. MS analysis found that the IPMK substrate Ins(1,4,5)P3 affinity enriched proteins that function in signaling, protein synthesis and degradation whereas the IPMK product Ins(1,3,4,5)P4 enriched proteins that function in energy metabolism. In BFs IPMK and IP5Pase knockdowns, respectively, increased ATP production and decreased pyruvate release. Importantly, this increased ATP production was blocked by oxphos inhibitors showing de novo production of a functional oxphos system. Thus, IPMK functions in the regulation of the metabolic switch between glycolysis and oxphos and affects differential editing of mt mRNAs that encode oxphos components. IPMK knockdown also increased sensitivity to citrate/cis-aconitate induced differentiation, whereas by affecting cellular metabolite levels. These data indicate that inositol phosphates are part of the cellular regulatory network that controls *T. brucei* differentiation and development in a coordinated fashion during its life cycle.

## 1977

### UNDERSTANDING THE TRYPANOSOME LYTIC FACTOR (TLF) MEDIATED KILLING OF *LEISHMANIA* SP.

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Trypanosome Lytic Factor (TLF) is an innate immunity factor originally discovered for its ability to protect against *Trypanosoma brucei* subspecies. Recently, we have shown TLF can ameliorate infections by cutaneous *Leishmania* sp. in macrophages and mice. Here, we investigated if TLF is effective at killing *Leishmania* sp. within neutrophils; one of the first cells to take up the parasites *in vivo*. We found that depleting neutrophils in TLF expressing mice exacerbates the infection indicating that TLF effectively kills parasites within neutrophils. *In vitro* experiments suggest that this TLF mediated killing of *Leishmania* sp. metacyclic promastigotes occurs due to the direct interaction of TLF and *Leishmania* within the acidic phagosome of phagocytic cells. *In vitro* we find that metacyclics are killed by a two step process designed to mimic neutrophil uptake or macrophage uptake: TLF is first incubated in acidic media (pH 5.6 mimics the phagosome), which promotes the association of APOL1, the pore-forming protein of TLF, to the metacyclics. Then parasites are switched to neutral media to mimic the escape from neutrophil phagosomes and release into the serum. Herein, we find the parasite number is reduced but not eliminated. Alternatively if parasites are further acidified to pH 4.5 to mimic the parasitophorous vacuole of macrophages that amastigotes reside in, the metacyclics are completely lysed. Yet *in vivo* in presence of TLF, *Leishmania* can grow and cause disease. We propose this is due to the timely differentiation to amastigotes, the form that proliferates in macrophages *in vivo*. We find that axenic amastigotes are completely resistant to TLF *in vitro*. Preliminary data suggest that difference in surface glycoproteins composition between the metacyclics and amastigotes leads to difference in susceptibility of parasites to TLF.

## 1978

### INTERFERING POLYAMINES METABOLISM IN TRYPANOSOMATIDS, A VALID APPROACH FOR DRUG DEVELOPMENT

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Chagas disease, leishmaniasis and sleeping sickness represent the major health condition with a wide range of disability for people who are infected with parasites from the family of trypanosomatids (*Trypanosoma cruzi* TC, *Leishmania donovani* LD, and *T. brucei* TB). Despite years of extensive research in this field, there is still no new therapeutic drug available in the market yet. Thus, for these neglected diseases a new era of antiparasitic medicine is required. To serve this purpose, we developed a strategy to build compounds in a very inexpensive synthesis process, with very high yields and Selectivity Index. In this present work we found that our compounds were very active against *T. cruzi* (N,N'-bis(isopropyl) bencyl-1,4 diamine, IC50 = 370 nM epi.) *L. donovani* (N,N'-bis(4-Bencyloxy-3-Methoxy)bencyl-1,10 diamine, IC50 = 16nM), or *T. brucei* (N,N'-bis(isopropyl)bencyl-1,4 diamine, IC50 = 29 nM bloodstream form). Interestingly, our studies showed that these compounds respond differently on each parasite and that is related to their structural features, like the selectivity toward TB or TC that could be rationalized in terms of certain physical-chemical properties. Additionally, the collection was also tested on the intracellular life stage of LD and TC. Having in mind the polyamine nature of the library prepared, different experiments related to polyamine metabolism and transport were conducted. Selected hits that also shown good parameters on ADME-Tox (in silico) were assayed against trypanothion synthetase of the three parasites studied and rescue experiment with polyamines metabolites were performed in LD. Finally, we designed and synthesized a fluorescent analog of the most active compounds and the incorporation and localization was studied in the TC amastigotes. We can conclude through analysis of this library that we have found some serious candidate for LD, TB and TC chemotherapy. We all the information collected, the action mechanism is not clear yet, but the compounds are multitarget including the transport process. More studies will be required to find the molecular target/s and action mechanism.

## 1979

### INCIDENCE OF SALMONELLA BACTEREMIA AMONG YOUNG CHILDREN IN SUB-SAHARAN AFRICA: MAL055 RTS,S/AS01 SALMONELLA ANCILLARY STUDY

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Salmonella enterica is a major cause of bacterial bloodstream infections in Africa, manifesting as typhoid fever and invasive nontyphoidal Salmonella (iNTS) disease. Robust up-to-date epidemiological data to support the development and implementation of new interventions including vaccines are currently lacking. Our aim was to determine incidence of Salmonella bacteremia in children under five years of age across sub-Saharan Africa using data from the phase 3 RTS,S/AS01 malaria vaccine trial (NCT20866619). Children aged 6-12 weeks (n=6537) and 5-17 months (n=8922) from 11 sites were recruited and randomized to receive RTS,S/AS01 or comparator vaccines. They were followed up for a median of 38 and 48 months respectively between 2009 and 2014. Blood cultures were performed for all children with fever leading to hospital admission. 257 microbiologically-confirmed cases of Salmonella bacteremia were detected during 50,280 person years of observation (PYO), with an overall incidence of 534 cases/100,000 PYO (95%CI 471-604). Incidence of bacteremia due to Salmonella Typhi (n=32, 12.5% of Salmonella bacteremias) was 66.5 (95%CI 45.5-93.9), and that for nontyphoidal Salmonella (NTS; n=222, 86.4%) was 461 (95%CI 402-526) cases/100,000 PYO. 90% (n=200) of the 222 cases of iNTS disease were due to two serovars of Salmonella, S. Typhimurium (n=136, 61%) and S. Enteritidis (n=64, 29%). Incidence of Salmonella bacteremia varied from 0 in Bagamoyo, Tanzania; Kilifi, Kenya; and Lamberene, Gabon, to 1280 (95%CI 961-1660) in Kintampo, Ghana,

and 1780 (95%CI 1420-2200) in Siaya, Kenya. NTS bacteremia, but not typhoid fever, was significantly associated with the number of malaria episodes during the trial, but NTS bacteremia was not reduced by RTS,S/AS01 compared with comparator vaccines (incidence rate ratio = 0.83 (95%CI 0.63-1.10)). These findings confirm Salmonella as a major and persistent cause of bacteremia among children under five years of age across sub-Saharan Africa with *S. Typhimurium*, Enteritidis and Typhi as the three commonest serovars. A vaccine able to protect against these serovars could have a major public health impact.

## 1980

### UNDERSTANDING THE POTENTIAL VALUE OF NEW DIAGNOSTICS FOR ENTERIC FEVER: INSIGHTS FROM DECISION ANALYTIC MODELING

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Enteric fever is responsible for an estimated 11.9 million illnesses and 129,000 deaths annually. Currently available diagnostics have limited accuracy, resulting in empirical management in which individuals with disease fail to receive appropriate antibiotics, while others receive unnecessary treatment. Several promising new typhoid diagnostics are in the development pipeline. Additionally, host immune biomarkers have demonstrated good accuracy in distinguishing bacterial from viral infections. To evaluate the potential clinical impact and cost-effectiveness of these new diagnostics, we developed a decision-analytic and cost-effectiveness model, simulating the treatment of individuals with acute febrile illnesses. We parameterized the model with disease, treatment, demographic, and cost data from studies in South Asia. In the base case, we assumed that 13% of patients had enteric fever; 26% had other bacterial infections (*Rickettsia*, leptospirosis, *E. coli*, *Strep* spp, etc); and 50% had viral infections. We use disability weights from the Global Burden of Disease study and mortality estimates from recent meta-analyses. We compared five diagnostic and treatment strategies: no diagnostics; Tubex TF (a rapid typhoid serodiagnostic); new rapid typhoid diagnostics; C-reactive protein (CRP); a new bacterial/viral biomarker. Multiway sensitivity analysis and probabilistic uncertainty analysis were performed, with variable importance evaluated by calculating partial rank correlation coefficients. In a population of 10,000 patients with acute febrile illnesses, Tubex TF and a new typhoid diagnostic would avert 132 and 525 DALYs, respectively, and averting \$23,000 and \$103,000 in healthcare costs. However, CRP or a new bacterial/viral biomarker would avert additional disability and costs compared with typhoid-specific diagnostics. In conclusion, while new typhoid diagnostics could yield health gains in endemic settings, biomarkers that distinguish bacterial from viral infections would have greater clinical impact and potentially yield cost-savings due to averted healthcare expenditures.

## 1981

### COMPARISON OF STRATEGIES AND THRESHOLDS FOR VI CONJUGATE VACCINES AGAINST TYPHOID FEVER: A COST-EFFECTIVENESS MODELING STUDY

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Typhoid fever remains a major public health problem globally. While new Vi conjugate vaccines hold promise for averting disease, the optimal programmatic delivery remains unclear. We aimed to identify the strategies

and associated epidemiologic conditions under which Vi conjugate vaccines would be cost-effective. We developed a dynamic, age-structured transmission and cost-effectiveness model that simulated vaccination strategies with a typhoid Vi conjugate vaccine. We simulated a 10-year vaccination program through: (i) routine immunization of infants (<1 years) through the Expanded Program on Immunization (EPI) at 85% coverage; and (ii) EPI plus a catch-up campaign in school-aged children (5-15 years) at 75% coverage. Direct vaccination costs were estimated at a total of US\$ 3.50 per child for EPI and US\$ 4.00 per child for school catch-up based on literature and the recently approved rotavirus vaccine. We assumed a 1% case fatality rate for typhoid fever. The incremental cost-effectiveness ratio (ICER) was calculated in 2016 US\$ per disability-adjusted life year (DALY) averted. We defined strategies as highly cost-effective if the ICER was less than the GDP per capita of a low-income country (US\$1,035). Vi conjugate typhoid vaccines were highly cost-effective by routine immunization through EPI in settings with an annual incidence above 40 cases per 100,000, and for the combined strategy (EPI plus a catch-up campaign) in settings with an annual incidence above 100 cases per 100,000. The incidence threshold was highly sensitive to the typhoid-related case fatality rate, which contributed the majority of disease burden. Typhoid Vi conjugate vaccines would be highly cost-effective in low-income countries in medium incidence settings (40 annual cases per 100,000). These results were sensitive to case fatality rates, underscoring the need to consider rising antimicrobial resistance in the global vaccination strategy.

## 1982

### RICKETTSIAL INFECTIONS AS A MAJOR ETIOLOGY OF ACUTE FEBRILE ILLNESS: A PROSPECTIVE STUDY IN NORTHERN SABAH, BORNEO, EAST MALAYSIA

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Serosurveys have documented rickettsioses in Malaysia, with *Orientia tsutsugamushi* (OT, the agent of scrub typhus) most frequent in urban and spotted fever group rickettsiosis (SFGR) in rural areas. However, SFGR and related typhus group rickettsioses (TGR) are not nationally reported and OT rarely (2 in both 2014 and 2015, incidence of 0.1 cases/million). In Sabah, 1 OT case in 2015 suggests an incidence of 0.3 cases/million. Rickettsioses remain unsuspected and untreated by regimens for acute febrile illness (AFI). We enrolled patients in 2 district hospitals in northern Sabah, Borneo, East Malaysia (Kudat, 2014 est. pop. 86,634 and Kota Marudu, 2014 est. pop. 69,163) from Dec 2013-Jan 2015. Acute and convalescent (paired) sera were obtained from febrile (38°C) patients without malaria. Sera (convalescent and if positive, acute) were screened for IgG (1:80) to OT, SFGR (*Rickettsia conorii*), and TGR (*R. typhi*) by IFA and then titrated to 2,560. Unpaired and selected paired acute-phase sera were screened (1:40) for IgM by IFA. An IgG titer  $\geq 160$  defined seroprevalent infection. A 4-fold IgG titer rise to  $\geq 160$  confirmed acute infection; those IgM-positive alone were probable. Of 123 convalescent sera, 21, 8 and 4 had acute infections with OT, SFG, and TG (12, 1 and 3 seroconversions) and 21, 8, and 9, respectively, seroprevalent infections; none were IgM-positive. Of 148 unpaired acute-phase sera, 1 was IgM-positive for OT, 3 for SFG, and 5 for TG. Overall, 33/123 (27%) had confirmed acute rickettsioses and 38 (31%) seroprevalent infections; 71 (58%) had confirmed acute or past rickettsioses (42 [34%] OT, 16 [13%] SFGR, 13 [11%] TGR). For OT, this results in an adjusted incidence of ~141 cases/million population in the region studied, and in Sabah of 6.5 cases/million (23-fold increase from Ministry of Health estimates). Acute-phase IgM was insensitive for the detection of acute rickettsioses. Since distinguishing acute and past infections requires convalescent sera and rickettsioses require specific therapy, further prospective studies that include paired serology are needed to determine the toll of untreated rickettsioses across Malaysia.

### TIMING AND SPATIAL HETEROGENEITY OF LEPTOSPIROSIS TRANSMISSION IN NORTHEAST THAILAND

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Thailand experienced an explosive country-wide outbreak of leptospirosis in the late 1990s, followed by high endemic transmission. The key barrier to effective control has been a lack of knowledge about the factors driving the timing and spatial distribution of this persistent transmission. We obtained data on weekly leptospirosis incidence in the 320 districts of northeastern Thailand between 2000 and 2014 from the Thai passive notifiable disease surveillance system (R506). We modeled incidence using a spatiotemporally explicit Poisson model and first examined the effects of current and lagged rainfall and temperature (Thai Meteorology Department). We then collected data on environmental covariates—land use (Thai Land Development Department), livestock and irrigation (FAO), NDVI, NDWI, and elevation (Google Earth Engine)—and evaluated their effects on spatial variation in incidence. Between 2000 and 2014, 53,719 cases of leptospirosis were reported in northeastern Thailand. The timing of peak incidence varied between early August and mid-October and did not coincide with periods of rice planting or harvesting. Instead, weekly incidence was strongly associated with rainfall and temperature in the current and two prior weeks. Districts with high flooding propensity (NWDI, OR = 95.24 per 0.01 index point), a high percentage of rice paddies (OR = 1.057 per %), and low cattle density (OR = 0.98 per head) had significantly higher leptospirosis incidence. We also encountered significant spatiotemporal residuals in our model that appear to represent focal outbreaks. Our study found that rainfall and temperature, not specific events in the agricultural cycle, were the main determinants of peak transmission. We also identified specific environmental features associated with persistent high transmission which may serve as targets for prevention. However, in addition to this endemic pattern, outbreaks contribute to the burden of leptospirosis. Understanding the sources of these epidemics will be important for leptospirosis control in this region.

### 1984

### DNA AND RNA SEQUENCING-BASED METAGENOMICS FOR UNBIASED PATHOGEN DETECTION AMONG TANZANIAN ADULTS WITH UNDIFFERENTIATED FEBRILE ILLNESS

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In cohort studies of febrile illness in the tropics, conventional infectious disease diagnostics achieve a laboratory-confirmed diagnosis for only 40–50% of participants. We assessed the ability of unbiased next-generation sequencing-based metagenomics to identify an etiologic pathogen in Tanzanian patients with undifferentiated febrile illness (UFI, defined as the subset of febrile patients enrolled into our fever study who had no cough, diarrhea, stiff neck, or symptoms localizing to skin, soft tissue, joint or bone). Utilizing plasma samples from 65 UFI patients (median age 37 [interquartile range 23–45] years, 41 [63%] females) who had negative aerobic blood culture and negative malaria blood smear results, we performed RNA and DNA sequencing on the Illumina NextSeq and analyzed the sequencing output with Taxonomer, an ultra-rapid, web-based metagenomics data analysis tool. This unbiased metagenomics

approach detected an etiologic pathogen in 17 (26%) patients. *Plasmodium falciparum* was detected in 2 patients. Bacterial pathogens were detected in 8 patients: Enterobacteriaceae (n=3), Legionella spp. (n=2), and Mycobacterium sp., Pantoea sp., and Stenotrophomonas maltophilia (n=1 patient, respectively, for the latter 3 bacteria). Parvovirus B19 was detected in 7 patients. HIV was identified in 6 patients (subtype D, n=3; subtype C, n=2; subtype A1, n=1), and viruses of unclear pathogenicity were detected in 11 patients (anellovirus, n=7; and GB virus C, n=4). Independent molecular testing is underway to confirm the presence of each etiologic pathogen in the original plasma sample. Our findings indicate that among patients presenting with febrile illness in the tropics, unbiased metagenomics can increase the proportion of patients with a laboratory-confirmed diagnosis. Identification of unsuspected parvovirus B19 illness is consistent with findings from a metagenomics study of febrile illness in Kenya. Metagenomics pathogen detection has the potential to become an important diagnostic modality for epidemiologic surveillance of fever in the tropics.

### 1985

### SYSTEMIC INFLAMMATION AND NEURODEVELOPMENTAL OUTCOMES IN BANGLADESHI INFANTS GROWING UP IN ADVERSITY

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Hundreds of millions of children who grow up in poverty do not meet their full developmental potentials, which in turn affects their academic performance and future earnings. The identification of biomarkers that predict future neurodevelopmental outcomes offers a promising approach toward allowing for early identification of at-risk children. We previously published a study linking systemic inflammation to the neurodevelopment of children from a slum community in Dhaka, Bangladesh. We have validated our initial findings of systemic inflammation and neurodevelopment in a second cohort in Dhaka. We have also implemented advanced neuroimaging testing in these children via EEG, NIRS, and fMRI. We sought to determine whether elevated levels of the inflammatory markers CRP and soluble CD14 (sCD14) are associated with neurodevelopmental outcomes in Bangladeshi children. 422 infant-mother pairs from an urban slum in Dhaka, Bangladesh were enrolled at birth and followed prospectively. Inflammation was measured with sCD14, IL-1 $\beta$  and IL-6 at 18 weeks, and CRP at 6, 18, 40, and 53 weeks. Psychologists assessed cognitive, language, motor, and social emotional development using the Bayley Scales of Infant and Toddler Development at 78 and 104 weeks of age. We tested for the ability of inflammatory markers to predict developmental outcomes, independent of known predictors. Every 10 pg/mL increase in sCD14 was associated with a 1.1 to 2.0 decrement in cognitive and motor scores at 78 weeks and in all domains at 104 weeks. The cumulative number of CRP elevations that a child experienced in the first year of life, as well as IL-1 $\beta$  and IL-6 at 18 weeks of age, were also negative predictors of Bayley Scales results (all  $p < 0.05$ ). In conclusion, elevated CRP, sCD14, IL-1 $\beta$  and IL-6 were associated with lower neurodevelopmental outcomes. Our findings implicate a role of inflammation in the neurodevelopment of children growing up in adversity and identify a strategy to predict children at-risk for developmental impairment. Further studies are needed to identify cut-off levels of these biomarkers at which targeted interventions should be implemented.

## 1986

**SURVEILLANCE OF MALARIA AMONG UNITED STATES PEACE CORPS VOLUNTEERS USING ELECTRONIC MEDICAL RECORDS**

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The United States Peace Corps has conducted surveillance for malaria and other diseases among Peace Corps Volunteers (PCVs) since 1985. In 2015, the surveillance system was incorporated into an electronic medical records (EMR) system. Peace Corps Medical Officers (PCMOs), who provide local medical support, chart clinical diagnoses and report surveillance data in the EMR, which is also retrievable at headquarters. We evaluated the utility of this new integrated system for malaria surveillance. We administered an email survey targeting all PCMOs and conducted semi-structured phone interviews with seven headquarters staff working with the EMR. We assessed the utility of four surveillance case definitions for malaria, including their sensitivity and positive predictive value. We compared clinical information in the EMR for all malaria cases captured between January and June 2016. Of 131 PCMOs, 77 (59%) responded to the survey. Of 53 respondents who work in malarious nations, 98% believe that most PCVs contact them when concerned that they have malaria. However, there was a gap between the number of cases documented in the EMR charts and the number of cases reported through the surveillance system. Of 134 cases with a malaria clinical diagnosis between January and August 2016, only 58 (43% sensitivity) were reported to the surveillance system by PCMOs. Headquarters staff estimate that 60% of all malaria cases in the surveillance system are identified by PCMOs, while 40% are added during data cleaning. However, cleaning is labor intensive (requiring up to 50 hours per month). Among the 48 cases in the EMR identified as malaria during the review period, positive predictive value was 67%. The EMR is an important tool for surveillance of malaria among PCVs and, with refinements in data quality and case definitions, could serve as a model for other multinational organizations wishing to monitor and protect the health of their staff.

## 1987

**CHARACTERIZATION OF PHYSIOLOGICAL SIGNATURES OF PLASMODIUM INFECTIONS IN NONHUMAN PRIMATES USING A CONTINUOUS TELEMETRY SYSTEM**

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The clinical symptoms of malaria in naïve individuals has long been known to be associated with changes in multiple physiological parameters, such

as temperature and respiration. To better assess the clinical impact of malaria infections, an integrative analysis of multiple physiological signals was conducted using a stringent experimental animal model system as a part of the Host Acute Models of Malaria to study Experimental Resilience (HAMMER) project. Time series data including blood pressure, temperature, activity, and ECG was collected from nonhuman primates (*Macaca mulatta* and *Macaca fascicularis*) infected with *Plasmodium knowlesi* using customized telemetry implants. The customized telemetry devices allowed nearly uninterrupted *in vivo* collection of time series data during pre-infection, liver, and blood stages at high frequencies ranging from 1 to 1,000 Hz based on data type. This type of continuous data capture has not been previously used to study malaria infections. Multiple methods were used to conduct feature selection for pattern classification between stages of infection using the high-resolution time series data collected in the study. Our analysis reveals that there were perturbations to the physiological signals and circadian rhythms during the liver and blood stages. Changes in activity, temperature and ECG between pre-infection and the liver stage were detected highlighting novel characteristic physiological signals that may be unique to the liver stage development. However, the underlying physiological mechanisms remain unclear. Our study highlights and quantifies the association of various physiological signals with malaria progression in nonhuman primates providing the basis for a predictive model identifying the onset of malaria symptoms in humans.

## 1988

**A SPATIAL DECISION SUPPORT SYSTEM APPROACH TO IMPLEMENTING MALARIA SURVEILLANCE AS A CORE INTERVENTION ACTIVITY IN HIGH PRIORITY AREAS OF VIETNAM**

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A bespoke spatial decision support system (SDSS) was piloted in Phu Yen Province, Vietnam to support the implementation of malaria surveillance as a core intervention activity. This paper examines the development and user acceptance of SDSS surveillance tools to address key challenges to elimination in Vietnam and the Greater Mekong Subregion (GMS) including locating active malaria transmission, guiding targeted response interventions, and supporting the identification and investigation of suspected forest transmission sites and associated high risk populations. A customized SDSS was developed for three communes in Phu Yen. Village health workers conducted household geographic reconnaissance (GR) operations to map and enumerate all households in the study site. Detected malaria cases were recorded in the SDSS from 2015–2016 and auto-georeferenced to household residence locations. Case data were utilized in the SDSS to guide and map village level targeted response interventions. Remote area sleeping site surveys were also conducted during the study period and analyzed in the SDSS. User acceptability survey and in-depth interviews were conducted with SDSS stakeholders in March 2017. A total of 4,667 households with 17,563 people were mapped during baseline GR operations. During the study period, 128 malaria cases were reported and automatically mapped in the SDSS. Twelve village level targeted response interventions were conducted, testing a total of 872 people. Of those, 361 were investigated during remote-area sleeping site assessments. Intervention and remote-area sleeping site data were mapped and analyzed in the SDSS. Overall, very

high acceptability (100%) of the SDSS was reported by Vietnamese malaria personnel (n=16). Key areas identified for improvement included data collection form standardization and further training and support. The pilot SDSS provided an effective and acceptable operational tool for Vietnam's malaria personnel to implement the surveillance. Study findings call for further operational research, streamlining and wider rollout of SDSS surveillance approaches throughout Vietnam and the GMS.

## 1989

### CLIMATE VARIABILITY AND MALARIA TRANSMISSION IN ETHIOPIA: APPLICATION OF A NEW CLIMATE DATASET FOR DISTRICT-BASED MALARIA ELIMINATION STRATEGY

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In Ethiopia, the influence of climate variability on malaria transmission and relationship of El Niño Southern Oscillation (ENSO) with malaria epidemics in the past is well documented. The National Malaria Control Program (NMCP)/Ministry of Health has been collaborating with the National Meteorological agency (NMA) since 2001 and demanding better tools to use climate information for decision-making at local level. However, the use of climate information for decision-making was impractical due to the complex nature of climate and its association with malaria in Ethiopia. The recent impetus of researchers in developing a new climate dataset known as Enhanced National Climate Services (ENACTS), a collaborative initiative between the NMA and International Research Institute for Climate and Society (IRI), helped to address the expectations of NMCP for a malaria elimination strategy. This strategy relies on a strong surveillance system at the lower administrative and planning units (districts). Thus, a district-based malaria surveillance suite was developed to inform the malaria community about climate. The proxy surveillance suite for malaria is designed to support implementation of malaria elimination through online information on (1) significant trends in three distinct seasons (March-April-May, July-August-September, October-November-December); (2) current and recent rainfall (decadal and cumulative); (3) climate suitability for malaria transmission; and (4) probabilities of temperature and rainfall occurrence associated with ENSO events. The development of the current proxy suite is a vital step for NMA in addressing the expectations of malaria community.

## 1990

### IN-HAND, IN-FIELD, IN-TIME DATA: EFFECTIVELY DIRECTING MOP-UPS IN AN INDOOR RESIDUAL SPRAY CAMPAIGN

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Household-level interventions are necessary for many health and development challenges, and their success relies on informed decisions using timely and accurate data. Indoor Residual Spraying (IRS) is a key control intervention for malaria, and, with billions of dollars invested by the President's Malaria Initiative, is an enormous household level intervention. What's more, WHO recommends reaching 85% of households with IRS in a given area to successfully protect populations. Such significant investment and strict metrics for success, demand strategic technical approaches to optimizing implementation. mSpray, is an electronic data capture tool that improved data availability in the 2016 IRS campaign in Luapula Province. mSpray incorporates satellite enumeration in planning IRS, geospatial tracking in spray activities, and monitoring by district officers in daily planning. The key to mSpray's success is the use of

the technology in the field, in real-time, to uncover, correct, and respond to otherwise invisible enumeration errors. These corrections informed district officers in their daily planning for deployment of IRS spray teams. Officers reviewing coverage data for spray areas could see which spray areas had not reached adequate coverage and needed to be revisited. The number and success of revisits, then, acts as a proxy indicator for the effect of data-based decision-making. In 2016 in Luapula, 66.5% of spray areas were revisited. In these areas, spray effectiveness increased, on average, by an absolute 29.4 percentage points. This is incredibly significant in an intervention that requires reaching 85% coverage to deliver any effect. While mop-ups are recommended practice, they do add costs due to additional spray days. Additional investigation is needed to understand the cost-benefit of mSpray to guide decision making, as well as the costs of revisits and the level of communal protection additional spray coverage actually affords. This presentation will discuss the use of the tool, the data use culture it propagated, the results, and potential future and further applications.

## 1991

### TOWARDS INCORPORATION OF MALARIA CONTROL INTO PLANNING AND MANAGEMENT OF WATER INFRASTRUCTURE

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There is growing acceptance that dams generally lead to elevated malaria risk in Africa. However, the aggregate effect of such dams on malaria - as well as the variables that enhance or attenuate this effect - are not thoroughly understood. This report highlights our work identifying the cumulative impact of large dams on malaria at present, as well as future impacts in the context of climate change, in Africa. The report also identifies key risk factors for elevated malaria transmission around dams and outlines how such risk factors can strengthen approaches to water resources planning and management. Methodologically, the work overlays locations of large and small dam reservoirs with data from Malaria Atlas Project (MAP) to determine impacts in communities close to v. far from reservoir shorelines. Population projections and malaria incidence estimates were used to predict future population at risk of malaria around large dams in IPCC Representative Concentration Pathways (RCP). A set of additional attributes are then considered to determine the degree to which they explain evidenced levels of malaria transmission around the reservoirs. Such additional parameters will include: i) dam size, ii) average slope along reservoir shoreline, iii) poverty levels in surrounding communities, iv) level of malaria transmission stability in which a dam is located. Results show that: i) at least 1.1 million people contract malaria each year due to their proximity to reservoirs, and as many as 15 million are at risk, ii) the size of the currently-affected population is likely to triple in the current century as a function of population growth and climate change - without even considering effects of new dam construction. Ultimately, formulation of control strategies that optimally respond to the elevated risk of malaria around dams will rest on identification and comprehension of how various risk factors combine with the presence of water reservoirs to affect malaria transmission. This report therefore reflects on work underway that assigns importance to key risk factors and describes relationships according to which their impact is manifested.

1992

### BED NET EFFECTIVENESS VARIES BY INSECTICIDE ACROSS AFRICA: A LARGE, POPULATION-BASED OBSERVATIONAL STUDY

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Insecticide-treated bed nets (ITNs) are critical for malaria control, and the World Health Organization estimates 68% of cases averted between 2001 and 2015 are due to ITNs. Continued success of ITNs depends on the continued efficacy of insecticides. However, resistance to pyrethroids has emerged across Africa. Monitoring resistance and developing countermeasures presents challenges. For example, insecticide resistance is geographically heterogeneous, and monitoring activities occur at only a small number of sites, limiting generalizability to a country's population. Further, resistance monitoring is dependent on bioassays, which cannot detect mosquito behavioral changes, or control for other factors important to transmission. Here, we address some of these limitations. Our aim was to assess the effect of bed nets treated with different insecticides on malaria risk across sub-Saharan Africa. We used data from 168,118 children younger than 5 years of age from 33 Demographic and Health Surveys (DHS) and Malaria Indicator Surveys (MIS) across sub-Saharan Africa from 2009 to 2016. We coded bed net use according to the insecticide used in the net, and used mixed effects logistic regression to estimate the association between sleeping under a net treated with different insecticides and odds of malaria infection, adjusting for potential confounders and stratifying by country and year. The two most widely used insecticides are deltamethrin (n=45,401) and permethrin (n=31,111). Sleeping under a deltamethrin-treated net was associated with odds of malaria infection ranging from 0.45 (95% CI 0.25 - 0.80, Gambia 2013) to 1.12 (0.92 - 1.35, Burkina Faso 2010). For permethrin-treated nets, odds of infection ranged from 0.40 (0.18 - 0.88, Burundi 2012) to 1.23 (0.86 - 1.77, Nigeria 2015). In conclusion, bed net effectiveness depends on insecticide used, and varies between countries and over time. Government and non-governmental organizations need to consider which insecticide is used in making decisions regarding bed net distributions to reduce malaria transmission. DHS and MIS surveys are valuable tools that can aid in such decision-making.

1993

### PTEX COMPONENT EXP2 IS REQUIRED FOR PROTEIN EXPORT AND SMALL MOLECULE TRANSPORT ACROSS THE MALARIA PARASITE VACUOLE MEMBRANE

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Intraerythrocytic malaria parasites reside within a parasitophorous vacuolar membrane (PVM) generated during host cell invasion. Erythrocyte remodeling and nutrient acquisition requires export of effector proteins and import of small molecules across this barrier between the parasite surface and host cell cytosol. The Plasmodium translocon of exported proteins (PTEX) is crucial for protein export across the PVM, however the molecular basis of an observed PVM nutrient channel remains unknown. PTEX consists of three core proteins including the AAA+ ATPase chaperone HSP101 and two novel proteins known as PTEX150 and EXP2, hypothesized to serve a structural role and to form a membrane-spanning channel, respectively. While the critical importance of HSP101 and PTEX150 to protein export has been demonstrated, the contribution of EXP2 to parasite biology has remained obscure. To interrogate EXP2 function in *Plasmodium falciparum*, we employed the recently developed TetR-DOZI-apptamer system to achieve regulatable EXP2 translation. EXP2

knockdown revealed a critical role in protein export and blood-stage parasite survival. The expression timing and protein levels of HSP101 and PTEX150 are closely mirrored, peaking early in the ring stage. In contrast, EXP2 expression peaks in trophozoites, corresponding to an increased ratio of EXP2 to other PTEX components in the later part of the developmental cycle and implying a stoichiometry mismatch that suggests EXP2 may serve multiple roles in PVM biology. Accordingly, cell-attached patch clamp recordings on the PVM of extruded parasites revealed that the presence of the PVM nutrient channel is also dependent upon EXP2 expression levels. Importantly, inactivation of HSP101 blocks protein export but does not impact this channel. Collectively, our results suggest that EXP2 serves dual roles as a protein-conducting pore in the context of PTEX and as a channel to facilitate small molecule passage across the PVM independent of HSP101. Our data may indicate a seemingly unprecedented dual functionality for a pore operating in its endogenous context.

1994

### LINKING EPCR-BINDING PFEMP-1 TO BRAIN SWELLING IN PEDIATRIC CEREBRAL MALARIA

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Brain swelling is a key pathogenic process in pediatric cerebral malaria (CM), but the underlying mechanisms are incompletely understood. It is thought that sequestration in the brain microvasculature mediated by the variant parasite antigen PfEMP-1 is essential for disease. Prior var/PfEMP1 expression studies have identified a subset of parasites in severe pediatric malaria that bind endothelial protein C receptor (EPCR), a receptor on endothelial cells involved in blood-brain barrier regulation. We combined extensive clinical data, including MRI imaging, retinal funduscopy, and Pfhyp2-based estimates of parasite biomass, with var expression analysis and machine learning approaches to identify host and parasite factors associated with (1) stringently defined cerebral malaria [WHO criteria plus malarial retinopathy (Ret+CM)] and (2) pediatric CM cases with brain swelling. Increased brain volume/swelling was independently diagnosed and scored by two radiologists blinded to the clinical status of each case, and an onsite ophthalmologist performed retinal examinations to identify malarial-specific retinopathy. Using the aforementioned approaches, EPCR-binding PfEMP-1, high parasite biomass, and low platelet counts were strong indicators of Ret+CM and brain swelling. Of the EPCR-binding PfEMP-1 measured, CIDR $\alpha$ 1.1 (DC8) was highly expressed in Ret+CM cases, and CIDR $\alpha$ 1.7 (group A) had the strongest association with severe swelling and fatality. We then identified a CIDR $\alpha$ 1.7 domain expressed in brains of fatal (Malawian) CM cases that blocks EPCR's natural ligand (APC) from binding and inhibits the barrier protective properties of EPCR in human brain endothelial cells. Next generation sequencing of expressed var tags confirmed the contribution of CIDR $\alpha$ 1.7 in severe/swelling CM cases. Overall, our study suggests that *P. falciparum* interference with EPCR function is associated with Ret+CM and brain swelling in pediatric CM. This strengthens the rationale for targeting adjunctive treatments on restoring EPCR protective pathways in pediatric CM.

1995

### THE BONE MARROW AS A MAJOR RESERVOIR FOR *PLASMODIUM VIVAX* INFECTION

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*Plasmodium vivax* is a major cause of malaria morbidity within and outside of Africa. Malaria transmission stages, or gametocytes, appear in blood circulation 3-5 days after the first asexual parasites are detected microscopically, and therefore transmission can occur well before the patient is symptomatic. It is assumed that both *P. vivax* asexual and gametocytes stages do not require tissue sequestration for development and therefore are present in circulation throughout their cycle. However, this hypothesis has not been rigorously tested. We recently discovered a significant bone marrow and spleen reservoir for *P. falciparum* gametocytes and the rodent malaria parasite *P. berghei*, suggesting that the reticulo-endothelial system may represent a conserved parasite reservoir across *Plasmodium* spp. The goal of the present study was to systematically assess *P. vivax* circulation dynamics and sequestration sites both in the non-human primate model and in human infections. Comparative transcriptional analysis of *P. falciparum* versus *P. vivax* gametocytes demonstrated a conserved cascade of stage specific gene expression until maturity despite significantly different cycle length. A subset of conserved asexual and gametocyte stage markers was successfully validated by quantitative Real-Time PCR (qRT-PCR) and antibody assays in peripheral blood samples from infected Aotus monkeys. To investigate possible tissue specific sequestration of *P. vivax* parasites during infection, we performed detailed histological analyses of asexual and gametocyte stages across organs. Analysis across 13 infected primates provided strong evidence for major accumulation of both asexual and gametocyte stages in the hematopoietic system of the bone marrow. Parallel transcriptional analysis of parasites in patient blood samples confirmed absence of these stages in circulation. These data suggest that the bone marrow plays an essential role in proliferation and transmission of malaria parasites.

1996

### FUNCTIONAL CONSERVATION OF AN ESSENTIAL HOST CELL INVASION LIGAND BETWEEN *PLASMODIUM FALCIPARUM* AND *P. VIVAX*, AND A PLATFORM TO ACCELERATE VACCINE DEVELOPMENT.

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Malaria caused by *Plasmodium vivax* is a major global health problem and the predominant form of malaria in many regions. Knowledge of key molecular events of host-cell invasion by *P. vivax* to guide vaccine and therapeutic development is limited, and lags behind that of *P. falciparum*, partly due to an inability to readily culture *P. vivax* *in vitro*. Apical Membrane Antigen 1 (AMA1) is a leading *P. falciparum* vaccine candidate and an orthologue is present in *P. vivax*, however, whether its function is conserved, or how it differs, has not been established. We report that AMA1 of *P. vivax* can functionally complement *P. falciparum*

AMA1 in invasion of erythrocytes. We generated novel chimeric parasites in which Pf-AMA1 was replaced with PvAMA1 in *P. falciparum* parasites; we observed efficient invasion with no observable change in growth or invasion phenotype. Antibodies raised against recombinant PvAMA1 specifically inhibited invasion of the chimeric parasites expressing PvAMA1, confirming that PvAMA1 plays an important role in parasite invasion. Furthermore, this established the approach as a powerful platform to accelerate vaccine and drug development. We established that the interaction of AMA1 with RON2 protein is involved in invasion for both *Plasmodium* species, and identified key structural determinants for invasion. Results indicate significant molecular flexibility in the AMA1-Ron2 interaction that enables conserved function despite substantial sequence divergence. These insights open new opportunities for vaccine and therapeutic development against *P. vivax*.

1997

### NANO-SCALE IMAGING REVEALS HOST CELL REMODELLING AND KNOB ASSEMBLY MECHANISMS IN *PLASMODIUM FALCIPARUM*

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Survival of the human malaria parasite *Plasmodium falciparum* within the host relies on its ability to drastically alter its red blood cell (RBC) host. Parasite derived modifications of the RBC membrane skeleton alter the deformability properties of the RBC. These modifications underpin cytoadherence based virulence and severe disease states such as cerebral and placental malaria. This adhesion is driven by the assembly of a parasite derived structure at the RBC membrane skeleton called the knob which acts as a scaffold for the presentation of the major virulence antigen PfEMP1 at the RBC surface. The knob is composed of the knob associated his-rich protein (KAHRP) and together with PfEMP1 they form the virulence complex. In this work, we have defined the protein trafficking networks and the structural remodelling events that underpin parasite virulence. Using a mass spec based approach, we identify the protein composition of important PfEMP1 trafficking structures called Maurer's clefts. We GFP tagged 9 of the most abundant proteins and using an immunoprecipitation and mass spectrometry approach we assembled a comprehensive protein interaction network of proteins at the Maurer's clefts, which includes the virulence protein PfEMP1. We have developed a correlative light and electron microscopy technique, which combines dSTORM super resolution microscopy and scanning electron microscopy, to visualise host cell remodelling events. Using this technique, we have tracked the trafficking of KAHRP to the RBC membrane skeleton and its sequential assembly into donut shaped structures which insert into the membrane forming the knobs. Further to this we also track the delivery of PfEMP1 to the virulence complex.

1998

### ROLE OF THE AMP-ACTIVATED PROTEIN KINASE (AMPK) PATHWAY IN SCHISTOSOME DEVELOPMENT AND HOST-PARASITE INTERACTIONS

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Schistosomes are obligate parasites, exhibiting auxotrophy for many compounds that must be acquired from the host in order to support parasite growth and development. In addition to key nutrients such as fatty acids, cholesterol and glucose, schistosome development appears to

require other signals from the host, as parasite growth and reproductive capacity are significantly attenuated in certain lines of gene-targeted mice. We previously found that impaired growth and reproduction of *Schistosoma mansoni* in immunodeficient mice correlated with reduced expression and activity of the parasite cAMP-dependent protein kinase (PKA). As PKA is a central regulator of cell metabolism and schistosomes utilize a combination of glycolysis, fatty acid oxidation and oxidative phosphorylation to meet their bioenergetic needs, we hypothesized that schistosomes from immunodeficient mice might also exhibit alterations in signaling pathways that regulate energy metabolism. First, we show that adult schistosomes express an AMP-activated protein kinase (AMPK), a heterotrimeric enzyme that is central to regulating energy metabolism at the cellular level in other eukaryotes. Second, we provide evidence that expression of the catalytic alpha subunit is developmentally regulated during the life cycle. Third, we show that schistosome AMPK activity is sensitive to changes in the worm's environment, suggesting a mechanism by which schistosome metabolism may be responsive to host factors. Finally, we provide evidence that AMPK expression is attenuated in parasites isolated from immunodeficient mice, suggesting the host exerts significant influence over the regulation of schistosome energy metabolism. Studies are underway to determine how schistosome AMPK expression and activity are modulated by environmental signals. Elucidating how schistosome energy metabolism is regulated and how external factors influence parasite energy metabolism may reveal opportunities to disrupt transmission of these important pathogens.

## 1999

### ATYPICAL PHARMACOLOGY OF SCHISTOSOME TRPA1-LIKE ION CHANNELS

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Praiquantel is effectively the only drug currently available for treatment and control of schistosomiasis, and there is an urgent need for new antischistosomal. Ion channels are validated targets for current anthelmintics. Transient receptor potential (TRP) channels comprise a diverse family of ion channels that are critical for transduction of sensory signals, as well as a variety of other functions. However, the properties of TRP channels remain largely unexplored in schistosomes and other parasitic helminths. Schistosome genomes predict representatives of most TRP channel subfamilies, but not TRPV, the vanilloid receptor family involved in nociception, inflammatory signaling, thermoreception, and other sensory functions. Despite the absence of TRPV channels, both adult and larval schistosomes display dramatic hyperactivity in response to selective activators of mammalian TRPV1 such as capsaicin and resiniferatoxin. Capsaicin also induces rapid, long-lasting separation of paired adult males and females, perhaps indicating a nociceptive effect. Capsaicin-induced worm hyperactivity exhibits TRPV1-like pharmacology and is virtually eliminated by knockdown of SmTRPA, a schistosome TRPA1-like channel (other TRPA1 channels are not sensitive to capsaicin). The effect of SmTRPA knockdown is selective, with no significant effect on hyperactivity induced by serotonin. The TRPA1 activator AITC also elicits significant hyperactivity that is abolished by knockdown of SmTRPA1, indicating that SmTRPA exhibits mixed TRPV1/TRPA1-like pharmacology. Consistent with our results on whole schistosomes, both capsaicin and AITC elicit an influx of Ca<sup>2+</sup> in mammalian cells expressing SmTRPA, as measured by Ca<sup>2+</sup> imaging. These results indicate that at least one schistosome TRP channel has atypical pharmacological sensitivities that could potentially be exploited for selective targeting by new antischistosomal. We further hypothesize that some sensory functions normally mediated by the missing TRPV channels may be fulfilled instead by schistosome TRP channels from other subfamilies.

## 2000

### DECODING GONAD-SPECIFIC AND PAIRING-DEPENDENT GENE EXPRESSION IN *SCHISTOSOMA MANSONI* BY COMPARATIVE TRANSCRIPTOMICS DELIVERED MOLECULAR INSIGHTS RELEVANT FOR BASIC AND APPLIED RESEARCH

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Schistosomes are exceptional trematodes that live dioeciously. To achieve sexual maturation and reproduction, female worms have to be constantly paired with males. Although males are sexually mature before pairing, we assumed that the male-female interaction of schistosomes is a bidirectional process, which is supported by former studies investigating pairing-induced gene expression. However, not much is known about the complexity of transcriptional processes following pairing, especially with respect to the gonads. Based on a recently established organ-isolation approach, we performed comparative transcriptomics with RNA of ovaries and testes from both paired and unpaired adult *S. mansoni*. By RNAseq we identified >7,000 transcripts in the gonads of both sexes. Although transcript levels of the majority of these genes (4,100) were pairing-unaffected in both gonads, 243 (testes) and 3,600 (ovaries) transcripts occurred pairing-dependently. Among these, 309 and 42 differentially expressed genes (DEGs) showed ovary-specific and testis-specific transcription, respectively. Furthermore, 436 transcripts occurred testis-specifically but pairing-independently. KEGG pathway mapping confirmed roles of these DEGs in transcription/translational, cellular, and signal transduction processes as well as energy metabolism. Among others we identified genes with preferential or specific expression in the gonads, and whose functions are associated to stem-cells/neoblasts or neural processes. Comparisons to work on neuropeptidergic signaling in planarian showed interesting parallels but also remarkable differences highlighting the importance of the nervous system for flatworm gonad differentiation. In-depth analyses exhibited new aspects of GPCRs, which represent the largest receptor family in schistosomes. Furthermore, GPCRs are interesting as potentially druggable targets for novel intervention strategies that are urgently needed. Overall, our datasets reveal yet unknown aspects of schistosome reproductive biology and will be relevant for basic as well as applied, exploitable research initiatives.

## 2001

### THE MICROBIOME IN THE COURSE OF URINARY SCHISTOSOMIASIS AND INDUCED PATHOLOGIES

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Research on the molecular mechanisms influencing the outcomes of bladder pathologies by *Schistosoma haematobium* is urgently needed to develop new diagnostics, therapeutics and infection prevention strategies. We sought to determine the microbiome features and changes in urine during urogenital schistosomiasis and induced bladder pathologies. Study participants were recruited from Eggua, southwestern Nigeria and screened for schistosomiasis infection and bladder pathologies by urine microscopy and ultrasonography respectively. Microbiome sequencing, pre-processing of microbiome sequences and data analytics of microbiome sequence data collection were performed using relevant bioinformatic tools and analytical software. Seventy study participants (36 males and

34 females, between ages 15 and 65) were categorised into four groups according to status of schistosomiasis infection and bladder pathology. Data analytics of the next-generation sequencing reads revealed that Proteobacteria and Firmicutes dominate and influence microbiome structure of urine samples from non-infected persons and persons with urogenital schistosomiasis. Furthermore, gender and age could influence taxa abundance independent of infection or bladder pathology. Several taxa distinguished *Schistosoma haematobium* induced bladder pathologies from infection alone and from healthy persons, including known immune-stimulatory taxa such as *Fusobacterium*, *Sphingobacterium* and *Enterococcus*. Two of these significant taxa, *Sphingobacterium* and *Aerococcus* were projected as markers of infection, while several genera including potentially beneficial taxa such as *Trabulsiella* and *Weissella*, were associated with the non-infected. Finally, expected changes in protein functional categories were observed to relate to cellular maintenance and lipid metabolism. The urinary microbiome is a factor to be considered in developing biomarkers, diagnostic tools, and new treatment for urinary schistosomiasis and induced bladder pathologies.

## 2002

### HELMINTHS INDUCE PERSISTENT EPIGENETICALLY-MEDIATED PERTURBATIONS IN THE TUBERCULOSIS IMMUNE RESPONSE

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Helminth infection is associated with an increased rate of TB progression and decreased TB immunity but the mechanism and duration of helminth-induced immune perturbation remains uncertain. There is burgeoning knowledge that the epigenetic code, post-translational modifications of DNA and its surrounding chromatin structure, determines immune phenotype. Murine models show that Schistosoma-infected mice undergo epigenetically mediated immune perturbations. DNA methylation epigenetically silences immune function by coiling DNA into a transcriptionally inactive state. In a cohort of TB-exposed children, we performed urine and stool ova and parasite (O&P) exam, helminth PCR and serology to characterize participants' helminth status. DNA methylation of 94 immune regulatory genes was measured using methylation-sensitive endonuclease digestion followed by qPCR. Helminth and DNA methylation results were correlated with downstream flow-cytometry based immune profiling. Compared to uninfected controls, active or remote Schistosomiasis was associated with a  $\geq 20\%$  increase in DNA methylation of 4 pioneer or transcription factors as well as 4 surface receptors ( $p < 0.005$ ; Sidak-Bonferroni). The increased DNA methylation persisted for 6 months after empiric anti-helminth therapy. One intracellular protein and 1 transcription factor were hypo-methylated  $\geq 10\%$  compared to healthy-controls. Schistosoma infection and DNA methylation correlated with decreased mycobacteria induced CD4+ T cell production of IFN- $\gamma$ ; and TNF and increased CD4 IL-4 and PD-1 ( $p < 0.005$ ). In addition, schistosomiasis was associated with decreased Natural Killer cell (CD56+) perforin and increased monocyte IL-10 production ( $p < 0.0001$ ). In conclusion, Schistosomiasis induces persistent and profound epigenetic changes characterized by DNA hypermethylation that is correlated with a perturbed mycobacteria-specific immunity including decreased cell-mediated CD4+ T cells and Natural Killer cells with an increase in immune tolerant response in CD4+ cells and monocytes.

## 2003

### DICHOTOMOUS EFFECTS OF IL-4 AND IL-10 ON HUMAN SCHISTOSOMIASIS IMMUNE RESPONSES

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IL-4 and IL-10 have dichotomous effects on immune responses in human schistosomiasis and in other chronic inflammatory diseases. In general, IL-4 pathways are associated with resistance to infection with schistosomes, whereas those driven by IL-10 are associated with reduced protection. This dichotomy is reflected in immune responses, such as IgE regulation and CD23 expression by B cells, which are positively and negatively regulated by IL-4 and IL-10, respectively. We thus sought to define the global effects of IL-10 and IL-4 on B cell activation and differentiation to better understand the mechanisms of host immunity. Reflecting global effects on resistance to infection, we report that IL-4 and IL-10 have highly dichotomous effects on human B cells. Relatively high doses of IL-4 decrease expression of homing receptors associated with secondary lymph node entry and exit, including CCR7, CXCR5, and CXCR4, whereas IL-10 increased levels. In contrast, IL-4 increased molecules associated with enhanced antigen capture and antigen presentation, while IL-10 generally reduced these receptors. In addition, IL-4 exposed cells responded to *E. coli* LPS by increasing direct  $\mu$  to e class switch. Overall, these results suggest that *in situ* B cell differentiation, as well as T cell activation, may take place under conditions with high concentrations of IL-4. IL-4 appears to favor an environment for B cells to activate a higher repertoire of T cells through enhanced antigen capture and presentation towards a more complex response. In addition, IL-4 mediated rapid low affinity IgE production likely aids in the amplification of antigen capture and production of high affinity and broader range of IgE later in the response. In contrast, despite rapid migration to the lymph nodes, IL-10 may result in reduced local antigen capture, leading to an overall reduced affinity of IgE, which has been shown to be associated with susceptibility to schistosome infection.

## 2004

### DEVELOPMENT OF AN INTERLEUKIN-4-INDUCING PRINCIPLE OF SCHISTOSOMA MANSONI EGGS (IPSE)-SPECIFIC PCR PLATFORM TO QUANTIFY EGG BURDENS ASSOCIATED WITH SCHISTOSOMIASIS

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Schistosomiasis is a neglected tropical disease (NTD) affecting between 200-500 million people worldwide. The two species causing most human cases of schistosomiasis are *Schistosoma mansoni* and *S. haematobium*. The gold standard for diagnosis is detection of parasite eggs in stool using the Kato-Katz method. Counting eggs passed in stool is labor-intensive with poor sensitivity. Other alternatives like the circulating anodic (CAA) and cathodic (CCA) antigen detection assays do not quantify egg burdens, which remain an important indicator of individual morbidity associated with schistosomiasis. The interleukin-4-inducing principle of *S. mansoni* eggs (IPSE) is the most abundant secreted protein from schistosome eggs. We hypothesize that the mRNA transcripts of IPSE may be found in the stool, urine, body fluids, and host tissues, and that these transcripts can be specifically targeted as a quantitative molecular biomarker of egg burdens in individuals affected by schistosomiasis. Liver tissue and stool samples were collected from *S. mansoni* and *S. haematobium* infected mice and hamsters, respectively. A standard curve was generated by qPCR

amplification of IPSE mRNA from known concentrations of parasite egg RNA. The resultant proportional equation was applied to assess correlation between concentrations of parasite RNA in samples with egg counts by microscopy. Preliminary results showed a positive correlation between increasing concentrations of IPSE RNA and the number of eggs counted in both stool and liver tissue samples of *S. mansoni* infected mice. Our next steps are to optimize this assay using *Schistosoma haematobium* infected hamsters, and to develop this assay as a morbidity assessment tool in field settings.

## 2005

### ESSENTIAL ASPECTS OF RNA METABOLISM FOR *P. FALCIPARUM* BLOOD-STAGE SURVIVAL

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Post-transcriptional and translational regulatory mechanisms have been shown to play important roles in *Plasmodium* gene expression, particularly during the sexual stages and the mosquito-to-human host transition. The role of these mechanisms during the blood stage has been less certain though mounting evidence suggests their utilization, particularly for invasion-related genes that appear to be regulated in a just-in-time fashion at the protein level. We have recently achieved *P. falciparum* blood-stage saturation-level mutagenesis with the recovery of ~38,000 single-insertion *piggyBac* transposon mutants, allowing us to assign probability scores for the essentiality of nearly all *P. falciparum* genes based on whether or not the coding sequence could be disrupted. We found that the essential genome is highly enriched for RNA-metabolic processes; >40% of the previously identified *P. falciparum* blood stage RNA-associated proteome are highly likely to be essential, with essentiality scores of RNA-binding proteins associated with centers for RNA degradation (P-bodies), translational regulation (stress granules), the exosome (involved in RNA surveillance and processing), or splicing being among the top quartile of all *P. falciparum* genes. In addition to conserved RNA metabolism genes, we identified ~50 uncharacterized potential RNA-binding proteins with no human orthologs that are likely to be essential. We report further validation and characterization of one such putative RNA-binding protein via complementary molecular techniques. Taken together, these observations suggest the essentiality of post-transcriptional regulatory mechanisms to ensure the parasite's survival in the blood stage, and further interrogation of these putative essential regulatory proteins promises important insights into *Plasmodium* biology and possible avenues for intervention.

## 2006

### FUNCTIONAL ANALYSIS OF RED BLOOD CELL DETERMINANTS OF PLASMODIUM INVASION

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The two major malaria parasite species, *Plasmodium falciparum* and *P. vivax* have distinct red blood cell (RBC) tropisms: *P. falciparum* can invade RBCs of any age while *P. vivax* is restricted to invading the youngest RBCs known as reticulocytes. Parasites make use of invasion ligand proteins to target and bind to proteins on the RBC surface known as host receptors, many of which have yet to be identified. *P. falciparum* has two essential host receptors: basigin/BSG and CD55. There is only one known host receptor for *P. vivax*, the Duffy Antigen Receptor for Chemokines (DARC), but this gene does not mediate reticulocyte tropism. Reticulocytes and erythrocytes do not have nuclei precluding direct genetic analysis, therefore we exploited an immortalized cell line, JK-1, to produce nucleated RBCs (that we term jkRBCs) that enable production of *in vitro* genetic mutants to identify and validate candidate host receptors. We were able to enrich a synchronous population of jkRBCs using a combination of epigenetic modifiers and density gradient sedimentation. Via quantitative surface proteomics and flow cytometry we demonstrate that all known *P. falciparum* host receptors are found at levels comparable to RBCs, and that *P. falciparum* invasion into jkRBCs is on par with invasion into RBCs. In order to validate the requirement of DARC for *P. vivax*, we overexpressed this gene under the control of the  $\beta$ -globin promoter, resulting in >3-fold higher invasion using *P. vivax* isolates from Brazil and India. Using the CRISPR/Cas9 system, we have been able to generate gene knockouts in the JK-1 cells. We generated gene mutants for a candidate *P. vivax* host receptor, transferrin receptor/TFRC/CD71. Mutant TFRC JK-1 cells are strongly inhibited for *P. vivax* invasion but do not show any defects in *P. falciparum* invasion. In addition, we also observed that knockouts of BSG and CD55 had no effect on *P. vivax* invasion, suggesting that the two parasite species use different invasion pathways. We have identified reticulocyte-specific genes by quantitative surface proteomics and we are in the process of generating and screening gene knockouts in order to identify novel *P. vivax* host receptors.

## 2007

### METABOLISM AND WHIPWORM INFECTION: MTOR, AND THE LARGE NEUTRAL AMINO ACID SLC7A5, INFLUENCE RESISTANCE TO THE INTESTINAL DWELLING NEMATODE TRICHURIS MURIS

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*Trichuris trichiura* (whipworm) is a gastrointestinal dwelling nematode that infects approximately 500 million people worldwide. *T. muris* occurs naturally in mice and is very closely related to the human whipworm, making it a great model to dissect the immune response against the parasite. In wild type mice, infection with a high dose of *T. muris* eggs leads to resistance and worm expulsion, which is dependent on a CD4+Th2 response and production of interleukin (IL)-13. Parasitic infection places metabolic demands on the host and it is well established that metabolic changes are essential in promoting T cell activation and effector function. T cells up-regulate glycolysis, glucose and amino acid uptake to support the new metabolic demand. The large neutral amino acid transporter Slc7a5 has been shown to be necessary for activation of mTORC1, a nutrient/energy/redox sensor critical for T cell differentiation into effector cells. We have found that during the early stages of a high dose *T. muris* infection (up to day 21), mice that lack Slc7a5 in CD4+ T cells have significantly

delayed worm expulsion, impaired production of antibodies, and lower levels of IL-13. Their CD4+ T cells also have reduced glycolytic rates when compared to cells from cohoused infected wild type mice. However, at later stages of infection (day 30+), antibody and IL-13 levels recovered alongside the ability to expel parasites. The critical role of mTOR in CD4+ T cells was shown in mice lacking mTOR in CD4+ T cells that failed to expel a high dose of parasites and developed long term chronic infection. Also, *in vitro* incubation with mTOR inhibitors blocks the production of most cytokines (including IL-10, IL-13, IL-17A and IFN- $\gamma$ ) in CD4+ T cells from infected animals. Our study shows that mTOR is essential for the proper functioning of T cells during *T. muris* infection and importantly, that amino acid uptake by CD4+T cells can profoundly influence the development of effective resistance to this parasite.

## 2008

### BONE MARROW-DERIVED MONOCYTES MEDIATE HOST PROTECTIVE RESPONSES TO *TRICHINELLA SPIRALIS*

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Helminth parasites are amongst the most abundant of all chronic human infections. Protective immunity to helminths is associated with the development of CD4+ T helper type 2 (TH2) cell responses and production of type 2 cytokines. Recent studies have found that alternatively activated (M2) macrophages play a crucial role in promoting both anti-helminth immunity and wound healing responses following infection. However, the contributions of tissue-resident versus inflammatory monocyte-derived macrophages remain unclear. Here, we show that mice depleted of macrophages or bone marrow-derived monocytes exhibit reduced TH2 cytokine-mediated immunity to *T. spiralis*. Specifically, both the nonspecific depletion of macrophages via treatment with clodronate liposomes as well as the depletion of CCR2+ bone marrow-derived monocytes via a diphtheria toxin inducible system resulted in reduced TH2 responses, a loss of intestinal M2 macrophages, a dramatic decrease in weight, and increased mortality. This increase in mortality and morbidity was not the result of either increased gut pathology or liver dysfunction. However, mice depleted of CCR2+ monocytes exhibited increased expression of tumor necrosis factor (TNF)- $\alpha$  in their brains. In conclusion, these data suggest that CCR2+ bone marrow-derived monocytes perform essential host protective responses and regulate inflammatory cytokine production in the central nervous system following *T. spiralis* infection.

## 2009

### SINGLE-CELL RNA-SEQ REVEALS HIDDEN SIGNATURE OF SEXUAL COMMITMENT IN MALARIA PARASITES

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*Plasmodium falciparum* parasites have to balance continuous asexual replication within red blood cells with differentiation into non-replicating sexual stages, called gametocytes. Commitment to either fate is already determined during the preceding cell cycle that begins with invasion by a single, asexually-committed merozoite and ends, 48-hours later, with a schizont releasing newly formed merozoites, all of which are committed to either continued asexual replication or differentiation into gametocytes. Sexual commitment requires activation of *ap2-g*, the transcriptional master regulator of sexual development, from its epigenetically silenced state

during asexual replication. Expression of AP2-G during this "commitment cycle" poises gene expression in nascent merozoites to initiate sexual development through a hitherto unknown mechanism. In order to maintain a persistent infection, *ap2-g* expression is limited to a sub-population of parasites (1-30%, depending on genetic background and growth conditions). As sexually-committed schizonts comprise only a sub-population and are morphologically indistinguishable from their asexually-committed counterparts, defining their characteristic gene expression has been difficult using traditional, bulk transcriptome profiling. To determine the transcriptional changes induced by AP2-G within this sub-population, we applied highly-parallel, single-cell RNA sequencing (scRNA-seq) to malaria cultures undergoing sexual commitment. In this first application of scRNA-seq to eukaryotic pathogens, we surveyed over 19,000 single parasite transcriptomes from a conditional AP2-G knockdown (AP2-G-DD) line and NF54 wildtype parasites in multiple stages of development and found that sexually committed, AP2-G<sup>+</sup> mature schizonts specifically up-regulate additional regulators of gene expression, including AP2 transcription factors, histone modifying enzymes, and regulators of nucleosome positioning. These epigenetic regulators likely act to poise the expression of genes necessary for initiation of gametocyte development in the subsequent cell cycle.

## 2010

### PLASMEPSINS IX AND X ARE ESSENTIAL AND DRUGGABLE MEDIATORS OF MALARIA PARASITE EGRESS AND INVASION

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Proteases of the malaria parasite *Plasmodium falciparum* have long been investigated as drug targets. The *P. falciparum* genome encodes ten aspartic proteases called plasmepsins, which are involved in diverse cellular processes. Most have been studied extensively but the functions of plasmepsins IX and X (PMIX and X) were unknown. Here, we show that PMIX is essential for erythrocyte invasion, acting on rhoptry secretory organelle biogenesis. In contrast, PMX is essential for both egress and invasion, controlling maturation of the subtilisin-like serine protease SUB1 in exosome secretory vesicles. We have identified compounds with potent antimalarial activity targeting PMX, including a compound known to have oral efficacy in a mouse malaria model.

## 2011

### SPECIES SPECIFIC TRYPANOSOME RESISTANCE IS GOVERNED BY APOL1 VARIATION IN BABOONS

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Some primates maintain resistance to infection by *Trypanosoma brucei* via the protective effects of the primate-specific protein apolipoprotein L-1 (APOL1). APOL1 circulates in plasma on high-density lipoprotein complexes called trypanosome lytic factors that are endocytosed by the

parasites. Once inside the parasite, the APOL1 forms cation selective pores in membranes leading to ion dysregulation followed by osmotic lysis. The human infective *T. b. rhodesiense* and *T. b. gambiense* have independently evolved APOL1 resistance mechanisms. All screened baboon (*Papio spp.*) APOL1s lyse *T. b. rhodesiense* by evading the parasites resistance mechanism, whereas *T. b. gambiense* has variable resistance to baboon APOL1s. *Papio cynocephalus* baboons are susceptible to *T. b. gambiense* infection *in vivo*, while *P. papio* and *P. hamadryas* baboons are resistant. Whole genome sequencing has recently revealed that the APOL1 gene is highly polymorphic in the human population, with some African-specific variants of APOL1 providing increased resistance to trypanosomes. The differential response to *T. b. gambiense* infection among baboon species may be a result of APOL1 polymorphism in the baboon lineage. By analyzing baboon genome sequencing data, we have identified putative APOL1 sequences in all baboon species, revealing a high degree of APOL1 variation within the *Papio* genus. Indeed, recombinant *P. cynocephalus* APOL1 is significantly less lytic to trypanosomes *in vitro* than *P. hamadryas* APOL1, and transiently transgenic mice expressing APOL1 constructs that resemble *P. cynocephalus* APOL1 are more susceptible to infection than mice expressing *P. hamadryas* APOL1. These data suggest that APOL1 variance governs susceptibility/resistance to trypanosome infection in primate hosts.

## 2012

### TCMCS-PARASITEPRESSURE GAUGE: REGULATORY ROLE OF A MECHANOSENSITIVE CHANNEL IN *T. CRUZI* PHYSIOLOGICAL MECHANISMS

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*Trypanosoma cruzi* faces various environmental challenges as it propagates from an insect vector to a mammalian host during its life cycle. To cope with these environmental challenges, *T. cruzi* has developed robust compensatory mechanisms; however, the sensory machinery utilized to detect variations in the extracellular conditions remains unknown. In all cell types, mechanosensation is responsible for detecting changes in pressure, osmolarity, and fluidity of the membrane. In addition mechanosensation is associated with increase in biofilm formation, quorum sensing and activation of virulence-related genes. In *T. cruzi*, we have identified and characterized a mechanosensitive channel (TcMCS) that shares structural and functional traits with the small conductance mechanosensitive channel, MscS of *E. coli*. The presence of homologues suggests a conserved mechanism for osmoadaptation in other protozoan parasites. Single-channel electrophysiological characterization of TcMCS using *E. coli* spheroplast showed a tension-dependent activation within the range similar to bacterial MscS channels. A linear non-rectifying conductance of 0.4 nS suggests a pore that is permeable to ions, amino acids and compatible osmolytes that can be transported across membranes in the course of adaptation. Immunofluorescence studies show that TcMCS is differentially localized in the three main life stages of *T. cruzi* with localization on the contractile vacuole and the plasma membrane. Gene knockout mediated by CRISPR-Cas9 caused severe defects in morphology and impaired growth and infectivity of the parasites. Under hyposmotic stress, cells overexpressing TcMCS swell significantly less and knockout mutants swell significantly more and are not able to recover. Known mechanosensitive channel blockers including gadolinium, cause significant differences in the parasite's ability to detect and compensate its volume under osmotic stress. TcMCS knockdown mutants showed major defects in metacyclogenesis and intracellular replication. Overall, our results support the physiological role of TcMCS in *T. cruzi* osmoregulation and infectivity.

## 2013

### A NATURAL MOUSE MODEL FOR CRYPTOSPORIDIOSIS

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Cryptosporidiosis is a leading cause of diarrhea and an important contributor to global infant mortality. There are no efficacious drugs or vaccines available and our knowledge of *Cryptosporidium* biology to drive their development is scant. *Cryptosporidium* research is greatly hindered by the lack of a continuous tissue culture system and poor animal models. To develop a more facile mouse model of *Cryptosporidium* infection, we have isolated a strain of *Cryptosporidium tyzzeri* (*C. parvum* mouse genotype I) from naturally infected *Mus domesticus*; strains which we now maintain continuously in our animal facility. De novo assembly of the *C. tyzzeri* genome shows 96% overall nucleotide identity to *C. parvum* and *hominis* and a high degree of synteny. The highest burden of *C. tyzzeri* infection is found in the distal ileum of the mouse small intestine, yet there is also significant infection of the jejunum and duodenum, similar to what is seen in human cryptosporidiosis. Perhaps of greatest importance, *C. tyzzeri* produces significant infections in healthy C57/BL6 mice. These infections produce high parasite burden but are self-limiting, and mice that have cleared *C. tyzzeri* appear resistant to future infection. Using CRISPR directed homology repair we have genetically engineered *C. tyzzeri* strains to express reporter genes for *in vivo* imaging and localization. In summary, we now have access to a natural mouse model that closely resembles the human infection in which both host and parasite are genetically tractable. We envision this model will lead to better understanding of cryptosporidiosis susceptibility, resolution, and subsequent protection in the context of a functioning immune system.

## 2014

### THE TRICHOMONASVAGINALIS HOMOLOGUE OF HUMAN MACROPHAGE MIGRATION INHIBITORY FACTOR INDUCES THE PARASITE SURVIVAL DURING NUTRIENT STARVATION

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*Trichomonas vaginalis* is responsible for the most prevalent non-viral sexually transmitted disease worldwide, yet the mechanisms used by this parasite to establish and maintain infection are poorly understood. We and collaborators have previously identified a *T. vaginalis* homologue (TvMIF) of a human cytokine, the macrophage inhibitory factor (HuMIF). TvMIF has been shown to "mimic" HuMIF by activating signaling pathways stimulated by HuMIF to increase cell growth and inhibit apoptosis in human host cells. To interrogate a possible role of TvMIF in parasite survival during infection, we asked whether overexpression of TvMIF (TvMIF-OE) confers an advantage to the parasite under nutrient stress by comparing the survival of TvMIF-OE parasites relative to wild type parasites. We found that TvMIF-OE survive significantly better than wild type parasites, when starved of serum, the source of lipids and other nutrients required by the parasite. We also observed that addition of exogenous recombinant TvMIF increases the survival of the TvMIF-OE parasites in the absence of serum. Recombinant HuMIF likewise increases the parasite survival in the absence of serum, indicating that the parasite may hijack this host survival factor to resist its own death. We hypothesize that TvMIF inhibits parasite apoptosis under stress conditions to maximize survival awaiting more favorable conditions. Using two methods to measure apoptosis, TvMIF-OE parasites were found to undergo less apoptosis than wild type parasites grown without serum. Reactive oxygen species (ROS) were also found to

be inhibited by the overexpression of TvMIF under serum starvation. We are currently attempting to identify the pathway(s) activated in *T. vaginalis* by TvMIF to compare these with known apoptosis or other signaling pathways. These studies are likely to reveal both similarities and differences in survival mechanisms used by this unicellular eukaryotic parasite and its multicellular human host.

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