on the IC50 was found with PYR (71% of isolates with an IC50 > 2000 nM), followed by MEF (35.5% with an IC50 > 30 nM), LUM (22.6% with an IC50 > 150 nM), CQ (22.6% with an IC50 > 100 nM) and DHA (16.1% with an IC50 > 12 nM). In contrast, only 6.5% of the *P. falciparum* isolates had an IC50 > 60 nM for AMQ, an IC50 > 800 nM for QN or an IC50 > 30 nM for ATM. The 71% frequency of *P. falciparum* isolates with Pyrimethamine IC50s > 2000 nM indicates this resistance is common in Dioro and suggests that PYR resistance may limit the efficacy of IPTp for pregnant women because IPTp is based on preventive treatment with SP during pregnancy. In addition, the 22.5% frequency of isolates resistant to LUM poses a threat to ACTs with LUM as the partner drug and potentially increases the risk of late recurrences after the initial parasite clearance due to the artemisinins.

1499

COMBINATORIAL GENETIC MODELING OF PFCRT-MEDIATED DRUG RESISTANCE EVOLUTION IN *PLASMODIUM FALCIPARUM*

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The emergence and spread of drug resistance poses an ongoing threat to the effective treatment and control of *Plasmodium falciparum* malaria. A critical parasite determinant is PfCRT, the primary mediator of CQ resistance (CQR) and a pleiotropic modulator of susceptibility to first-line artemisinin-based combination therapy (ACT) partner drugs. Aside from the validated CQR molecular marker K76T, *P. falciparum* parasites have acquired at least three additional pfcrt mutations, whose contributions to resistance and fitness have remained elusive. Focusing on the quadruple-mutant Ecuadorian PfCRT haplotype Ecu1110 (K76T/A220S/N326D/I356L), we genetically modified the pfcrt locus of isogenic, asexual blood stage *P. falciparum* parasites using zinc-finger nucleases (ZFNs), producing all possible combinations of intermediate pfcrt alleles. Our analysis included the related quintuple-mutant PfCRT haplotype 7G8 (Ecu1110+C72S) that is widespread throughout South America and the Western Pacific. Drug susceptibilities and *in vitro* growth profiles of our combinatorial pfcrt¬-modified parasites were used to simulate the mutational trajectories accessible to parasites as they evolved CQR. Our results uncover unique contributions to parasite drug resistance and growth for mutations beyond K76T and predict critical roles for the CQ metabolite monodesethyl-chloroquine and the related quinoline-type drug amodiaquine in driving mutant pfcrt evolution. Modeling outputs further highlight the influence of parasite proliferation rates alongside gains in drug resistance in dictating successful trajectories. Our findings suggest that *P. falciparum* parasites have navigated constrained pfcrt adaptive landscapes by means of probabilistically rare mutational bursts that led to the infrequent emergence of pfcrt alleles in the field. We recently extended this in an analysis of pfcrt resistance alleles that distinguish the evolution of CQR in Asia and Africa.

1500

COMPARISON OF HIGH RESOLUTION MELT (HRM) ANALYSIS TO TA CLONING AND SEQUENCING FOR THE ANALYSIS OF A CLINICAL TRIAL USING AN INVESTIGATIONAL AMINOQUINOLINE, AQ-13, TO CIRCUMVENT CHLOROQUINE RESISTANCE IN SUBJECTS WITH UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN MALI

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Chloroquine resistance, which was first described in Southeast Asia and South America, has now complicated malaria control for more than 50 years. To address this problem, we have developed an analogue of chloroquine (CQ) which is active *in vitro* against CQ-resistant parasites and is safe in human subjects (AQ-13). To test the efficacy and therefore the potential clinical value of this investigational antimalarial, it has been compared for efficacy with the current first-line treatment for patients with uncomplicated *Plasmodium falciparum* malaria (Coartem = Artémether + Luméfantrine, A+L) in a randomized, blinded clinical trial. As part of that process, the efficacy of A+L and AQ-13 has been examined in subjects infected with CQ-resistant vs. CQ-susceptible parasites, based on the K76T single nucleotide polymorphism responsible for CQ resistance. The HRM analyses performed in Mali have shown that parasite isolates obtained from subjects in both groups had specimens with only K76 parasites, only T76 parasites and mixtures of K76 and T76 parasites. Because all subjects in the A+L and AQ-13 treatment groups cleared all asexual parasites from the blood within 3 days, both A+L and AQ-13 were efficacious against CQ-resistant and CQ-susceptible parasites. Based on that information, these samples are now being cloned and sequenced in order to compare the codon present at position 76 of the *Plasmodium falciparum chloroquine resistance transporter* gene (*pfcrt*) in these samples to the results of HRM analyses for the same samples. We anticipate that these results will be available within 2 months and that they will add to the information now available on comparisons of HRM with sequencing for important loci such as position 76 of *pfcrt*.

1501

ARTEMISININ-COMBINATION THERAPY VERSUS CHLOROQUINE FOR THE TREATMENT OF *PLASMODIUM MALARIAE* IN SABAH, MALAYSIA: A RANDOMIZED CONTROLLED TRIAL

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Background. Human infection with *Plasmodium malariae* is uncommon but remains present in the Asia-Pacific region, Africa and South America, and can cause severe anaemia. There have been no previous randomised trials to evaluate the optimal treatment for uncomplicated malaria due to *P. malariae*. Methods. An open-label, randomised controlled trial was conducted at three district hospitals in Sabah, Malaysia. Patients aged 1 year or older with uncomplicated *P. malariae* on screening microscopy were randomly assigned to receive oral artesunate–mefloquine (ASMQ; target dose 12 mg/kg artesunate and 25 mg/kg mefloquine) or chloroquine (CQ; target dose 25 mg/kg). The primary endpoint was parasite clearance at 24 h. Analysis was by modified intention to treat. Secondary analysis

incorporated additional patients separately randomised to artemether-lumefantrine (AL) or CQ. Findings. Between Jan 14, 2013, and Sep 20, 2014, admitted patients with PCR-confirmed *P. malariae* infection were allocated treatment with either ASMQ (n=6) or CQ (n=4). 24 h after treatment, we recorded parasite clearance in 3 (50% [95% CI 12-88]) of 6 patients in the ASMQ group versus none (0% [45–64]) of 4 patients in the CQ group (p=0.091). At 48 hours all ASMQ treated patients were negative for parasites versus none in the CQ arm (p=0.002), with this difference remaining at 72 hours with only 1 patient (25% [0-81]) in the CQ arm demonstrating parasite clearance (p=0.011). Fever clearance appeared faster in the ASMQ arm (median 6 hours [0-18]) versus 18.8 (3.8-42) following CQ (p=0.319). 1 patient in the ASMQ arm developed anaemia at day 28 during follow-up compared to none in the CQ arm (p=0.389). All patients had an adequate clinical and parasitological response to treatment at day 28 of follow-up. There were no serious adverse events due to either study medication. Results were consistent with the larger secondary analysis of patients treated with either ASMQ or AL (n=10) vs CQ (n=10). Interpretation. Artesunate–mefloquine demonstrated a rapid therapeutic response for *P. malariae* malaria, supporting a unified ACT treatment policy for for all *Plasmodium* species in co-endemic areas.

1502

THE BLOOD SCHIZONTICIDAL ACTIVITY OF TAFENOQUINE IS IMPORTANT FOR ITS PROPHYLACTIC EFFICACY

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Tafenoquine is being developed by the US Army and 60 Degrees Pharmaceuticals for malaria prophylaxis. Recent retrospective analyses of clinical data from the Phase II/III program suggest that tafenoquine at the intended dose exhibited similar efficacy to mefloquine, with 100% PE in non-immune subjects and 93% PE in semi-immune subjects, as reported previously. It has been generally assumed that the prophylactic efficacy of tafenoquine was due to the known causal (elimination of developing Pv or Pf hepatic schizonts) or antihypnozoite (killing of latent Pv liver forms) modes of action of the drug. This has led to the suggestion that because of the known association of primaquine relapses with cP450 2d6 polymorphisms, individuals with genetic polymorphisms may also be at greater risk from contracting symptomatic malaria while taking tafenoquine. However, non-clinical studies demonstrating blood schizonticidal effects the similarity of field of efficacy of tafenoquine to mefloquine (per above), the apparent lack of association of *P. vivax* relapses with cP450 2D6 polymorphisms (as reported previously), and findings from clinical and non-clinical studies suggesting merozoite escape from the liver (as reported previously) imply an alternate hypothesis: Tafenoquine also exhibits blood schizonticidal effects that may be important for its prophylactic efficacy in humans.

1503

EVALUATION OF *PLASMODIUM FALCIPARUM* ARTEMISININ RESISTANCE IN WESTERN THAILAND AS PART OF A DOD MULTI-CENTER TRIAL II

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Artemisinin-resistant *Plasmodium falciparum* threatens the effectiveness of all artemisinin-based combination therapies. A multi-center artesunate-mefloquine (A+M) efficacy trial is on-going in three US DoD laboratories in Peru, Kenya, and Thailand, to compare parasite clearance rates at 72 hours after artesunate initiation. Here we report the results of the Thailand site in Sangkhlaburi district near the Thai-Myanmar border. Participants received 4 mg/kg artesunate at 0, 24, and 48 h, 15 mg/kg mefloquine at 72 h, and, at 84-96 h, 10 mg/kg mefloquine plus 0.5 mg/kg primaquine for transmission blocking. To investigate presence of artemisinin resistance (ART-R) by WHO criteria, we calculated parasite clearance half-life (PC1/2), by microscopy per-formed every 4h for the first 12h after first artesunate dose and every 6h for 72h or until two consecutive negative smears. Mutations in the Kelch propeller gene (K13) and 42 day efficacy outcomes were also assessed. Between Oct 31, 2013, and Oct 7, 2015, we enrolled 48 subjects 46 of which were evaluable for at 72h. Of these, 33 (72 %) had K13 wild type (WT) genotypes while 13 subjects (28%) harbored K13 mutations at enrollment, including the commonly de-tected C580Y mutation. The median PC1/2 of the mutant K13 group was significantly longer than the K13 WT group (4.91h 95% CI 4.4-6.5 vs. 3.3h, 95% CI 2.9-4.2, p=0.0003), thus meeting the WHO definition of confirmed ART-R. We also found polymorphisms the MAL genes associ-ated with delayed parasite clearance in nine subjects, including a single subject with both K13 and MAL mutations. Despite this, 100% of subjects achieved adequate clinical and parasitologi-cal response (ACPR) at 42 days. While our data suggest that clinical effectiveness of the tradi-tionally employed 3 day A+M regimen has yet to be compromised, ART-R is now confirmed in this area of western Thailand.

1504

PREVENTION OF MALARIA IN PREGNANCY: QUANTIFICATION OF TARGET CONCENTRATIONS OF DIHYDROARTEMISININ - PIPERAQUINE

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In a recent randomized trial comparing intermittent preventive therapy (IPTp) regimens to prevent malaria in 300 pregnant women in Tororo, Uganda, dihydroartemisinin-piperaquine (DP) given once a month (DPqm) or every 2 months (DPq2m) was superior to sulfadoxine-pyrimethamine (SP) given every 2 months (SPq2m), and DPqm was superior to DPq2m for several outcomes. The aim of this analysis was to quantify preventive (target) concentrations of piperaquine (PQ) using population pharmacokinetic (PK) –pharmacodynamic (PD) analysis, with the presence of circulating malaria parasites during pregnancy (PD endpoint) detected by a highly sensitive loop-mediated isothermal amplification (LAMP) assay. All women contributed longitudinal PD data (2260 observations obtained at monthly routine visits and at the time of clinical malaria), and women receiving DP contributed longitudinal PK data (1293 venous and capillary measurements obtained at monthly routine visits). Nonlinear mixed effect modeling was used for joint continuous (population PK) data and repeated binary measurements (LAMP) analysis. The probability of parasitemia was 46% (relative standard error RSE, 39%), 22% (RSE 9%) and 11% (RSE 5%) in the SPq2m, DPq2m and DPqm arms, respectively. More frequent DP dose (DPqm) was associated with absence of malaria parasites ($p<10^{-21}$), however PQ plasma levels were far superior to DP dose interval ($p<10^{-95}$) as a predictor of parasitemia; PQ plasma concentrations of 5.5 ng/mL, 8.7 ng/mL and 13.3 ng/mL were found to provide 95%, 99% and 99.9% protection from parasitemia. Modeling target concentrations, 90% of women in the DPq2m arm, but only 15% of those in the DPqm arm had PQ levels below 5.5 ng/mL for at least 25% of time receiving IPTp. Population clearance of PQ in Ugandan women was 3140 L/day (RSE 6%), with modest between subject variability (CV % 35 (RSE 13%). There were no changes in PQ PK during the course of pregnancy. In conclusion, our

analysis provides evidence to define the plasma level of PQ that prevents malaria parasitemia during pregnancy, and offers a rational framework for further optimization of dosing strategies of DP in IPTp regimens.

1505

USING POLYPHARMACOLOGY TO IDENTIFY NOVEL DRUGS AND DRUG TARGETS AGAINST MALARIA INFECTION

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Malaria control and eradication efforts are hindered by drug resistance, difficulty targeting liver-resident hypnozoites and a prolonged process of de novo drug development. Chemotherapeutics which target host proteins within liver-stage infected hepatocytes have the capacity to overcome each of these roadblocks. We have recently demonstrated that targeting the hepatocyte P53 and Bcl-2 pathways can eliminate multiple species of Plasmodium parasites, including *P. falciparum*, from the liver. To expand the range of host-targeted drugs, we have applied a systems biology approach that identifies critical host kinases for the development of Plasmodium liver stage. Our approach takes advantage of 37 broad-spectrum kinase inhibitors and their measured activity against 301 human kinases. By assessing the efficacy of these 37 inhibitors against liver stage infection, and training a model based on machine learning algorithms, we predicted 33 kinases with the largest role in liver stage infection. These kinases include several Receptor Tyrosine Kinases, and members of the Protein Kinase C cascade. This also includes five out of eight of the kinases identified by Prudêncio and colleagues in their siRNA screen (P=0.0005) for host kinases involved in liver stage infection. Moreover, this platform allows for the prediction of novel kinases inhibitors, including those already tested in the clinic for other indications. If effective, these compounds might provide a rapid path to new anti-malarials for prophylaxis and radical cure. Since this approach identifies key signaling networks from a condensed data set, it's compatible with freshly isolated parasites from the field, as well as *in vivo* screening approaches. Our ongoing efforts include applying this approach to rapidly identify novel inhibitors against a variety of intracellular parasites and bacteria and also to emerging infections such as Dengue and Zika viruses.

1506

DOXYCYCLINE TARGETS THE BACTERIA-LIKE SMALL SUBUNIT RIBOSOMAL RNA IN THE *PLASMODIUM FALCIPARUM* MALARIA PARASITE

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Doxycycline is a tetracycline-class antibiotic that is highly effective for malaria chemoprophylaxis and is also used in combination with quinine for malaria treatment. In bacteria, like other tetracyclines, targets of doxycycline include the small subunit ribosomal RNA (SSrRNA) molecule and proteins within the 30S small subunit ribosome, which are involved in protein synthesis. However, the mechanism of action of doxycycline against the malaria parasite is uncertain. Evidence suggests that doxycycline inhibits protein synthesis within the plasmodial apicoplast, whose extranuclear genome contains homologues to bacterial genes. As a means to identify a molecular target(s) and the mechanism of action in *Plasmodium falciparum*, we attempted to select for doxycycline resistance. After several months of *in vitro* culture under continuous incremental doxycycline pressure, we generated resistant parasites. Clonal parasite lines showed stable and significant increases in the IC_{50} to doxycycline, determined by a 96-hour growth inhibition assay necessary to capture the slow acting schizonticidal activity of the cyclines. Sanger sequencing of the apicoplast small subunit ribosomal RNA gene (*pfssrRNA*; PFC10_API0057) revealed novel SNPs in resistant parasites. In the absence of drug pressure, doxycycline resistant parasites exhibited slower growth compared to controls, suggesting a fitness cost accompanies resistance. Our results suggest that in malaria parasites, doxycycline targets the plastid-encoded, bacterial-like SSrRNA. Similar to the action of cyclines against bacteria, doxycycline may prevent binding of an incoming aminoacyl-transfer RNA to the A site of the ribosome and thus block the elongation step of protein synthesis in the parasite apicoplast.

1507

ENHANCING TRANSLATIONAL SIGNIFICANCE OF *PLASMODIUM FALCIPARUM* MOUSE MODEL

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Malaria continues being a major global disease and current therapies are threatened by spread of resistant parasites. This situation has prompted antimalarial community to identify new molecules suitable to be used as part of new treatments that can overcome current issues. The last few years have seen unprecedented progress in the identification and early clinical testing of novel antimalarial drug candidates. However effective progression of candidate antimalarials depends on selecting optimal dosing for clinical studies. Understanding and determining efficacy parameters using preclinical models is critical to estimate effective human doses. One of the most important parameters is to estimate the parasite reduction rate (PRR) to determine how long efficacious drug concentrations should be present to fully eliminate blood parasites and cure the patients. Significant advances have been done in this field during the last years with the development of *in vitro* assays that can determine the killing profile of antimalarial compounds (1). However, a quantitative *in vivo* assay to determine rate of parasite killing was missing. We have adapted the *Plasmodium falciparum* mouse model protocol to allow determining simultaneously rate of clearance and killing *in vivo* by antimalarial drugs. These studies provide invaluable results that can be used for a robust estimation of PRR in patients to inform human dose predictions. Such data can inform clinical trials required for effective deployment of novel antimalarial treatments.

1508

DETECTING ANTIMALARIALS IN BLOOD FROM COMMUNITY SURVEYS IN TANZANIA

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The assessment of the impact of diagnostic or treatment strategies on antimalarial drug use often rely on histories of drug intake in community surveys. Estimating accurately the levels of circulating antimalarials in a population allow an unbiased measure of drug consumption. Here, we explored the relationship between endemicity, health facility practices and blood drug concentration in malaria-endemic communities. The study took place in three regions of Tanzania (Mwanza, Mbeya and Mtwara) in

2015. In each region, surveys were conducted in three districts. All health facilities from randomly selected wards were visited to assess treatment and diagnosis practices. Information on demographics, health seeking behavior and drug use was obtained through community surveys, while health facility based surveys collected information on diagnosis and treatment practices. Finger-prick blood samples were obtained for on-site testing as well as for collecting samples on filter paper. Antimalarial blood concentration including Lumefantrine was measured later by LC-MS/MS. Parasite prevalence was 20% (506/2463) in Mwanza, 4% (84/1985) in Mbeya and 26% (559/2152) in Mtwara. Participants with any antimalarial in the blood were 34% (844/2463), 20% (399/1985) and 26% (554/2152) respectively in Mwanza, Mbeya and Mtwara. Individual who tested positive with a RDT had a marginally higher frequency of being detected with an antimalarial (29.7% vs 26.8%). Indeed, the association of RDT positivity and presence of drug was relatively poor (OR=1.15, 95% CI:1.00-1.33). Results from health facilities showed that amongst the febrile patients, 67% (151/226) were tested for parasite. Prevalence of persons with residual antimalarials in the blood was relatively high, especially in Mbeya where endemicity was low. This indicates poor use of diagnostic testing and inappropriate prescription of antimalarials. This might result in high levels of circulating drug in a population, probably the most important driver of the development of drug resistant pathogens. Effort should therefore be made to reduce these poor practices and prevent emergence of resistance.

1509

ACTIVE DETECTION OF ASYMPTOMATIC MALARIA BY LOOP MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) IN NORTHWEST ETHIOPIA

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Despite the scale-up of universal intervention measures, asymptomatic malaria is one of the challenges that needs to be addressed in malaria endemic sub-Saharan Africa. Due to the poor sensitivity of common laboratory diagnosis methods of malaria like rapid diagnosis tests (RDTs) and microscopy, field deployable rapid molecular techniques need to be available to accurately diagnose malaria. This pilot study aimed to assess the performance of loop mediated isothermal polymerase amplification (LAMP) for the detection of asymptomatic malaria in North Gondar, Ethiopia. A community based cross-sectional study was conducted from February to May 2014 in North Gondar, Ethiopia. A total of 802 study participants were enrolled. Data on socio-demographic profile and associated risk factors for asymptomatic malaria were collected using interview-based questionnaire. Capillary blood was collected and blood films and dried blood spots (DBS) were prepared for malaria parasite detection with giemsa microscopy, nested polymerase chain reaction (nPCR) and LAMP using a non-instrumented nucleid acid amplification (NINA) device. In this study, 45.3% of the study participants had access to combined universal intervention measures of malaria. LAMP and nPCR were performed on 160 DBS samples. The overall prevalence of asymptomatic malaria using giemsa microscopy, LAMP and nPCR was 3.75%, 5% and 4.375%, respectively. In conclusion, LAMP is able to identify two extra asymptomatic malaria carriers per 100 study population. This study indicated that active diagnosis of asymptomatic malaria with low-cost techniques like LAMP can support malaria elimination through enhanced active case detection.

1510

EVALUATING THE COSTS AND COST-EFFECTIVENESS OF MICROSCOPY COMPARED TO LAMP USED DURING REACTIVE CASE DETECTION IN ACEH PROVINCE, INDONESIA

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Reactive case detection (RACD) is an active surveillance method where households and neighbors of a passively identified case (an index case) are screened to identify and treat asymptomatic infections with the goal of reducing further transmission. Despite being a resource-intensive activity, RACD is recommended and implemented widely in malaria-eliminating countries. However, little is known about the cost to conduct RACD and the cost effectiveness of the various diagnostic methods used during RACD to identify otherwise undetected cases. The aim of this study was to determine the cost of conducting RACD, and compare the cost effectiveness of microscopy (currently standard practice) to the more sensitive loop mediated isothermal amplification (LAMP) as a diagnostic method. The study site included five subdistricts in Aceh Besar District, Aceh, an eliminating province located on the westernmost tip of Indonesia. Costs and effects were recorded for a 20 month study period between May 2014 and December 2015. The cost of all inputs used in conducting RACD (capital, consumables, personnel, services) was recorded. A total of 38 passively-detected malaria cases were recorded during the study period. Using standard procedures, these index cases were traced back to their community and an additional 1,495 individuals were screened. The average cost of conducting RACD using microscopy and LAMP was $2,481 per event with personnel being the main cost driver. The average cost of screening each individual during RACD was $46.96, with diagnostic testing costs $0.54 and $12.26 per person for microscopy and LAMP, respectively. Among the actively screened people, two new cases were detected by microscopy, confirmed by LAMP, and an additional four cases were detected only by LAMP. The incremental cost effectiveness ratio of LAMP versus microscopy was estimated to be $4,383 per case detected. LAMP was more costly, but more sensitive for the detection of additional cases in RACD and may play an important role in detecting and treating the asymptomatic reservoir and reducing onward transmission in eliminating settings.

1511

A FIELD-BASED POINT-OF-CARE ASSAY TO DETECT ANTIMALARIAL DRUGS FROM FINGERSTICK BLOOD SAMPLES

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Malaria parasites with reduced sensitivity to several of the current first-line antimalarial drug therapies - artemisinin-based combination therapies (ACTs) - have recently emerged in Cambodia. Preventing the spread of drug resistant parasites through Southeast Asia and to Africa is a top priority for global malaria elimination campaigns. The ability to detect these small molecule drugs in malaria patient samples at the point-of-care would allow healthcare workers to identify previous treatment failures and adjust future treatment to improve efficacy and reduce the spread of resistant parasites. A simple, point-of-care assay to detect ACT partner

drugs would also allow for real-time mapping of drug use and distribution. We aim to develop a low-cost, field-based test to detect several slow-clearing ACT drug compounds from fingerstick blood samples. We will select drug specific aptamers via an inverted SELEX protocol in which we immobilize a DNA library and isolate structure-switching sequences that are released upon binding drug target in solution. Our assay will filter out blood cells and provide a colorimetric readout of drug levels in recovered plasma via the interaction of drug, aptamer, and colloidal gold. In order to remove subjectivity associated with user interpretation of the assay, we will develop a smartphone application to quantify colorimetric readout, storing results along with location and patient information. We will design this device with constant input from clinicians and healthcare workers to ensure its feasibility for use in rural clinics in malaria-endemic settings.

1512

FIRST NATIONAL INTEGRATED COMMUNITY CASE MANAGEMENT (ICCCM) ONSITE TRAINING AND SUPPORTIVE SUPERVISION ASSESSMENT IN GHANA IN JANUARY-FEBRUARY 2015

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Integrated Community Case Management is an intervention to get sick children under five years of age treated at the community level as quickly as possible for malaria, cough or difficulty in breathing and diarrhoea. An Integrated Community Case Management (ICCM) Onsite Training and Supportive Supervision (OTSS) assessment was carried out in the months of January and February 2015.This became necessary because there were a number of challenges relating to community-based agents (CBAs) with some districts not reporting at all or on time. This had led to the inability to meet set targets of the program for the year 2014. Community health nurses and officers (CHNs/CHOs) (direct supervisors of CBAs) were trained and sent on the field. In the field CHNs/CHOs carried out training and supportive supervision using the OTSS checklist. After the assessment, members of the district health management team (DHMTs) with National teams and supervisors discussed findings and planned possible solutions for the challenges encountered. At the national level, the data was entered using epi info software, collated, cleaned and analysed. In all 10,393 CBAs were assessed. CBAs were able to diagnose and treat malaria and diagnose diarrhoea effectively using symptomatic diagnosis. Most CBAs, who had been trained on malaria rapid diagnostic tests (mRDTs), could not carry out the test. CBAs also had difficulty in correctly assessing for cough and difficulty in breathing in terms of counting breaths. There was erratic availability of artesunate-amodiaquine for malaria treatment. There were little or no medicines managing cough and difficulty in breathing and diarrhoea; even in regions that were expected to have supplies. Referrals by CBAs were done but caregivers generally refused to send their children to the next level of care. Most CBAs require training, as a lot of them had their training. In conclusion, CBAs are able to diagnose and treat malaria and diagnose diarrhoea symptomatically. They however lack the capacity to use mRDTs. They also need more training on diagnosis of cough and difficulty in breathing. The CBAs also need to be updated.

1513

THE ROLE OF MOBILE DEVICES IN RAPID DIAGNOSTIC TESTING QUALITY CONTROL ON COMMUNITY HEALTH VOLUNTEERS IN WESTERN KENYA

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Community health volunteers (CHVs) are trained to provide basic disease prevention and health promotion at the household level. In order to improve early diagnosis of malaria amongst rural populations, CHVs are an avenue to provide basic malaria testing with rapid diagnostic tests (RDTs) and treatment for uncomplicated cases. Monitoring RDT performance of CHVs is vital in ensuring quality and accuracy of testing. Physical monitoring of CHVs in real time is complicated in large-scale programs, thus the need for innovative quality control approaches. As part of a larger study, we trained a total of 275 CHVs on how to perform malaria RDTs. We introduced Fio Corporation's android based mobile diagnostic devices (Deki™ Readers) to remotely monitor the performance of a sample of CHVs in real time. These Deki Readers provided technological support for quality control by identifying RDT processing errors (too much/too little buffer, sample or buffer in wrong well, too much/too little blood) and interpreting rapid diagnostic tests (positive/negative/invalid). For our study Deki Reader software was programmed to interpret the tests only after the CHV provided their interpretation allowing for comparison between Deki Reader and CHV interpretation and real-time feedback to the CHV. Both results were uploaded to a secure data server for real-time review by the study team. The CHVs requiring additional mentorship were identified and followed up. Ninety (90) CHVs were randomly selected and trained for two days. CHVs also received technical support from Fio support staff. A total of 1199 clients were tested where 89.66% (1075) of the tests were concordant, 5.17% (62) tests had interpretation errors while 5.17% (62) had processing errors. The mean age of the CHVs was 41.4 years (C.I 40.9 - 42.0). 53.42% (47) of the CHVs had secondary education and above. Error rates were not correlated with age and education level. The low error rates irrespective of age and education is encouraging and confirms the ability of CHVs to correctly perform RDTs. Mobile devices to monitor CHV performance are feasible and valuable in the quality control of malaria diagnosis in remote areas.

1514

DETECTION OF MALARIA INFECTION BY HEMOZOIN CONTENT COMPARED TO RDTS AND MICROSCOPY FROM PERUVIAN AMAZON SAMPLES

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Detection of the presence of malaria parasites primarily relies upon the use of RDT card tests 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 novel malaria diagnostic techniques to rapidly and accurately diagnose across all species and with HRP2 deletion. A multidisciplinary effort designed an inexpensive, rapid (one minute) malaria detection device that indicates the presence of hemozoin, a by-product of parasite digestion of hemoglobin. Polarized laser 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 laser light able to pass through it. This decrease in light is directly proportional to parasitemia (R2=0.996). A test of 69 patients in Peru comparing an early prototype to microscopy achieved 92% sensitivity and 100% specificity for *Plasmodium falciparum* and *P. vivax* infections. Comparing CareStart RDTs to microscopy for these same samples showed 67% sensitivity and 100% specificity. The decreased effectiveness of RDTs in Peru is likely because of known HRP2 deletions in Peruvian *P. falciparum* and higher prevalence of *P. vivax* which is difficult to detect with RDTs. Our long term goal is to translate this technology into a robust, low-cost device, which can be used in malaria-endemic regions to enable rapid malaria diagnosis at the point-of-care for all species of malaria.

1515

PLATFORM FOR *PLASMODIUM* DETECTION IN BLOOD DONORS FROM ENDEMIC AND NON-ENDEMIC BRAZILIAN AREAS: PROCESSING OF POOLED SAMPLES USING MOLECULAR AND SEROLOGICAL MARKERS

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Malaria transmitted by blood transfusion remains one of the most important infections for hemotherapy services. In Brazil the incidence of malaria by blood transfusion is unknown, and this event may contribute to the spread of the disease in cases of failure in the clinical and epidemiological screening or due to asymptomatic donors. Donors that caused transfusion malaria showed very low parasitemia with an estimated rate of 1 to 10 parasites per unit of blood, which requires sensitive methods for the diagnosis and prevention. This study included 147 Brazilian public or private blood banks located in endemic and non-endemic areas for malaria with 13,383 blood donors that were accepted by the local methods of screening. The samples were grouped into pools of 10 and were processed by three different real time PCR and one nested PCR. A rapid test It was used for antibody detection. Samples from the positive pools were tested individually by PCR to detect positive donors. Real-time PCR revealed amplification for Plasmodium in 43 pools with samples from Amazon Region (4.72%) and Extra-Amazon Region (3.19%). Nested PCR detected four pools with *P. vivax*, two pools with *P. falciparum* and one pool with *P. malariae*, all related to samples from Extra-Amazon Region. Samples from positive pools were processed individually and real-time PCR revealed amplification in 25 donors, showing a positivity rate of 6.94% in 360 individual samples. Nested PCR detected two donors, one harboring *P. malariae* and one *P. vivax*. The rapid diagnostic test was positive for *P. vivax* in 13 pools from non-endemic area and in three pools from endemic area. Real-time PCR from Lima showed the best performance and was able to identify Plasmodium in pools of 10 samples, reducing time and cost of processing. This study, which analyzed blood samples from donors from endemic and non-endemic areas revealed the risk of transfusion malaria in our country and the need for sensitive validated protocols for detection of low parasitaemia. These results may support the decision making of blood donor screening criteria by regulatory agencies, in order to reduce malaria transmission in Brazilian blood banks.

1516

REDUCING THE DIAGNOSTIC BURDEN OF MALARIA USING MICROSCOPY IMAGE ANALYSIS AND MACHINE LEARNING IN THE FIELD

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Microscopy remains the main technique for diagnosing malaria, despite the availability of Rapid Diagnostic Tests. Hundreds of millions of blood films are examined using microscopy every year for diagnosing malaria and quantifying parasite burdens. Processing this large number of slides consumes scarce resources. Microscopy technicians who read these slides in the field may be inadequately trained or overwhelmed with the volume of slides to process, leading to missed and incorrect diagnoses. To ease the burden for microscopists and improve diagnostic and quantitative accuracy, we have developed a smartphone application that can assist field microscopists in diagnosis of malaria. The software runs on a standard Android smartphone that is attached to a microscope by a low cost adapter. Images of thin-film microscope slides are acquired through the eyepiece of the microscope using the smartphone's built-in camera. The smartphone application assists microscopist in detecting parasites and estimating the parasitaemia. For each microscope field, the image processing software identifies infected and uninfected cells, and reports the parasite count per microliter of blood. The software was trained with more than 200,000 red blood cells from slides acquired at Chittagong Medical College Hospital in Bangladesh from patients with and without *Plasmodium falciparum* infection. These were manually annotated by an experienced professional slide reader. This is one of the largest labeled malaria slide image collections, enabling the application of new machine learning techniques such as deep learning. For each field-of-view image taken, an image processing pipeline is applied first to detect and segment cells before computing color and texture features for automatic machine classification to discriminate between infected and uninfected cells and other objects in the slide. Initial experiments show that our software correlates highly with both human experts and flow cytometry. We demonstrate the smartphone user interface, show the typical smartphone application work flow, and report on the diagnostic performance in field conditions in Bangladesh.

1517

REAL-TIME QUALITY ASSURANCE OF MALARIA SURVEILLANCE DATA IN MYANMAR AND ITS BORDER WITH CHINA

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Collection of high quality data in public health surveillance and research studies is essential to ensure data interpretability and applicability. We evaluated the feasibility of systematic quality assurance procedures and the impact of a real-time integrated approach for large-scale malaria surveillance in Myanmar. We will present the data from and experience with evidence-based integrated monitoring and evaluation (M&E) procedures, with real-time feedback and quality assurance, implemented during the preparation and conduct of a large malaria surveillance study. The study was conducted in 43 villages located in 13 rural malaria-

endemic townships of nine State and Regions of Myanmar, and by a network of seven research and non-research partners working in the public and private sectors on malaria control and/or surveillance towards malaria elimination. M&E procedures were developed and performed manually and electronically using an edit-check system. Training was provided before and again during the study, with quality assessments before and after mid-study retraining. Study documents were comprehensively and systematically reviewed. Sample quality was evaluated by a trained team of laboratory experts, using a pre-specified check list. The most common and critical errors were site- and partner-specific, regardless of the type of the study. Documentation errors were related to age, travel history, antimalarial treatment history, evidence of consent, sample labeling, the quantity of blood required, and sample contamination. Findings from a comprehensive evaluation of approximately 5,000 study documents and more than 12,000 blood samples, using three different types of study record forms, and the quality before and after re-training, will be presented. Systematic and comprehensive monitoring and evaluation of data and related samples can be effectively integrated within a surveillance system, and real-time evaluation and feedback early in the process may significantly improve the quality of the data and samples, therefore subsequent usefulness of public health interventions developed and deployed based on these data.

1518

PLASMA-QPCR FOR DIFFERENTIAL DIAGNOSIS OF *PLASMODIUM FALCIPARUM* AND *P. VIVAX*

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Accurate, sensitive detection of human malaria parasite, by species, is required for the treatment of the disease, and for assessing the parasite load in asymptomatic carriers. Microscopy and antigen-based rapid tests reliably detect upto 50,000 parasites per ml of blood. Newer nucleic acid amplification methods offer higher sensitivity and flexibility but use template preparations that are laborious, expensive and subject to carryover sample contaminations. We use as little as 4 µl of patient plasma as a source of parasite DNA, without a need for purification. This molecular diagnosis method is simple, rapid, sensitive and reliable. Brand new sets of primers for the detection of *Plasmodium falciparum* (Pf) and *P. vivax* (Pv) by qPCR were designed at a non-conserved region of 18S ribosomal RNA genes. Species specificity of designed primers were confirmed by bioinformatics, by biochemical validation and by Sanger sequencing of amplified products. Though the presence of plasma delayed Ct value by four cycles with each tested standard genome sample, the linear range of detection was identical to reactions without plasma. With more than 90% amplification efficiency, the limit of detection stood less than 50 copies of parasite genome per ml of blood. Over 200 diverse patient samples were tested in triplicates on a 96-well qPCR instrument in parallel using Pf or Pv primer sets, with positive and no-template control reactions. Around 84 % of the plasma-qPCR results agreed with both microscopy and RDT, 9 % agreed with one of the two methods, 6 % displayed higher sensitivity and 1 % of the samples did not agree any of the two methods. This is better than any published comparison of three different methods. Direct plasma-based molecular diagnosis opens new avenues for differential screening of suspected malaria samples.

1519

QUALITY OF FEVER CASE MANAGEMENT IN THE PRIVATE SECTOR IN KINSHASA: RESULTS FROM BASELINE EXIT INTERVIEW AND MYSTERY CLIENT STUDIES

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The private sector is the most common fever treatment source in Kinshasa. 70% of childhood fevers are treated in this sector and 97% of antimalarials are distributed through private outlets, including 89% through unregulated drug shops where diagnostic testing is not available. There is no published data on the quality of fever case management in private outlets, and a pressing need to fill this gap. As part of a project to increase RDT availability and correct use in this sector we conducted exit interview and mystery client surveys to benchmark standard quality of care indicators in late 2015. 123 mystery client visits by confirmed RDT-negative volunteers were conducted at 65 facilities with blood testing available. 1,655 eligible client interviews were conducted at 83 health facilities, 44 pharmacies and 60 drug stores. Eligible clients were adults seeking treatment for fever for themselves or on behalf of someone else. 79.3% of facility clients received a malaria test, with 26.7% tested by RDT and 11.4% tested by both RDT and microscopy. 82.8% of test-positive facility clients received any antimalarial. However, fewer than half received an ACT (43.9%) and a similar proportion received both an antimalarial and an antibiotic (43.7%). The most common non-ACT treatments were quinine and artemisinin-based injections. 19/46 (41.3%) test-negative clients received any antimalarial. Testing was uncommon in pharmacies and drug shops (<8%) and 4 out of 10 untested clients received any antimalarial (40.6%). Mystery clients experienced poor quality of case management for fever at facilities. In 63% of visits the provider reported the client was positive for malaria following testing, and only 10% of clients received the correct diagnosis (negative) and did not receive any antimalarial. Providers wore gloves for only 23% of tests and waste disposal was suboptimal (immediate disposal of lancet in sharps box: 65% of tests). These results confirm there is much scope for improving private sector fever case management in Kinshasa, including both the provision of testing and availability of quality-assured ACT treatment.

1520

ANTIBODIES TO *PLASMODIUM VIVAX* MSP1-19 RECOMBINANT ANTIGEN IN BLOOD DONORS FROM SAO PAULO BLOOD BANK

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Transfusion-transmitted malaria (TTM) is a challenge for blood banks due to asymptomatic *Plasmodium* infections. In non-endemic and low endemic areas, TTM is a rarely reported event. Sao Paulo state is not endemic for malaria, but sporadic autochthonous cases, mostly asymptomatic, have been reported and transfusional cases due to asymptomatic donors harboring *P. malariae* have been described. These donors involved in TTM were individuals who had visited the Atlantic Forest in different regions of the state and were unaware of their *Plasmodium* carrier status. The aim of this study was to evaluate the exposition of donor candidates to *Plasmodium* as a measure of TTM risk. Blood samples were collected from 6,328 candidates for blood donation that attended Fundacao Pro-Sangue/Hemocentro de Sao Paulo. IgG antibodies were surveyed in plasma samples by ELISA using *P. vivax* $MSP1_{19}$ recombinant antigen. The reactivity index (RI) was calculated for each sample and values of RI ≥ 1.0 were considered positive. Out of 6,381 donors, 51 were positive

for IgG anti-PvMSP1$_{19}$ (0.81% - IC 95% = 0.61-1.06). RI≥1.0 varied from 1.01 to 9.92 (media=3.15 and median=2.26). Among the 51 samples positive by serology, 68.63% were from donors with displacements to the Atlantic Forest biome in São Paulo State and/or who live in regions near the Atlantic Forest. Using molecular tools, researchers have detected that approximately 50% of *Plasmodium* infections related to Atlantic Forest biome are caused by *P. vivax* and 50%, to *P. malariae*, corroborating our results. The detection of anti-*P. vivax* IgG in blood bank donors in non-endemic area is a measure of the exposure of candidates to *Plasmodium* antigens, but not necessarily a marker of parasitemia or disease. These results constitute an alert as asymptomatic donors are currently missed by the clinical-epidemiological screening preceding donation and no laboratorial test is applied. This study is part of a project that evaluates the power of a few specific questions on donor's proximity to the Atlantic Forest in addition to laboratorial methods to disclose *Plasmodium* carriers, aiming to reduce the risk of TTM in this area.

1521

ASSESSMENT OF THE VARIABILITY IN THE INTERPRETATIONS OF MALARIA RAPID DIAGNOSTIC TEST (MRDT) RESULTS

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Malaria RDTs are accurate and relatively simple to use. However, correct processing and interpretations of the test results are critical for mRDTs to perform optimally in the field. Errors in the post-processing phase, properly interpreting the RDTs as positive, negative or invalid, remain frequent. Based on 4 field studies of FIonet, where a healthcare worker and an automated RDT reader provided mRDT result interpretations, discordance was estimated to occur in ~9% of mRDTs. Our aim was to investigate the discordant results, to assess the variation and repeatability of the interpretation of the mRDT results. We developed a questionnaire composed of high-resolution images of mRDTs conducted in the field by healthcare workers during the Fionet studies, where an image of each RDT processed was captured at the time for interpretation. A proportional stratified random sample of images was selected, where the healthcare worker and Fionet had discordant interpretations of the mRDT results. Additionally, 2 mRDTs were included as controls and 2 were repeated. Respondent characteristics, such as their level of experience were collected. So far 45 respondents have completed the survey, with ~26% being experienced RDT users and located across 3 continents. Repeated mRDT images demonstrated that over 10% of respondents changed their interpretation between the first and second appearance of the image in the survey (Kappa=0.73). A large amount of variability in the interpretations was observed, from complete agreement to almost complete disagreement, for example the respondents' results for one mRDT where 44% positive, 46% negative and 10% invalid. For >30% of the mRDT images, there was less than 75% agreement on the interpretation. The largest variation in interpretation was observed when there was a very faint line present. Interpretations were not statistically different between experienced and non-experienced RDT users. The reliability of the interpretations of the ~9% of mRDT test results, are variable and consequently sub-optimal to rely on user interpretations in the field and supports the implementation of an objective automated RDT reader.

1522

PUNCH CARD MICROFLUIDICS PLATFORM FOR MULTIPLEX MOLECULAR DIAGNOSIS OF *PLASMODIUM FALCIPARUM* AND *P. VIVAX*

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Malaria continues to cause massive devastation globally. Routine microscopy and RDTs often fall short in diagnosing cases with low parasite densities. Moreover, with current elimination efforts in countries that are prime for such, increasing global concerns surrounding antimicrobial resistance and presence of co-infections, there is a need for effective multiplex diagnostic tests that can detect low parasitemia levels. To address this need, we have invented a punch card-based microfluidic platform and are using it to implement multiplex molecular diagnostic assays for malaria. Specifically, we are implementing recombinase polymerase amplification (RPA) assays starting with *Plasmodium vivax* and *P. falciparum*. Our platform is a self-contained, completely integrated hand-crank powered, programmable microfluidic platform. A paper tape encodes information as a series of punched holes. A mechanical reader/actuator reads these paper tapes and correspondingly executes operations onto a microfluidic chip coupled to the platform in a plug-and-play fashion. Enabled by the complexity of codes that can be represented by a series of holes in punched paper tapes, we harness our 30 independently controlled pumps and valves to implement the assays. Unlike conventional lateral flow-based tests, our platform has the capacity to process larger sample volumes, run multiple steps with the capacity to incorporate wash steps and the capability of implementing quantitative results if needed. Nucleic acid extraction is achieved using Fusion 5, a glass microfiber membrane that is embedded in the microfluidic chip. To achieve the optimum temperature of 37 degrees Celsius for the RPA assays, a heating pad is implemented in the device using a method that is similar to the previously described non-instrumented nucleic acid amplification (NINA) approach. With its portable and robust design, low cost and ease-of-use, we envision punch card programmable microfluidics bringing complex control of microfluidic chips into field-based diagnostic applications in low-resource settings to help combat malaria.

1523

SUPPORTING THE IMPLEMENTATION OF MALARIA RAPID DIAGNOSTIC TESTS (RDTS): TOOLS FOR QUALITY CONTROL AND ASSESSMENT IN ENDEMIC SETTINGS

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The World Health Organization (WHO) recommends that every suspected malaria case be confirmed by parasitological testing using microscopy or malaria Rapid Diagnostic Tests (RDTs), and treatment of confirmed cases be done with artemisinin combination therapies (ACTs). Therefore there is a need for good quality RDTs to ensure access to prompt and accurate diagnosis, especially in remote settings where good quality microscopy may not be available. The need for RDT quality and durability, especially under transport and storage conditions typical in malaria-endemic regions, has received considerable attention. Early evidence has shown lot to lot variation of products and susceptibility to deterioration upon exposure to high temperatures and humidity which can be encountered along the supply chain. Moreover documented reports show that health worker's poor adherence to RDTs test results is partly attributed to lack of confidence in the quality of tests. Tools to monitor the quality of RDTs at the end-users level and manage any problems have been set up in the frame of an RDT implementation project, with inputs from various implementers as well as health care staff from public and private sectors. A protocol was developed for countries to have a framework in which RDT problems will be documented, cross-checked and reported from the end user to the national, regional and international stakeholders. A troubleshooting guide provides help to RDT users and their supervisors to address problems that occur during use of RDTs. Positive control wells (PCWs) have been made available for point-of-care (POC) users, to reassure them on the quality of the test kits. When reconstituted with water and applied to a good quality RDT, the PCW solution produces a

positive test result. PCWs can therefore be used as POC quality control tool by front-line health workers to test their RDT stocks and ensure their validity and accuracy. Preliminary results from a pilot study assessing the use and acceptability of PCWs, the troubleshooting guide, and the problems protocol by health worker supervisors in the private and public health sectors in Kenya and Tanzania will be presented.

1524

PERCEIVED VALUE OF MALARIA RAPID DIAGNOSTIC TESTS AMONG PRIVATE PROVIDERS IN MADAGASCAR AND UGANDA: A QUALITATIVE STUDY

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Malaria rapid diagnostic tests (RDTs) have become ubiquitous in public sector health facilities throughout the malaria-endemic world, but private sector use is still nascent. Little is known about private provider perceptions of RDT credibility, how RDT results affect treatment decisions, and whether providers see RDTs as an asset or liability from both a health and a business perspective. We conducted in-depth interviews (IDIs) with 36 private providers in Uganda and 36 in Madagascar, most enrolled in a private-sector mRDT promotion program. IDI topics included RDT use, perceived test credibility, alternative diagnostic strategies, treatment decisions, pricing strategies, and perceived effect on overall profitability. Most providers agreed that RDTs improved their profitability and standing in the community. However, both confidence in test results and treatment based on test results varied widely. This presentation will describe the range of private-sector provider perspectives about RDT credibility and situations in which providers do or do not base treatment on RDT results. It will also consider the implications of study findings for scaling up RDT use in the private sector. This qualitative study was part of a three-year initiative to pilot RDT use among private providers in Kenya, Madagascar, Nigeria, Uganda and Tanzania.

1525

FIVE YEARS INTO THE MALARIA DIAGNOSIS SCALE-UP, ARE ACTS REALLY GETTING TO INFECTED PEOPLE? ESTIMATING ACT MISUSE IN THE INFORMAL PRIVATE SECTOR

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In 2010, the World Health Organization broadened its recommendation to parasitologically confirm malaria before treatment is provided, catalyzing the increased availability and use of malaria rapid diagnostic tests (mRDTs). The use of mRDTs promotes appropriate treatment for febrile illness and prevents antimalarials, especially the first line artemisinin-based combination therapies (ACTs), from being wasted on patients without malaria. Even so, mRDTs are not always available in the private health sector, where a substantial proportion of fever patients seek treatment, contributing to the irrational use (or misuse) of antimalarials, especially in the informal private sector, i.e. drug shops. A model to quantify ACT misuse will help in setting priorities as further scaling-up of mRDTs occurs in endemic contexts. The following data inputs were used in the model to estimate ACT misuse in a given geography: malaria prevalence, number of fever patients seeking treatment, proportion of fevers receiving a malaria test, proportion of fevers with or without a test, and proportion of fevers receiving ACTs. These inputs were derived from raw data or modeled from the Demographic Health Surveys, ACTWatch Surveys, reviews of published literature, and data from the national malaria control programs. The number of ACTs misused was estimated as the number of ACTs given to fever cases who had not received a test but were assumed negative based on prevalence figures, and to those who had received a negative test. For the informal private sector across three endemic countries - Kenya, Uganda and Tanzania - irrational ACT use was estimated at approximately 10 million. At 64 cents a course, approximately $6.8 million worth of ACTs are being wasted on those without malaria. Given that this represents only small a subset of ACT misuse across sub-Saharan Africa, it is important that mRDT scale-up continues, especially in the informal private sector where a third of all treatment-seeking occurs. Further, drug shop owners must be properly trained on mRDT use and should be empowered to trust mRDT results in diagnosing malaria.

1526

EFFICACY OF DIHYDROARTEMISININ-PIPERAQUIN AND CHLOROQUINE IN THE TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM AND PLASMODIUM VIVAX* MALARIA IN VIETNAM

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Resistance to antimalarial drugs is a major public health problem, which may hamper the control of malaria. In order to deal with the growing resistance of both *Plasmodium falciparum* and *P. vivax*, surveillance of the first choice antimalarial drugs- DHA-PPQ and chloroquine, was conducted in the Central Vietnam during 2012 to 2015. The surveillance was conducted at 3 sentinel sites in Quang Tri, Gia Lai and Ninh Thuận based on the World Health Organization protocol (WHO, 2009). Standard total dosage of DHA-PPQ 40/320 mg (8 tablets, in 3 days, chloroquine 500 mg (10 tablets in 3 days) for adult patients, with 28 or 42 day follow-up. K13 gene mutation identification was also done at the Institute Pasteur of Phnom- Penh (2014) and the Department of Microbiology, University of Sassari, Italy (2015). During the period from 2012 -2015 the DHA-PPQ efficacy to *P. falciparum* malaria were high with APCR, (69/69, 100%) (46/46, 100%) in Quang Tri and Ninh Thuan province respectively, but APCR (57/60, 95%), ETF (2/60; 3.33%), LCF (1/60, 1.67%) in Gia Lai sentinel site. The mean parasite clearance time (PCT) was 48 hours except 11 cases in Gia Lai having blood smear positive rate on D3 of 18.3%. In those cases there were 9 mutations in Kelch 13 propeller gene (C580Y, R539T). The efficacy of CQ on the clearance of blood stage *P. vivax* (without primaquine) was still high with ACPR at 100% in Quang Tri province, Gia Lai province, in Ninh Thuan province. There was no recurrence with 28 days follow-up. Conclusions: DHA-PPQ and chloroquine remain efficacious for the treatment of uncomplicated falciparum and vivax malaria respectively in the Central of Vietnam. Further investigation as detection of artemisinin resistant markers and PK/PD are needed.

1527

PHARMACOKINETIC INTERACTIONS OF ARTESUNATE ON THE DISPOSITION OF AMODIAQUINE IN SUBJECTS WITH *PLASMODIUM FALCIPARUM* INFECTION AFTER ORAL ADMINISTRATION OF FIXED-DOSE COMBINATION OF AMODIAQUINE-ARTESUNATE

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Artemisinin-based combination therapy has been adopted by several African countries including Nigeria as first line treatment for uncomplicated falciparum malaria. There is a need to balance the

advantages of the combination against the possible effect of interaction between the component drugs. We investigated the pharmacokinetic interactions of artesunate (AS) on the disposition of amodiaquine (AQ) in subjects with *Plasmodium falciparum* infection after oral administration of fixed-dose combination of amodiaquine-artesunate (AQAS). This is a randomized, open-label trial in which twenty subjects with *P. falciparum* infection were assigned into two treatment arms namely, AQ or in combination with artesunate (AQ/AS). AQ (600mg) or a fixed dose combination of AQ/AS (AQ 306.3 mg/AS 100 mg × 2 tablets) was administered once daily for 3 days. Blood samples were collected at different sampling times. Subjects were followed up for 28 days to assess response to treatment; those who failed to respond to AQ or AQ/AS were treated with artemether/lumefantrine and quinine respectively. Plasma was obtained and assayed for AQ and desethylamodiaquine (DAQ) levels using hplc technique. The pharmacokinetics parameters of AQ and DAQ were determined and compared in the two arms. There are no statistically significant difference in the peak plasma concentration, Cmax (774.34 ± 146.94 vs. 763.19 ± 89.99 ng/ml), concentration on day 7, Conc day 7 (357.13 ± 45.06 vs. 390.88 ± 53.63 ng/ml), total drug exposure, AUC0-∞ (187,710 ± 14.110 vs. 197,960 ± 14, 6874 ngh/ml) and elimination half-life, T½ (212.81 ± 1.24 vs. 212.89 ± 1.20 h) (P >0.05) of DAQ in AQAS vs. AQ respectively. The pharmacokinetic parameters of AQ were also similar in both arms (P > 0.05). Parasites cleared in all subjects in the two arms except in a subject in AQ arm in whom parasites were seen on Day 14. Although not significant, the reduced total exposure of AQ in AQAS arm was a concern particularly in areas with reduced AQ sensitivity. Further studies are needed to assess the degree of reduction in total exposure of DAQ observed in this study so as to design optimal dosing/tolerability profile for AQ use.

1528

UNEXPECTED FALL IN HEMOGLOBIN VALUE DURING THE FIRST PHASE OF MALARIA PREVENTION TRIAL: PRELIMINARY FINDINGS FROM A DROUGHT PRONE AREA IN ETHIOPIA

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As part of a field trial to provide evidence on the combined use of LLINs and IRS for malaria prevention, we measured hemoglobin values among children less than five years old. Our hypothesis was that by preventing malaria, the mean hemoglobin values would increase by 0.5 gm/ dl. In this trial we followed about 3000 children 6 to 59 months old from August 2014 to December 2015. We did active (weekly home visit) and passive malaria case search. Hemoglobin (HB) concentration surveys were conducted after the major malaria transmission seasons in 2014 and 2015, and children with HB < 11 gm/dl were classified as having anemia. The mean HB value decreased from 11.6 gm/dl in 2014 to 11.2 g/dl in 2015 (mean difference 0.35 gm/dl (95% CI 0.27 - 0.43; P<0.001). In 2014, the prevalence of anemia was 28.2% (95 % CI; 26.6 - 29.8) and increased in 2015 to 36.8% (95 % CI; 35.1 - 38.5). Among 171 registered malaria cases, 88 (51.5%) were due to *Plasmodium falciparum*. Among these children, malaria incidence rate was 8.6 (95% CI; 6.5 - 11.3) in 2014, and 8.3 (95% CI 6.4 - 10.8) cases per 10,000 person weeks in 2015. The mean hemoglobin value as well as malaria incidence increased with increasing age of the child. Family wealth and educational status of the head of households were predictors of anemia, but malaria incidence was not associated with anemia. This study showed an unexpected fall in mean HB value between the two surveys. This occurred in spite of malaria prevention efforts. During the same period, the region experienced one of the most severe droughts in decades. Even if many children in the study area received supplementary feeding, and we believe the worsening food household insecurity may explain the increase in anaemia prevalence. This study demonstrated that doing field trials in drought prone areas may bring unexpected challenges.

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SAFETY AND TOLERABILITY OF ROSIGLITAZONE ADJUNCTIVE THERAPY FOR CHILDREN WITH UNCOMPLICATED MALARIA: A RANDOMIZED, DOUBLE BLIND, PLACEBO CONTROLLED TRIAL

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Studies have shown that the oral antidiabetic Rosiglitazone can improve malaria outcomes in adults, by decreasing the levels of Angiopoietin II (AGII), an independent and quantitative biomarker of disease severity in malaria. We present the first results of a pilot clinical trial assessing the safety and efficacy of rosiglitazone as adjunctive therapy for *Plasmodium falciparum* malaria in Mozambican children. Thirty children (1-12 years) with uncomplicated malaria were randomized (2:1) to receiverosiglitazone (0.045mg/kg/dose) or placebo (double blind) twice-daily for four days. ECG, Blood glucose levels, biochemical and haematological parameters were monitored for safety. AGII and other biomarkers of host response including endothelial activation, inflammation, coagulopathy, and neuroprotection were measured for efficacy. Results: No significant differences were found in terms of the incidence of biochemical, haematological or electrocardiographic abnormalities. Efficacy results will be presented. In conclusion, this study confirmed the safety of Rosiglitazone in Mozambican children with malaria. Evaluation of Rosiglitazone as adjuvant therapy for severe malaria is warranted.

1530

SAFETY AND EFFICACY OF REPEATED ADMINISTRATION OF PYRONARIDINE-ARTESUNATE OR DIHYDROARTEMISININ-PIPERAQUINE VS ARTESUNATE-AMODIAQUINE IN CHILDREN AND ADULT PATIENTS WITH ACUTE UNCOMPLICATED *PLASMODIUM* SP MALARIA OVER OF TWO YEARS PERIOD AT BANFORA/NIANGOLOKO SITE IN BURKINA FASO

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The safety and efficacy of repeated administration of three ACTs [(pyronaridine-artesunate (PYR) or dihydroartemisinin-piperaquine (DHA-PQ) vs artesunate-amodiaquine (ASAQ)] were evaluated in West African Countries, members of the West African Network of Clinical trials for AntiMalarial drugs (WANECAM). In the current study we present the preliminaries data of repeated administration of PYR or DHA-PQ vs ASAQ over a period of 2 year in children and adults with uncomplicated Plasmodium sp malaria at Banfora/Niangoloko sites in Burkina Faso. This study is a comparative, randomized, open label longitudinal clinical trial involving children and adults with uncomplicated *Plasmodium* sp. malaria. Each of participant enrolled received during their subsequent episodes the same drug and went through the same trial procedures as for the initial episode. A total of 1090 participants were screened from which, 763 were enrolled in ASAQ (315), DHA-PQ (224) and PYR (224) arm, from July 2012 to December 2013. As per age 342, 357 and 64 participants aged < 5 years, 5-14 years and ≥ 15 years were followed respectively during

the two years. The preliminaries results showed that 245 of 315 (77.8%) patients, 166 of 224 (74.10%); and 176 of 224 (78.6%) experienced at least 2 malaria episodes and 108 (34.3%), 63 (28.1%) and 82 (36.6%) experienced at least 5 malaria episodes in the ASAQ, DHA-PQ and PYR arms respectively. The average time between the first and the second malaria episode was statistically longer ($p<0.05$) in DHA (157 days) compared to ASAQ (135 days) and PYR (117 days) arms. Adequate clinical and parasitological response (ACPR) by day 28 was 93.0 %, 97.8% and 98.2% in ASAQ, DHA and PYR arm respectively during the first malaria episodes. The 42 day cure rate (not adjusted by PCR) was 80.3 %, 93.8% and 78.2% in ASAQ, DHA-PQ and PYR arms respectively during the first malaria episodes. Our preliminary results confirmed the two news drugs (DHA-PQ and PYR) are safe and their efficacy comparable to the ASAQ in uncomplicated malaria treatment in high malaria transmission region.

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RANDOMIZED, BLINDED CLINICAL TRIAL COMPARING AN INVESTIGATIONAL ANTIMALARIAL, AQ-13, TO ARTÉMETHER + LUMÉFANTRINE FOR TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA

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Chloroquine (CQ) was the treatment of choice for *Plasmodium falciparum* malaria until CQ-resistant parasites were identified in Southeast Asia and South America over 50 years ago. Although CQ-resistant *P. falciparum* have been an obstacle to malaria control since that time, recent studies have shown 4-aminoquinolines (4-AQs) with modified side chains are active against CQ-resistant parasites and that the lead compound (AQ-13) is safe in human subjects. To determine whether AQ-13 is effective for the treatment of uncomplicated malaria, we performed a randomized, blinded clinical trial in which 66 Malian men received oral treatment with AQ-13 or the current recommendation of artémether + luméfantrine (A+L) for 3 days. After all subjects completed the 42 day follow-up, the study was unblinded and results were compared for subjects randomized to A+L vs. AQ-13. There were no differences in the ages, initial parasite counts or Hb levels of subjects randomized to A+L vs. AQ-13. Likewise, High Resolution Melt (HRM) analysIs indicated that similar numbers of subjects with CQ-susceptible, CQ-resistant and mixtures of -susceptible and -resistant *P. falciparum* were randomized to A+L and AQ-13. Subjects treated with either A+L or AQ-13 cleared asexual parasites from their blood on or before day 7 and no serious, Grade 3 or Grade 4 AEs occurred in either group. There were 2 withdrawals for personal reasons from the AQ-13 arm on day 4 after asexual parasites had been cleared on day 3 and 2 recurrences in the A+L arm on follow-up days 17 and 21. These results indicate AQ-13 is efficacious and safe for the treatment of uncomplicated malaria caused by CQ-susceptible and –resistant *P. falciparum*. Based on this study, the efficacy of AQ-13 cannot be distinguished from the efficacy of A+L for uncomplicated *P. falciparum* malaria. Efficacies for A+L and AQ-13 were 94% (31/33) based on intent to treat and 94% and 100% (31/33 and 31/31) based on per protocol analyses.

1532

RANDOMIZED, BLINDED CLINICAL TRIAL COMPARING AN INVESTIGATIONAL ANTIMALARIAL, AQ-13, TO ARTÉMETHER + LUMÉFANTRINE FOR TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA

Ousmane A. Koita[1], Lansana Sangare[1], Haiyan Deng[2], Sahare Fongoro[1], Moctar Coulibaly[1], Aliou Sissako[1], Trevor A. Thompson[2], Youssouf Diarra[1], Mamadou Ba[1], Ababacar Maiga[1], Boubakar Diallo[1], Frances J. Mather[2], Asif Anwar[2], Donald J. Krogstad[3]

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Chloroquine (CQ) was the treatment of choice for *Plasmodium falciparum* malaria until CQ-resistant parasites were identified in Southeast Asia and South America over 50 years ago. Although CQ-resistant *P. falciparum* have been an obstacle to malaria control since that time, recent studies have shown 4-aminoquinolines (4-AQs) with modified side chains are active against CQ-resistant parasites and that the lead compound (AQ-13) is safe in human subjects. To determine whether AQ-13 is effective for the treatment of uncomplicated malaria, we performed a randomized, blinded clinical trial in which 66 Malian men received oral treatment with AQ-13 or the current recommendation of artémether + luméfantrine (A+L) for 3 days. After all subjects completed the 42 day follow-up, the study was unblinded and results were compared for subjects randomized to A+L vs. AQ-13. There were no differences in the ages, initial parasite counts or Hb levels of subjects randomized to A+L vs. AQ-13. Likewise, High Resolution Melt (HRM) analysIs indicated that similar numbers of subjects with CQ-susceptible, CQ-resistant and mixtures of -susceptible and -resistant *P. falciparum* were randomized to A+L and AQ-13. Subjects treated with either A+L or AQ-13 cleared asexual parasites from their blood on or before day 7 and no serious, Grade 3 or Grade 4 AEs occurred in either group. There were 2 withdrawals for personal reasons from the AQ-13 arm on day 4 after asexual parasites had been cleared on day 3 and 2 recurrences in the A+L arm on follow-up days 17 and 21. These results indicate AQ-13 is efficacious and safe for the treatment of uncomplicated malaria caused by CQ-susceptible and -resistant *P. falciparum*. Based on this study, the efficacy of AQ-13 cannot be distinguished from the efficacy of A+L for uncomplicated *P. falciparum* malaria. Efficacies for A+L and AQ-13 were 94% (31/33) based on intent to treat and 94% and 100% (31/33 and 31/31) based on per protocol analyses.

1533

A TRIAL OF SEASONAL MALARIA CHEMOPREVENTION PLUS AZITHROMYCIN IN AFRICAN CHILDREN

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Mass administration of azithromycin (AZ) on a single occasion for elimination of trachoma in Ethiopia was associated with a substantial reduction in overall child mortality in communities treated with AZ compared to untreated control communities. This reduction was sustained for 26 months after treatment (Rate Ratio 0.35; 95% C1: 0.17, 0.74). The impact of AZ on mortality could have been achieved through its action on pneumococci and other bacteria. As seasonal malaria chemoprevention (SMC) is implemented in countries of the Sahel and sub-Sahel, it would

be possible to add AZ to the antimalarial drug regimen given during the rainy season when the risk of both malaria and severe bacterial infections is highest. Thus, we are conducting a study in Mali and Burkina Faso to determine whether addition of AZ to SMC using sulphadoxine/pyrimethamine (SP)+amodiaquine (AQ) provides an additional reduction in deaths and severe illness in young African children. This is a double blind, randomised, placebo-controlled trial involving 19200 children aged 3 - 59 months who are randomised by household to receive four rounds of either SP+AQ+AZ or SP+AQ+ placebo at monthly intervals during the peak malaria transmission season over a three-year period. Administration of the first round of drugs started in August 2014 and the final round will be completed in November 2016. In 2014 and 2015, the proportion of children who received ≥3 monthly rounds of drug each year was >90%. We will present the study rationale and design, and preliminary results of blinded analysis of the incidence of primary (hospital admission or death during the transmission season) and selected secondary endpoints (incidence of clinical malaria, respiratory infection and diarrhoea, and the prevalence of malaria parasitaemia, markers of SP resistance, malnutrition and pneumococcal carriage at cross-sectional surveys). Final results will be available in 2017.

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A PROOF-OF-CONCEPT, RANDOMIZED STUDY IN NON-IMMUNE HEALTHY ADULT VOLUNTEERS TO INVESTIGATE THE SAFETY, TOLERABILITY, PHARMACOKINETIC PROFILE AND PROPHYLACTIC ACTIVITY OF A SINGLE DOSE OF DSM265 IN A CONTROLLED HUMAN MALARIAL INFECTION CHALLENGE EITHER BY DIRECT VENOUS INOCULATION OF *PLASMODIUM FALCIPARUM* SPOROZOITES (PFSPZ) OR A SINGLE EPISODE OF BITES BY MOSQUITOES CARRYING *P. FALCIPARUM*

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In the face of rising drug resistance, new anti-malarial drugs are needed for prophylaxis and radical cure. DSM265 is a novel triazolopyrimidine-based inhibitor of dihydroorotate dehydrogenase (DHODH), a key enzyme in the pyrimidine biosynthesis pathway. DSM265 demonstrated promising *in vitro* and *in vivo* activities against liver and blood stages in preclinical studies and recently advanced to clinical trials for blood stage infection. In collaboration with the Medicines for Malaria Venture and the University of Tübingen, the Seattle Malaria Clinical Trials Center initiated a study to assess the safety, tolerability, pharmacokinetic profile and prophylactic activity of a single dose of DSM265 in a controlled human malarial infection (CHMI) challenge either by direct venous inoculation (DVI) of *Plasmodium falciparum* sporozoites (PfSPZ) or a single episode of bites by mosquitoes carrying *P. falciparum*. This study is designed to determine the initial dosing interval for once-weekly chemoprevention. Three cohorts (n=6 drug-treated plus n=2 placebo controls) are planned to assess DSM265 dosing 3 or 7 days prior to PfSPZ DVI CHMI or 7 days prior to mosquito bite CHMI. Subjects will be followed using the standard CHMI model using a *Plasmodium* 18S rRNA molecular qRT-PCR-based treatment threshold to initiate rescue treatment after ≥250 estimated parasites/mL are detected. Pharmacokinetic data on DSM265, safety laboratory data, adverse events and parasite growth kinetics will be assessed. The study is ongoing, with data from completed cohorts to be presented.

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PHASE II STUDY OF ARTEFENOMEL (OZ439) AND PIPERAQUINE TO INVESTIGATE SINGLE DOSE TREATMENT FOR UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA

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We performed a clinical phase II study with single dose combinations of artefenomel (OZ439) and piperaquine phosphate (PQP) at three dose levels as part of the development of a single dose cure for uncomplicated *Plasmodium falciparum* malaria to improve patient compliance, reduce risk of drug resistance, and to support eradication campaigns. Patients (n=448) in Africa (n=365) and Asia (n=83) were recruited with 85% being children <5 yrs of age. OZ439/PQP combination demonstrated an acceptable safety profile and was well tolerated. The primary endpoint was Day 28 PCR-adjusted ACPR (ACPR28). ACPR28 for the combination of 800mg OZ439 with 640 mg PQP, 960 mg PQP and 1440 mg PQP was 68.4%, 70.8% and 78.6% respectively, in per protocol population. ACPR28 was lower in Asian than African patients despite achieving higher drug exposures. Only 1 early treatment failure occurred. The success of a single dose treatment is assumed to be dependent on adequate parasiticidal drug exposure for three parasite life-cycles (approximately 1 week). Population PK analyses using non-linear mixed effect modelling allowed estimation of concentration at Day 7 and identification of influential covariates in this study. The probability of achieving ACPR28 was a function of both OZ439 and PQP concentrations at Day 7, as well as baseline parasitemia, and region. Neither age nor presumed immunity was identified as a covariate. In Asia, >70% patients had parasites with mutations in the Kelch13 (K13) propeller gene. K13 genotype, known to alter parasite reduction rate for artesunate also impacted the parasite clearance by OZ439/PQP but had no significant impact on the cure rate measured as ACPR28. Furthermore, K13 genotype was not a significant covariate in the model for ACPR28. In conclusion, none of the treatment arms investigated in this study met the regulatory efficacy threshold defined as ACPR28 >95%. Given that there are increasing reports of PQP resistance in South-East Asia, we decided not to follow up on the potential of this combination for a 3-day regimen, instead we plan to investigate combinations with newer compounds where there is less risk of pre-existing resistance.

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TOLERABILITY OF SINGLE DOSE PRIMAQUINE IN G6PD-DEFICIENT ADULT MALES IN MALI WITHOUT MALARIA: AN OPEN-LABEL PHASE 2 DOSE-ADJUSTMENT TRIAL

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Single low dose primaquine (SLD-PQ) is recommended by the WHO to block the transmission of *Plasmodium falciparum* parasites to mosquitoes. However, uptake is limited due to concerns of hemolytic side effects in individuals with enzymatic glucose-6-phosphate dehydrogenase (G6PD) deficiency. We determined the safety of three, single-dose regimens of primaquine in G6PD-deficient adult males without malaria in Mali. We conducted an open-label, non-randomized, dose-adjustment trial of the tolerability of 0.40, 0.45, and 0.50 mg/kg of single dose primaquine in G6PD-deficient adult males in Mali without malaria. Adult males with a Carestart® qualitative G6PD-deficient test result were treated with a

0.40, 0.45, or 0.50 mg/kg dose of primaquine, followed by a 0.50 mg/kg control group of G6PD-replete men. The primary outcome was the within-person percentage change in hemoglobin concentration, assessed using a Hemocue system, from baseline levels between day 0 and day 10. All individuals who received a single-dose of primaquine and completed safety assessments--comprising hemoglobin concentration, urine color, and clinical assessment--daily on days 1-10, and on days 14 and 28 following primaquine administration, were included in the primary sample analysis (n=28). We enrolled 28 participants sequentially, from August 13 to December 19, 2015. Primaquine doses of 0.40, 0.45 and 0.5 mg/kg were all found to be safe and tolerable. A hemolytic dose response was not observed at these doses in any of the participants, no serious adverse events were reported, and adverse events were not associated with the treatment group. SLD-PQ up to 0.50 mg/kg was well tolerated in healthy G6PD-deficient populations in West Africa, and should be rolled out using 0.50 mg/kg as the upper bound for weight-based dosing bands.

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THE ETHICS OF USING A PLACEBO ARM IN RANDOMIZED CONTROLLED TRIALS: A CASE OF IN A PRIMAQUINE ANTIRELAPSE STUDY

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Clinical research involving randomised controlled trials is critical for advancing global health. However global health trials can evoke important ethical issues, particularly the use of placebo or non-intervention control arms. The issue has generated ethical consideration for many years, that hinges on the debate on "double standard of care". We explore this through evaluation of the use of a placebo arm in the specific example of a large multicentred placebo-controlled, double-blinded, randomized trial to determine primaquine antirelapse efficacy in vivax malaria. The trial involves almost 2000 patients enrolled in Indonesia, Vietnam, Ethiopia, Pakistan and Afghanistan. There are three arms - chloroquine or an artemisinin combination therapy treatment plus either: 7 days primaquine , 14 days primaquine or 14 days placebo. The need for the study is justified in view of the poor evidence for the current WHO recommended regimen of 14 days primaquine. The ethical rationale for including a 14-day placebo arm can be made on the grounds that the standard of care in most endemic countries does not include in reality widespread, routine use of primaquine. We argue that since there is equipoise among the study arms, the risk of being in the placebo arm is no greater than the risk of not being in the trial and that there are no double standards. This analysis complements others in literature with regards to the use of placebo or no intervention treatment arms, and highlights that such debate case be justified on its own merits rather than relying on general guidelines.

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INHALED NITRIC OXIDE IMPROVES NEUROCOGNITIVE OUTCOMES IN CHILDREN WITH SEVERE MALARIA AND LACTIC ACIDOSIS

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Severe malaria is a leading cause of neurocognitive impairment in African children. Low levels of bioavailable nitric oxide (NO) are associated with severe malaria. Supplementation with inhaled NO (iNO) has been shown to be neuroprotective in term or near-term infants with persistent pulmonary hypertension but the neuroprotective actions of iNO have not been documented in systemic infections. This randomized, double-blind, placebo-controlled trial compared the effect of inhaled nitric oxide (iNO) at 80ppm versus room air (placebo) on neurocognitive function in children with severe malaria treated with parenteral artesunate. Children aged 1 to 10 years received either iNO or placebo. Six months post-discharge, neurocognitive testing was performed to assess overall cognition, attention, associative memory, executive function, motor skills, language and visual reception. We compared test scores between the treatment arms and the frequency of impairment in all domains between the arms. At six months, 61 children in the iNO arm and 59 children in the placebo arm were evaluated. 35% of children had impairment (defined by a z-score ≤-2SD) in at least one domain. There were no significant differences in z-scores for overall cognition, attention, associative memory and executive function between iNO and the placebo group. Children receiving iNO were less likely to have multiple impaired domains (11.5% vs 25.4%, p=0.048) and to have fine motor impairment, relative to children receiving placebo (8.2% vs 22.0%, p=0.034). Subgroup analysis in children with acidosis at admission showed iNO was associated with improved attention (p=0.001), fine motor functioning (p=0.004), visual reception (p=0.028), receptive language (p=0.015), and overall cognitive function (p=0.009). Inhaled nitric oxide is associated with better cognitive outcomes in children with severe malaria presenting with acidosis.

1539

A RANDOMIZED TRIAL OF THE SAFETY OF LOW DOSE PRIMAQUINE IN THE TREATMENT OF G6PD NORMAL AND DEFICIENT ADULT PATIENTS WITH *PLASMODIUM FALCIPARUM* MALARIA IN SENEGAL

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WHO recommends the addition of a single dose of primaquine (0.25 mg base/kg) to artemisinin combination treatments (ACTs) as a component of pre-elimination or elimination programs. However, primaquine has been little used in Africa and there are concerns about its safety, as the drug can cause acute haemolytic anaemia in individuals with G6PD deficiency. This open randomised controlled trial was conducted to assess the safety of adding low-dose primaquine to the normal ACT regimen (AL, ASAQ, DHAP) in adult patients in Senegal. Patients with *P. falciparum* malaria (parasitaemia {1,000-100,000} trophozoites/µL) were randomized to receive treatment with ACT or ACT plus low-dose primaquine. Haemoglobin concentration was measured at enrolment, and on days 3, 7, 14, 21 and 28 post-treatment. G6PD status was determined for each patient using a qualitative field test (CareStart™). The primary

outcome was the change in haemoglobin concentration from day 0 to day 7, which was compared between trial arms using analysis of covariance. Secondary endpoints included haemoglobin variation from day 0 to day 28. Two hundred and seventy five patients (137 in the ACT arm and 138 in ACT plus primaquine arm) were randomized. At enrolment, gender, mean weight, parasitaemia, haemoglobin, and prevalence of G6PD deficiency were similar in the two arms. Mean haemoglobin concentration on day 7, was similar in primaquine and control groups (11.9 and 12.1 g/dL respectively). The difference in Hb concentration on day 7 in the primaquine group compared to controls after adjusting for Hb at baseline was -0.029 (95%CI -0.51,0.45) g/dL. Haemoglobin change at day 7 was significantly associated with haemoglobin at enrolment, weight and gender. There was no evidence of an association with treatment drug, G6PD status, and parasiteamia at enrolment. Haemoglobin concentrations recovered and exceeded baseline level by day 28. The administration of single low dose primaquine (0.25 mg/kg) in addition to ACT treatment, to adult patients with acute *P. falciparum* malaria, is safe and does not induced significant drop in haemoglobin level both for G6PD normal and deficient individuals.

1540

MODERATE AND SEVERE LFT ELEVATIONS IN CONTROLLED HUMAN *P. FALCIPARUM* MALARIA INFECTION MODEL: RECENT EXPERIENCE, LITERATURE REVIEW AND MECHANISTIC HYPOTHESES

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Controlled Human Malaria Infection (CHMI) in healthy subjects is a critical model in vaccine research and in profiling new chemical entities (NCEs). Documented laboratory changes induced by CHMI include mild benign liver function tests (LFT) elevations (<2.5xULN). In recent evaluations of antimalarial NCEs with the *Plasmodium falciparum* (P.f.) induced blood stage malaria (IBSM) model, we have observed cases of transient asymptomatic moderate/severe LFT elevations (2.6-10xULN). Among more than 174 healthy subjects tested in our P.f. IBSM studies with NCEs and approved antimalarials, 6 participants showed ALT/AST elevations of up to 10 x ULN. These cases were reported in two distinct studies (3/8 subjects each) with two NCEs that were not identified as hepatotoxic in the initial Phase 1 studies. A liver safety review of 13 completed P.f. IBSM studies (7 approved antimalarials and 6 NCEs) and 44 published sporozoite challenge studies was performed. For sporozoite CHMIs, moderate/severe LFT elevations were also reported in a mosquito-bite study with a NCE (pafuramidine) in 6/19 subjects (4 active/2 placebo). Most of these subjects (including placebo) received acetaminophen with a highest cumulative dose of 17.5g. ALT elevations were generally higher than AST. For IBSM studies, only one subject showed bilirubin > 2xULN (potential Hy´s law reported as serious adverse event). Review of these cases by Drug-Induced Liver Injury Experts suggest that these changes are likely to be multifactorial in origin with combined interaction of 3 possible causative factors: 1- Inflammatory state induced by CHMI, 2-Acetaminophen, 3-NCE and additional risk factors (undiagnosed condition such as liver steatosis or alcohol consumption). Because these laboratory findings are not uncommon, specific safety provisions for the conduct of CHMI studies with NCEs during drug development are proposed. The recommendations for IBSM studies include preclinical hepatotoxicity profiling of the NCE, strengthened eligibility criteria, use of a positive control and symptomatic treatment with NSAIDs (ibuprofen) as a substitute to acetaminophen.

1541

HARMONIZATION OF A MULTI-CENTER ARTESUNATE-MEFLOQUINE DOD CLINICAL EFFICACY STUDY FOR PATIENTS WITH UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN KENYA, PERU AND THAILAND

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Artemisinin-resistant *Plasmodium falciparum* is of growing concern in Southeast Asia, with potential to spread to other *P. falciparum* endemic regions. To conduct surveillance for emerging artemisinin resistance in geographically distinct regions, the AFHSB-GEIS leveraged DoD laboratories in Kenya, Peru, and Thailand to coordinate a multi-center harmonized trial for artesunate-mefloquine efficacy, based on parasite clearance rates for 72 hours after artesunate dosing, using a standardized approach based on WHO efficacy study methodology. Here we describe specific activities designed to improve synchronization and data comparability across study sites, as well as challenges and lessons learned. Participating DoD organizations jointly developed clinical and laboratory quality assurance programs and plans, study documents and case report forms, and a reciprocal monitoring strategy to facilitate harmonization across sites. Each site conducted self-monitoring and reciprocal monitoring of other sites' progress for adherence to the clinical protocol and quality assurance plans. Microscopy proficiency testing was conducted at the initiation and evaluated at multiple points throughout the study. Data management was centralized among sites to ensure data integrity, including the reception of raw data, cleaning, querying, and producing complete datasets for analysis. Site personnel were trained in correctly populating clinical data from site-specific case report forms into harmonized case report forms for submission to a clinical data management system used to provide quality control. Challenges incurred included the removal of a participating site, protocol enrollment following evolving *P. falciparum* transmission patterns, and data submission technical difficulties. Future coordinated efforts will be directed at harmonized *in vitro* drug efficacy testing and genetic studies for markers of artemisinin resistance.

1542

DETERMINATION OF CYTOCHROME P-450 ISOENZYME 2D6 (CYP2D6) GENOTYPES IN AN ACTIVE-DUTY U.S. MILITARY POPULATION AND THE PHARMACOGENOMIC IMPACT ON PRIMAQUINE METABOLISM

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Plasmodium vivax malaria is a leading cause of malaria in U.S. service members and radical cure therapy requires a 2-week course of primaquine (PQ) to eliminate the latent hypnozoites and prevent relapse infection. Cytochrome P450 isoenzyme 2D6 (CYP2D6) is a key enzyme involved in converting parent PQ into the active anti-malaria metabolite. Allelic polymorphisms determine CYP2D6 genotype, and these differences result in variations in enzyme activity or phenotype. CYP2D6 phenotypes are assigned based on expected impact of enzyme activity on drug metabolism: no activity (Poor Metabolizers (PM)), decreased activity (Intermediate Metabolizers (IM)), normal activity (Extensive Metabolizers (EM)), and increased activity (Ultra rapid Metabolizers (UM)). Given the risk of *P. vivax* infection in U.S. Service members deployed to endemic

areas, we sought to characterize the prevalence of CYP2D6 genotypes and associated phenotypes of the enzyme in this population. Approximately 450 active duty personnel underwent CYP2D6 genotyping by a multiplexed cytometric bead array assay, Luminex xTAG® CYP2D6 Kit v3 (Austin, TX) allowing for detection of the major alleles in the United States: 1,2,3,4,5,6,7,8,9,10,11,15, 17,29, 35,41. A subset of volunteers were administered a one-time oral dose of 30 mg PQ. Blood and urine were collected at various timepoints in order to measure concentrations of PQ, carboxyprimaquine, and phenolic metabolites by ultra-performance liquid chromatography with mass spectrometry (UPLC-MS) and determine the pharmacokinetic (PK) parameters. The results of CYP2D6 genotypes and the effect of CYP2D6 phenotype on PQ metabolism will be presented. Results from this study will inform both DoD force health protection policy for the use of primaquine and future prospective *in vivo* studies of anti-relapse treatment of *P. vivax* infection.

1543

THE ROLE OF PRIVATE HEALTH CARE PROVIDERS IN ACHIEVING MALARIA ELIMINATION IN ACEH BESAR DISTRICT

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Malaria elimination is a goal of the Government of Indonesia. The District of Aceh Besar has promulgated Regent's Regulation No. 23/2013 which formally commits to achieve elimination by 2015. However, the role of private health care providers in progressing towards malaria elimination has not been identified. A survey of six types of private health care providers through a simple random sampling method has been conducted. Primary and secondary data were collected from 153 providers from March to August 2014 on occupational characteristics, availability of malaria-related supplies, knowledge of malaria, and involvement in the malaria elimination program. Data analysis was done using Chi-Square test and logistic regression with EPI Info version 7. The result showed that educational background (p=0,045), participation in malaria training (p=0,004), occupational characteristics (p=0,004) and knowledge of malaria (p<0,001) were associated with involvement in malaria elimination program. Additionally, roles of private health care providers in malaria elimination were predominantly influenced by having good knowledge of malaria (OR 8.1; 95% CI 3.8-17.5) and participation in malaria training (OR 2.7; 95% CI 1.2-6.3). The contribution private providers to officially reported data for 2013 showed that, 13.5% of suspected malaria cases were laboratory-confirmed, 7.4% of malaria cases were treated by ACT, and 5.6% malaria cases treated were reported to government. At pharmacies and drug stores, an average of 4 people sought medication for malaria daily, with pharmacies selling a mean of 38 Chloroquine tablets monthly. Private health care providers play a pivotal role in diagnosis, treatment, prevention and recording-reporting of malaria in area moving toward malaria elimination. The private providers in Aceh Besar falls far short of standards for diagnosis, treatment and reporting set by the public section. This highlights the need for established of an effective public-private network to ensure adherence to standards, effective monitoring, and good communication.

1544

VILLAGE MALARIA WORKER PERFORMANCE KEY TO THE ELIMINATION OF ARTEMISININ-RESISTANT MALARIA: A WESTERN CAMBODIA HEALTH SYSTEM ASSESSMENT

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Village Malaria Workers (VMWs) and Mobile Malaria Workers (MMWs) are a critical component of Cambodia's national strategy to eliminate *Plasmodium falciparum* malaria by 2025. Since 2004, VMWs have been providing malaria diagnosis through the use of Rapid Diagnostic Tests (RDTs), and free-of-charge Artemisinin-based Combination Therapies (ACTs) in villages more than 5 kilometres away from the closest health facility. This study aimed to assess the job performance of VMWs/MMWs, and identify challenges they face, which may impede elimination efforts. *Methods.* A mixed-methods assessment was conducted in five provinces of western Cambodia. 185 VMW/MMW participants were surveyed using a structured questionnaire. Qualitative data was gathered through a total of 60 Focus Group Discussions (FGDs) and 65 In-depth Interviews (IDIs). Data triangulation of the qualitative and quantitative data was used during analysis. Overall, VMWs/MMWs met or exceeded the expected performance levels (80%). Nevertheless, some performance gaps were identified. Misconceptions regarding malaria transmission and prevention were found among workers. The recommended approach for malaria treatment, Directly Observed Treatment (DOT), had low implementation rates. Stock outs, difficulties in reaching out to Migrant and Mobile Populations (MMPs), insufficient means of transportation and dwindling worker satisfaction also affected job performance. VMW/MMW job performance must be increased from 80% to 100% in order to achieve elimination. In order to do this, it is recommended for the national malaria program to eliminate worker malaria knowledge gaps. Barriers to DOT implementation and health system failures also need to be addressed. The VMW programme should be expanded on several fronts in order to tackle remaining performance gaps. Findings from this evaluation are useful to inform the planning of future activities of the programme and to improve the effectiveness of interventions in a context where artemisinin drug resistance is a significant public health issue.

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DESIGNING MALARIA ELIMINATION STRATEGIES TO ACHIEVE HIGH COMMUNITY UPTAKE: FINDINGS FROM A FORMATIVE RESEARCH STUDY IN THE DEPARTMENT OF GRAND ANSE, HAITI

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The success of malaria elimination efforts depends on high community acceptance and uptake of effective interventions. This is particularly challenging in low transmission settings, such as Haiti, where malaria is one of many competing health issues. Haiti has taken on the ambitious goal of eliminating malaria by 2020. To do so, aggressive strategies such as targeted mass drug administration (MDA) will likely be used. We conducted formative research to inform the design of elimination strategies using qualitative methods. Key informant interviews (n=6), in-depth interviews (n=9), and focus group discussions (n=7) were conducted from December 7-19 of 2015 with purposefully selected health agents, traditional healers, community leaders, and community members. Data were transcribed, coded and analyzed in relation to emergent themes. Findings indicate that elimination strategies should include multiple actors from the Haitian pluralistic health system. Results from social influence mapping suggest formal healthcare providers, as well as 'Hougans' [voodoo priests], would influence community uptake of interventions. Incentives and disincentives for both groups related to the goal of malaria elimination, and for working collaboratively, should be addressed. It is important to leverage community resources including community leaders (teachers, priests) and organizations (churches, social aid clubs, schools) so that positive messaging is reinforced across multiple sources, and uptake is modeled by appropriate social influences. This is especially important for harder-to-reach populations including youth and men. The intervention should prospectively target misinformation and rumors that may develop concurrent with roll-out. Uptake may benefit from ongoing monitoring of community perceptions during implementation of elimination strategies, and coupling traditional social marketing and communication techniques with social network strategies. Results from Haiti will likely have broader implications for other low transmission settings aiming to eliminate malaria.

1546

TARGETED COMMUNITY SENSITIZATION TO REDUCE ANTICIPATED REFUSALS IN MALARIA MASS DRUG ADMINISTRATION TRIAL: LESSONS LEARNED FROM SOUTHERN ZAMBIA

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The Zambia National Malaria Control Programme has embarked on an ambitious goal of eliminating malaria in Southern Province of Zambia and nationally by 2020. One component to accelerate toward the elimination goal includes selective use of mass drug administration (MDA) with dihydroartemisinin piperaquine (DHAp). As part of a recent trial to evaluate the effectiveness of MDA, 20 randomized health facility catchment areas were wholly targeted for this community based treatment strategy. In anticipation of research-based field activities, several community sensitization activities were conducted to promote the uptake of MDA at community, household, and individual levels in 2014 and 2015. High coverage was deemed essential to the effectiveness of MDA and refusals were monitored closely through household surveys carried out during treatment campaigns associated with the trial interventions. Brochures and job aids were pretested and targeted to community members and local leaders who might influence trial participation and treatment adherence. Radio scripts were developed, translated into the local language, and aired on local community radio stations as a program with recognizable jingles. Community entry meetings scheduled through the chief's palace ensured community members could hear directly from their local MOH staff about the study, have their questions answered, and enjoy drama group performances which emphasized key messages with the audience. Campaign surveys indicated the perceived benefits of participation usually outweighed possible hesitation. Refusal rate among those found at the households at the time of the campaign visits was only 2%. The greatest share of any incomplete coverage was attributed to absent household members. Effective community sensitization is key for successful implementation of treatment campaigns. Understanding and working through local community structures which are respected by community members, engaging traditional leaders, and working through local district-level health staff for community meetings all played a central part in achieving high levels of participation.

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SCHOOL-AGE CHILDREN ARE DISPROPORTIONATELY IMPORTANT *PLASMODIUM FALCIPARUM* TRANSMISSION RESERVOIRS IN SOUTHERN MALAWI

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Growing evidence from highly endemic malaria settings shows that *Plasmodium falciparum* infection prevalence peaks among school-age children (5-15 years old). These infections are frequently low density and asymptomatic, with less frequent antimalarial treatment-seeking than for younger children. The importance of school-age children to transmission persistence remains unknown. We evaluated the transmission contribution potential of young children, school-age children, and adults with data from southern Malawi. Six cross-sectional surveys were carried out in ~900 households from three districts at the end of the rainy and dry seasons of 2012 to 2014. To estimate the relative age-specific contributions to *P. falciparum* transmission, we populated simple mathematical models with data on population age distribution, PCR-based parasite prevalence and densities, gametocyte presence and density by qRT-PCR, and mosquito biting risk as modified by ITN use. Approximately 74.6% of gametocyte infections during the dry season and 57.9% during the rainy season are estimated to be among school-age children. While young children were more likely to have higher density infections by microscopy, infections among school-age children were the most likely to be gametocytemic, and age group was not significantly associated with density of gametocytes among gametocytemic individuals. Even if only half of gametocyte infections among school-age children and adults are assumed to be infectious, school-age children represent 68% of the infectious human population during the rainy season, and 52% during the dry season. Furthermore, in our survey, school-age children were less likely to sleep under bed nets than either adults or young children, and thus less protected from *Anopheles* feeding. When incorporating heterogeneity in ITN use, school-age children represent 82% of the gametocyte infections that are available for biting during the dry season, and 66% during the rainy season. Interventions that do not reach school-age children are unlikely to interrupt transmission in this and other highly endemic settings.

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MALARIA ELIMINATION CHALLENGES IN MESOAMERICA: EVIDENCE OF SUBMICROSCOPIC MALARIA RESERVOIRS IN GUATEMALA

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Although Guatemala remains one of the countries with higher malaria transmission in Mesoamerica, its incidence has decreased substantially since 2000. Guatemala is committed to eliminating malaria as part of the Malaria Elimination in Mesoamerica and the Hispaniola Island (EMMIE) initiative and is still in the control phase. During the past decade, the government strengthened malaria control activities, including mass distribution of long lasting insecticide impregnated bed nets, early diagnosis and prompt treatment. This study aimed to determine the prevalence of malaria in three areas of Guatemala using active case detection and molecular diagnostic tools that are more sensitive than microscopy. Cross-sectional surveys were conducted in three departments with varying transmission intensities; Escuintla, Alta Verapaz and Zacapa. Blood samples from 706 volunteers were screened for malaria using microscopy and quantitative polymerase chain reaction (qPCR) which was also used to determine the prevalence of gametocytes among infected individuals. Malaria was only diagnosed by microscopy in 2.8% (4/141) of the volunteers from Escuintla. By contrast, qPCR detected a prevalence of 7.1% (10/141) in the same volunteers, 8.4% (36/429) in Alta Verapaz, and 5.9% (8/136) in Zacapa. Overall, 7.6% (54/706) of the screened individuals were positive, with an average parasitemia level of 40.2 parasites/µl (range 1-1133 parasites/µl), and 27.8% (15/54) carried mature gametocytes. A total of 57.4% (31/54) of qPCR positive volunteers were asymptomatic and out of the 42.6% (23/54) of symptomatic individuals, only one had a positive microscopy result. A considerable number of asymptomatic *P. vivax* infections, mostly submicroscopic and with a proportion harboring mature gametocytes, was found in Guatemala. This pattern is likely contributing to the maintenance of transmission across the region. Robust surveillance systems, molecular diagnostic tests and tailored malaria detection activities in each endemic site may prove to be imperative in accelerating malaria elimination in Guatemala and possibly across all of Mesoamerica.

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ZAMBIA'S NATIONAL STRATEGY TO MOVE FROM ACCELERATED BURDEN REDUCTION TO MALARIA ELIMINATION BY 2020

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Zambia has made substantial progress addressing malaria and in many districts, malaria incidence has been reduced to levels where interruption of transmission may be feasible. This has been achieved through the commitment of national and local governments, keen interest among partners, momentum of scientific advances, and establishment of mechanisms to ensure proper coordination of activities with neighboring countries. Through a multi-stakeholder engagement process, Zambia has developed its first-ever National Malaria Elimination Strategy with the vision of eliminating malaria by 2020. All districts will be covered through a stratified step-wise approach based on a package of interventions addressing local transmission levels. The cornerstone of the strategy is the use of real-time, sensitive surveillance systems to detect, characterize and monitor all cases, at the health facility and community levels. As the first step, through a strong routine surveillance system, Zambia classified all districts according to their level of malaria transmission based on confirmed cases per 1000 population (Levels 0, 1-49, 50-199, 200-499,>500 case per 1000). The next step relies on optimizing vector control and case management—addressing current and local transmission intensities with the most appropriate and targeted population coverage of available interventions. To accelerate to elimination in selected areas, population-wide strategies to clear parasites using effective antimalarial drugs (such as mass drug administration) will be used in a time-limited manner, with the objective of bringing transmission down to a level where individual cases and small transmission foci can be appropriately managed through case investigation and community case management. Modeling suggests that this strategy will significantly reduce malaria across the country by 2020 and that regional collaboration will be key to sustaining success. Cost estimates will be presented for different scenarios, making the financial case for investing in malaria elimination in Zambia.

1550

TRANSITIONING AN EVIDENCE-BASED MALARIA MASS DRUG ADMINISTRATION (MDA) RESEARCH STRATEGY TO PROGRAM/ROUTINE MODE: FACTORS FOR CONSIDERATION

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The Zambia National Malaria Control Programme conducted a large-scale (60 health facility catchment areas serving nearly 330,000 participants) community randomized controlled trial to evaluate the effectiveness of treatment strategies on accelerating malaria elimination. The trial strategies included: mass drug administration (MDA), where all eligible individuals were treated with dihydroartemisinin-piperaquine (DHAp), and focal MDA (fMDA) where all eligible individuals residing in a household with at least one RDT positive member were treated with DHAp. Four treatment campaign rounds (two each year) were conducted during late dry season and early rainy season between December 2014 and February 2016. Informed by successful implementation of the trial, the new National Malaria Elimination Strategy 2016–2020 includes MDA as a key intervention. Reflecting on the successes and challenges of the recent MDA campaigns, this poster presents essential elements that implementers should consider when planning malaria MDA interventions. Trainings, logistics, and procurements were organized by health facility catchment area for decentralized operational management. Catchment teams were trained with household lists and maps for spatial orientation to maximize work flow and population coverage. Adverse event monitoring and post-marketing pharmacovigilance were conducted. Community mobilization was a prerequisite to maximize local participation. Diverse stakeholder groups were engaged for appropriate community authorization and access. Despite exclusion of children less than 3 months of age and women in early pregnancy, the trial achieved coverage rates as high as 87% and refusal rates among individuals present of less than 2% during house-to-house visits. Results from programme implementation experience will be presented in comparison to the trial methods.

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INCLUDING MOST-AT-RISK POPULATIONS IN HEALTH PROGRAM PLANNING AND IMPLEMENTATION: AN INTERCULTURAL COMMUNICATION FRAMEWORK TO SUPPORT MALARIA ELIMINATION AMONGST INDIGENOUS PEOPLES IN THE AMERICAS

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Central American countries need to improve malaria interventions with indigenous communities in order to eliminate malaria by 2020. A 2014 Amazon Malaria Initiative needs assessment found that national malaria control programs (NMCPs) in Central America had difficulty reaching these most-at-risk populations. Three cases in which malaria incidence had decreased in indigenous communities over the past 10 years were selected for further study. From 2015-2016, a literature review and in-depth, semi-structured interviews were conducted to examine intercultural approaches that may have contributed to lower malaria incidence in the selected communities. Approval to conduct interviews was granted by the Pan-American Health Organization and NMCPs of the Guatemalan, Honduran, and Panamanian ministries of health. In a Miskito community in Gracias a Dios Department, the Honduran NMCP worked with community members to achieve 100% coverage of a campaign to distribute and install long-lasting insecticidal nets. From June 2010 to June 2011, a reduction from 337 to 60 malaria cases was observed. In Bisira, in the Ngöbe-Buglé *comarca* of Panama, after the implementation of indigenous participation in health decision-making and environmental sanitation work, malaria cases fell from 71 in 2004 to zero cases by 2008. In the department of Alta Verapaz, Guatemala, local health authorities used a mobile phone system to improve surveillance, timely diagnosis, and treatment of malaria with the Q'eqchi Maya population. Findings were that the interventions studied prioritized community awareness and participation, integration of local languages, intercultural capacity development of health professionals, and adaptive design and implementation of culturally appropriate malaria interventions. The authors developed an intercultural communication framework based on best practices from the three cases. The resulting framework enables the adaptation of best practices to other contexts, in order to collaborate effectively with indigenous populations on malaria and meet the 2020 elimination target.

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ADVERSE EVENT REPORTING FROM MALARIA MASS DRUG ADMINISTRATION ROUNDS CONDUCTED IN SOUTHERN ZAMBIA

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The National Malaria Control Center of the Ministry of Health of Zambia is conducting a large-scale mass drug administration (MDA) community randomized controlled trial to evaluate the effectiveness of different MDA strategies on reducing malaria infections. The trial involved two strategies: MDA, where all eligible individuals were treated with dihydroartemisinin—piperaquine (DHAp), and focal MDA (fMDA), where all eligible individuals residing in a household with at least one RDT-positive member were treated with DHAp. Implementing MDA at such a large scale provides an opportunity to document the extent to which potential safety issues are reported or adverse events occur given the level of exposure to treatments. Field teams composed of community health workers, enumerators, and adherence monitors, and supervised by facility-based staff, received standardized training on the treatment campaign procedures, use of DHAp for eligible consenting participants, adverse event monitoring, grading of events, and emergency and event handling procedures.. Adverse events were recorded on standard forms and in line with recommendations from national pharmacovigilance network recommendations. The principle aim of this data collection activity was to document and follow up on all adverse events (AEs) and serious adverse events (SAEs) occurring during the course of implementing the MDA trial for individuals taking DHAp and reporting an adverse event to a catchment team member or local health facility. During 4 rounds of MDA community-based teams recorded all adverse events related to taking of DHAp. During the first two intervention rounds, over 280,638 participants were tested and 159,696 were treated with DHAp in 40 health catchment areas. A total of 687 AEs (0.24% of participants and 0.43% of treatments) were reported; one was recorded as a serious AE. The most common AE reported during the campaigns was stomach pains, followed by dry cough and vomiting; details and characteristics of persons with AEs will be reported. During this large MDA trial, the use of DHAp for malaria treatment was generally well tolerated.

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THE ROLE OF THE PRIVATE SECTOR IN SURVEILLANCE FOR MALARIA ELIMINATION IN HISPANIOLA: A CASE STUDY

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Hispaniola, an island in the Caribbean home to Haiti and the Dominican Republic (DR), is targeting malaria elimination by 2020. If accomplished, this binational goal would create a malaria-free zone across the Caribbean. Haiti bears the majority of the malaria burden with >95% of the total malaria cases on the island. A key priority for achieving elimination will be ensuring that all malaria cases are diagnosed and reported in a timely fashion. However, it is thought that many individuals with fever first seek care in both the formal and informal private health sectors, and there is limited information on how best to engage the private sector in Hispaniola in effective malaria case management and reporting. This project aimed to gain a better understanding of the private sector to inform both governments and partners on how to effectively integrate the private sector in malaria case reporting systems, and align private sector activities with national policies. A mixed-methods research design was used, comprised of a literature review, focus groups, and semi-structured interviews with key informants, private providers, and patients seeking care for fever. Private health sector is diverse and includes formal private, non-governmental, and mission hospitals and clinics, and informal shops, street vendors, and traditional healers. Preliminary results suggest that while the informal private sector is more utilized for care in Haiti than in the DR, in neither country does this sector have sufficient access to rapid diagnostic tests to confirm malaria cases, and to the national treatment strategy. In Haiti, care-seeking behavior is strongly influenced by spirituality, and those with more severe symptoms will often visit a traditional healer before they go to the formal sector. In the DR, visiting a traditional healer for a fever is not common, unless the cause is unable to be determined by a doctor. Traditional healers in both countries report referring patients with fever to formal healthcare facilities and would like to be more formally linked.

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COMPARING DATA FROM A MALARIA ROUTINE SURVEILLANCE SYSTEM TO HEALTH FACILITY SOURCE RECORDS IN ETHIOPIA AND SENEGAL

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The Governments of Ethiopia and Senegal are working toward achieving malaria-free zones in specifically targeted regions. Accurate, routinely reported information on malaria case rates is required to direct appropriate strategies to achieve this goal and to monitor progress. We assessed the accuracy of a rapid reporting system, with weekly malaria case rates reported to DHIS 2, in 2013 and 2014 at 20 health posts in Amhara Region, Ethiopia and at 13 health posts in Kanel, Linguère, and Ranérou districts in northern Senegal. Data on RDT-confirmed and treated malaria cases were extracted from paper registers at the health posts, including date of diagnosis, malaria RDT result, age, and area of residence Data from source records were compared to data reported in DHIS 2 during the 2013 and 2014 transmission seasons. In Ethiopia according to source records, the average weekly incidence of malaria per 1000 population across all 20 health posts was 0.85 (95% CI, 0.29-1.41) and 0.61 (95% CI, 0.02-1.21) during the major transmission seasons (September-November) in 2013 and 2014, respectively. The mean difference between DHIS 2 and source records in the number of malaria cases reported per week across all health posts was 1.33 (95% CI, 0.79-1.87) in 2013 and 0.17 (95% CI, -0.52-1.82) in 2014. In Senegal according to source records, the average weekly incidence of malaria per 1000 population across all 13 health posts was 53.08 (95% CI, 30.4-75.76) and 23.58 (95% CI, 12.94-34.22) during the major transmission seasons (July-January) in 2013 and 2014, respectively. The mean difference between DHIS 2 and source records in the number of malaria cases reported per week across all health posts was 1.86 (95% CI, 0.42-3.29) in 2013 and 0.22 (95% CI, -1.1-0.66) in 2014. Malaria cases by age (<5 and ≥5 years) and species in DHIS 2 had varying degrees of accuracy to source records. Travel history could not be ascertained from source records for most cases. Routine monitoring of the discordance between DHIS 2 data and source records combined with targeted retraining in health posts with higher levels of discordance may result in substantial improvement in the accuracy of DHIS 2 data.

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PRELIMINARY RESULTS OF THE THIRD MALARIA INDICATOR SURVEY IN ETHIOPIAN (MIS-2015)

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Malaria is among the major health problems in Ethiopia. Two Malaria Indicator Surveys (MISs) were conducted in 2007 and 2011 to measure the coverage and utilization of key malaria interventions, malaria parasitemia, and anemia. These surveys assessed the progress on scale-up of malaria prevention and control interventions. A follow up MIS 2015 was conducted between September and December 2015 to measure attainment of goals set in the 2011-2015 national malaria strategic plan. MIS 2015 was a population-based cross-sectional household (HH) survey. Two stage cluster probability sampling was used to select 555 enumeration areas from all malarious areas of Ethiopia. The survey followed standardized MIS guidelines that included the household and women's questionnaires that were uploaded on to smart phones using the Open Data Kit platform with GPS capability. A total of 100,159 HHs were mapped and 13,875 HHs were randomly selected. Overall, 64% of HHs had at least one long-lasting insecticidal net (LLIN) with an average of 1.8 LLIN per household; 32% of HH achieved universal coverage (1 LLIN per 2 persons). IRS had been conducted in 29% of HHs in the 12 months preceding the survey and 71% of HHs in malarious areas were protected by either a LLIN or IRS. Of children less than five years of age (U5), 45% slept under a LLIN the night before the survey, and 70% slept under a LLIN if the HH owned at least one LLIN. These figures were 43% and 71%, respectively, for pregnant women. Sixteen percent of children U5 had history of fever in the two weeks preceding the survey; of these, 38% sought medical attention within 24 hours of fever onset and 89% took an antimalarial drug. Malaria parasite prevalence in areas <2,000m was 0.6% by microscopy blood-slide examination and 1.4 % by rapid diagnostic test with regional variation. The results of the current survey document the sustained gains in malaria control in Ethiopia while highlighting gaps in current utilization of interventions.

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A LONGITUDINAL COHORT TO MONITOR MALARIA INFECTION INCIDENCE IN THE CONTEXT OF A COMMUNITY RANDOMIZED TRIAL OF MASS DRUG ADMINISTRATION IN SOUTHERN PROVINCE, ZAMBIA

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The Zambian National Malaria Control Center (NMCC) has embarked upon an elimination strategy in Southern Province and has recently conducted a community randomized trial to compare the effectiveness of mass

drug administration (MDA) and focal mass drug administration (fMDA; treating all persons in households with any rapid diagnostic test (RDT) positives) using dihydroartemisinin-piperaquine (DHAp) to standard of care interventions. In this setting we enrolled individuals into a prospective cohort in December 2014 during the first mass treatment round to assess community infection incidence over a 12 month follow-up period and evaluate the impact of MDA and fMDA. After clearing all identified malaria infections, the cohort consisted of monthly follow-up on 2,250 individuals from 60 health facility catchment areas and collection of parasite infection samples using RDTs and dried blood spots for molecular testing, fever and travel history, intervention coverage, and other risk factor data. Monthly entomological data were collected at a sample of cohort households, and monthly climate and environmental data linked to each cohort-month. A total of 1,388 individuals under 20 years of age and 750 20 and older were successfully enrolled. Cumulative infection incidence by RDT was highest for children under 5 (0.060 infections per person-month), and lowest for individuals 20 years and older (0.028 infections per person-month). Infection prevalence was highest in December 2014 preceding the start of the trial (7.2%) and lowest in October 2015 following the third MDA and fMDA round (1.4%). Cumulative infection incidence following trial implementation was lowest in the MDA arm (0.031 infections per person-month), highest in the control arm (0.048 infections per person-month), and intermediate in the fMDA arm (0.037 infections per person-month). Infection incidence in Southern Province has been reduced to the point where case-based elimination surveillance strategies are warranted: health facilities can now move to community case management and case and foci investigations and response to sustain these gains and seek local elimination.

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SURVEILLANCE SYSTEMS FOR ELIMINATION: LESSONS FROM RAPID REPORTING ACROSS FOUR COUNTRIES

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Data-driven decision-making in national malaria control programs in Africa is critical for the efficient use of resources across countries with diverse malaria burden. Despite the need for improved information, routine malaria surveillance throughout sub-Saharan Africa is known to have many challenges including under-representation of the true burden of malaria circulating in communities, as well as lacking quality and timely data reporting. As malaria control programs pursue malaria elimination, timely, reliable data becomes crucial to respond to potential resurgence and to target malaria transmission foci with appropriate interventions. During the past five years our project team worked with national malaria control programs in Ethiopia, Kenya, Senegal and Zambia to support different forms of electronic, paper-based and blended malaria surveillance systems. In Ethiopia the system covered 213 health facility catchment areas (HFCAs) in eight woredas; In Kenya the system covered 25 HFCAs in one sub-county; in Senegal the system covered 212 HFCAs in 3 regions; and in Zambia the system covered 446 HFCAs in three provinces. We assessed different attributes and characteristics of deploying each of the systems, looking at the barriers and facilitators to improving system functionality (quality and timeliness), the performance and ultimately the use of data by national and subnational decision makers in each country over a two year period. We explored different drivers of system deployment including organizational culture, technical infrastructure, data collection, storage and reporting processes and existing talent and skills of users in each country context. Our findings suggest that the selection of open source platforms such as DHIS2 and ODK and the training and mentoring of local staff at all levels can lead to a well-supported system that can produce quality information for decision making; we will further delineate the critical system components in the presentation.

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DEVELOPMENT OF A CLOUD-BASED DISEASE SURVEILLANCE AND RISK MAPPING (DISARM) PLATFORM FOR MALARIA ELIMINATION SETTINGS - CHALLENGES FROM AN IT PERSPECTIVE

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In 2015, there were around 214 million cases of malaria and 438 000 deaths. Malaria control efforts have cut the number of deaths in half. Despite the enormous burden of on sub-Saharan Africa, 90% of all malaria deaths in 2015, a number of countries on the continent are pursuing malaria elimination efforts.1 The ability to track and target malaria transmission is vital to the success of malaria elimination programs, especially in elimination settings, where transmission is rare and clustered.2 The DiSARM platform pulls surveillance and intervention data in real-time, combines it with climate and environmental data, automates analyses by running spatial models in Google Earth Engine, and produces risk & decision-support maps. A key feature of the platform is that non-experts can run spatial models to produce risk maps. After piloting DiSARM in Swaziland and Zimbabwe, the goal is to introduce the platform to all Elimination Eight countries in southern Africa. The development

of DiSARM involves a number of steps, which have already taken place or are scheduled for this year, an in-depth evaluation of current malaria surveillance data processing, in-country implementation in pilot countries and development of platform based on pilot findings. A major barrier to implementing DiSARM is that malaria surveillance systems are set up for reporting and monitoring & evaluation. Data are often transferred to the surveillance system in aggregate and transfer intervals are long and often manual. Both proprietary surveillance systems and widely used surveillance platforms like District Health Information Software are common. A process for implementing DiSARM in both scenarios has to be developed. The group of potential end-users for DiSARM is large and with diverse needs. The interface has to be customizable to the different tasks by the different users. The potential of DiSARM in supporting malaria elimination efforts is huge. Pilots will give valuable insight for the roll-out in more countries.

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DETECTING HIGH TRANSMISSION FOCI OF MALARIA IN A LOW TRANSMISSION SETTING: RESULTS OF A PILOT MALARIA MAPPING SYSTEM IN HAITI

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The National Malaria Control Program (NMCP) in Haiti is committed to eliminating malaria by 2020. National malaria parasite prevalence is <1%, but areas of relatively high transmission (foci) are heterogeneously distributed across the country. To detect malaria foci across space and time in this low endemic setting, the NMCP established a pilot system to identify laboratory-confirmed RDT and smear positive malaria cases from health facilities in Grande-Anse, Sud, and Sud-Est Departments and to geo-locate their residences. From October 2014 to September 2015, 1,419 (58%) of 2,462 confirmed cases from 57 health facilities were identified, mapped, and a surveillance questionnaire was administered. Less than 1% of all geo-located cases traveled outside their communes in the month before seeking health care. Mean age of malaria cases was 22 years (range: 0-97) and mean number of days with symptoms before care seeking was 2.9 days (range: 0-32). Case distribution was compared to a completely random spatial distribution using the Global Moran's I test; clustering was present in two of ten analysis areas ($p<0.01$). Two space-time permutation models were created in SatScan 9.4 to search for smaller significant transmission foci with parameters of a maximum cluster radius of 3km and two minimum time spans of 14 days and 1 month. In total, 13 statistically significant ($p<0.05$) space-time case clusters were detected; average radii was 844 meters (118 cases) for the 14 day clusters and 1.14 km (170 cases) for the 1 month minimum clusters. Cases were aggregated and joined to the 2014 LandScan dataset and malaria incidence rates were calculated for 1km2 areas. Local incidence rates were compared to a global rate using the Getis Ord-Gi* analysis and to regional rates with the Anselin's Local Moran's I analysis. The global method identified 43 1km2 foci ($p<0.05$) and the regional method detected 69 foci with five outlier foci (isolated areas with unusually high incidence rates). This system detected foci at differing time and spatial scales contributing to targeting of malaria control and elimination activities.

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EPIDEMIOLOGICAL AND OPERATIONAL LESSONS LEARNED FROM A MALARIA ELIMINATION CAMPAIGN IN ZAMBIA'S LAKE KARIBA REGION

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The Zambia National Malaria Control Centre is conducting an elimination campaign in the Lake Kariba region in Southern Province, with widespread scale-up of drug campaigns, via mass screen and treat (MSAT) and mass drug administration (MDA), and vector control. The survey data collected during the course of the campaign are highly informative with regards to the many factors that affect disease transmission; for example, analysis of local prevalence in the context of insecticide treated nets (ITNs) and indoor residual spraying (IRS) allowed the quantification of both individual and community-level protection for each method of vector control. Not to be disregarded, however, is the added value of the data for understanding the challenges of implementing surveillance and interventions across a large, dynamic population, spread out over a substantial and geographically varied region. Many of the features discovered in the data, such as a strong demographic dependence of coverage rates, and seasonal patterns of migration between smaller villages and larger centers, have major implications for the feasibility of elimination under existing protocols and suggest operational checks useful for similar programs across Sub-Saharan Africa.

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EMERGING INCURABLE MALARIA IN SOUTHEAST ASIA - A CALL FOR TARGETED, DECISIVE ACTION IN THE REGION

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Clinically nearly incurable strains of malaria have now reached Vietnam from Cambodia. Historically drug-resistant strains emerge in Cambodia and then spread to Africa. The new strains are not just artemisinin-resistant; they are resistant to nearly all antimalarial drugs. From 2001 to 2015, approximately 6.2 million malaria deaths were averted through massive global investments. Malaria mortality dwarfs that of highly publicized recent outbreaks (11,325 for Ebola, 774 for SARS and very few for Zika virus). The impressive gains with malaria are at risk of being reversed; decisive action must be taken to eliminate them. Published reports demonstrate dihydroartemisinin-piperaquine (DHA-PIP)-resistant malaria was present in Cambodia in 2010. Unfortunately, attempts to contain these parasites have failed - new approaches are urgently needed. In Binh Phuoc Province, Vietnam, in 2015, a PCR-corrected 32% (14/44) late clinical treatment failure rate was documented (K13 genotyping, drug levels pending). A confirmatory trial with early similar results is on-going. Current results and available data from Cambodia will also be presented. To address this emerging threat regionally, current M&E reports, strategies and recommendations for malaria elimination and epidemic preparedness are being critically reviewed and compared. Our team has also developed a simple red light-green light system for cross-border emergency operations centers to be able to visualize, target and monitor real-time rapid responses and on-going adherence. The same solutions and methods needed to eliminate malaria will enhance the infrastructure and

provide indispensable experience in preparation for the next global threat. We will discuss why we believe these new malaria strains should be part of the Global Health Security Agenda. In conclusion, DHA-PIP treatment failures are now in Vietnam; the first reported spread beyond Cambodia. We recommend emerging incurable malaria be addressed and resourced as a crisis. Malaria and any threat agent can be rapidly addressed using the same new approaches.

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MALARIA ERADICATION: ARE REGIONAL INITIATIVES CRITICAL TO ITS SUCCESS

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There has been major progress towards global malaria elimination within the past decades, with an estimated 1.2 billion cases and 6.2 million deaths averted globally since 2001. However, there is increasing evidence that individual ministries of health and national malaria programs may face major challenges in 'getting to zero' in isolation. As such regional cooperation is critical for continued progress; however there is limited guidance for the structure, planning or functional roles of regional cooperation. To address this, we review a range of global malaria initiatives, and provide practical guidance for future programs.

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VIETNAM MALARIA STATUS UPDATE AND PLAN TO ELIMINATE EMERGING INCURABLE MALARIA STRAINS

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Clinically artemisinin combination therapy (ACT)-resistant of *Plasmodium falciparum* (Pf) has now emerged in Vietnam. New WHO guidelines recommend their elimination by 2020. Since 1991, Vietnam has successfully reduced malaria cases by >97%. So that these impressive gains are not reversed, elimination of the emerging resistant strains is imperative. In 2015, Pf decrease by 47% nationwide as a result of many factors. The major exception was Binh Phuoc Province, where clinical ACT-resistance emerged, which likely caused the 32% increase in incidence (the same pattern was seen in Cambodia). A country plan to eliminate ACT-resistant Pf has been developed. The major challenges identified are as follows: 1) drug resistance, 2) forest-goers/seasonal workers, 3) financial support, 4) access to timely, essential information, 5) cross-border and intersector collaboration. Potential solutions to the challenges will be presented. Some examples are as follows. Interagency meetings are being conducted to raise domestic funding. The US Navy sponsored "enhanced surveillance and operations research" has identified unmet needs which can guide new intervention programs. Lastly, partnerships with the military are being explored to utilize the existing network of health care facilities and military personnel operating in and near malaria endemic zones. In conclusion, we present a path to resource ACT-resistant Pf for rapid elimination. New tools and collaborations now make elimination possible. As malaria disappears, the same enabling factors can be applied to other health challenges related to poverty (e.g. malnutrition). We can both lead the elimination of ACT-resistant malaria and the next global health security issue that emerges.

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U.S. NAVY-NIMPE COLLABORATIONS AND OPERATIONS RESEARCH SUPPORT OF THE MALARIA ELIMINATION PROGRAM IN VIETNAM

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US Navy collaborations with the Vietnamese National Institute of Malariology, Parasitology, and Entomology began in 2014 with an Enhanced Surveillance and Operations Research for Malaria Elimination project. Phu Yen Province in central Vietnam, near areas of highly drug-resistant malaria, is the lead study site. To better understand how to accelerate malaria elimination, our team conducted surveys in preparation for future operations research. In 2015 a survey was completed of 100 households with and 100 households without confirmed malaria in three communes of Western Phu Yen. The survey included data collection on known risk factors, occupation, use of bed nets and preferences for potential interventions. The malaria burden by self-reported occupation was: paper planation work (47%), agarwood harvesting (15%), farming (13%), charcoal production (9%), trapping (6%), timber harvesting (4%) and hunting (3%). Although total cases were lower, greater proportions of farmers, charcoal producers and hunters suffered from the disease suggesting they may be an important part of the transmission reservoir. Overall, treated net use was low (19% in risk areas), despite households having treated nets (mean = 2.8). Households reporting not using a treated net had a higher risk of malaria (OR 2.6, p=0.05). A majority of forest-goers (85%) reported dislike of nets provided by public health programs, e.g. Global Fund long lasting insecticide-treated nets; 82% of forest-goers indicated a desire for hammocks with a zip-in treated net. When asked about preferences for future interventions, 94% were willing to use malaria prophylaxis and 90% mosquito repellent. In conclusion, the majority of individuals at greatest risk of malaria in our study area did not report routine use treated bednets or other malaria prevention products. Based on these preliminary findings an updated survey is planned for 4800 households and all transmission hotspots in 2016 to confirm the 2015 findings. These activities will provide an evidence base to plan operations research to tailor interventions for malaria elimination.

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AN INNOVATIVE INFORMATION SYSTEM TO ELIMINATE EMERGING INCURABLE MALARIA

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Malaria resistant to nearly all drugs is again emerging in Southeast Asia, which must be rapidly eliminated. The goal of surveillance for malaria elimination is to capture every malaria case and execute a prompt and effective response. Here we describe the real world evaluation of an effective information system for rapid reporting, case investigation and response. Three communes in western Phu Yen (PY) Province, central Vietnam were selected as the lead study area. Sony smart phones, KLL collect data, and the Ona server were down-selected for data capture and management. MapInfo Professional® was utilized for enhanced mapping.

Five data capture forms were developed and iteratively improved during the study period of January to September 2015. GPS coordinates in the transmission sites were captured for 89% of 64 cases from January to September 2015. Fourteen transmission foci were identified, defined as more than one case within a one km radius, which accounted for 80% of cases. The majority of cases (97%) were from people living in PY. Forest (86%) and forest fringe (14%) were identified as the probable transmission sites. Only 16% of cases reported sleeping under a treated net, 30% an untreated net and 54% without any net. The sleeping sites were classified as houses (10%), huts (60%) and hammock only (25%). Routine follow-up forms were tested in 20 households; 45% were probably using, 35% were possibly using and 10% were not using the net provided to their household. By self-reporting, 10% reported not regularly using their net, which increased to 25% when the picture evidence of non-use was included. Weekly (zero) and monthly quality reporting forms were also successfully evaluated. New results and plans to expand to high-priority areas also will be presented. In conclusion, the system was found to be user-friendly. The use of pictures revealed discordance between self-reporting and actual net use. The described transformative technology will help National Malaria Control Programs and partners improve the quality and targeting of interventions. This new approach will facilitate rapid elimination of malaria.

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THE FORMULA "AVERAGE PLUS TWO STANDARD DEVIATIONS" APPLIED TO THE FOLLOW-UP OF THE MALARIA EPIDEMIC WARNING THRESHOLD OF THROUGH 24 SITES SENTINELS OF SURVEILLANCE IN SENEGAL

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To predict and manage malaria epidemics, the Senegal National Malaria Control Program (NMCP) created an epidemiological surveillance system of rapid detection. This malaria sentinel system, set up in 2008, includes 24 sentinel sites, 18 of which are distributed in low to moderate transmission zones at risk for epidemics. Using a standard Excel spreadsheet, sites report the total patients; suspected malaria cases, patients tested, and confirmed malaria cases every week. The quality of the transmitted data is assured through quarterly supervision of sites integrating on-site data verification. Since 2009, 100% of suspected malaria cases have been tested by rapid diagnostic tests, and 100 % of positive cases received artemisinin-based combination therapy. In 2015, the NMCP introduced a method of calculating epidemic threshold using historical data for each site dating back five years. For every site, a standardized Excel worksheet is used to calculate and draw a curve of the epidemic threshold. The following formula is used for calculation of the epidemic threshold: weekly average of the cases for a given epidemiologic week over the previous five years plus two standard deviations. During the 45 epidemiologic weeks of 2016, the reported cases were systematically compared with the epidemic threshold curve weekly. This formula proved to be very sensitive, with detection of 99% of potential epidemic situations during 2016, compared to 25% using a threshold based on a formula of simple averages. In every sentinel site if the threshold is met or exceeded, a systematic documentation of the reported cases is made. In zones of moderate or high transmission, specific actions are taken based on the results of this documentation. In zones of low transmission, the threshold is one case, and every case is systematically documented and investigated. Since implementation, four sites reached or surpassed the warning level more than four times. Investigations showed that 80% of cases were imported, and 20% of autochthonous cases did not sleep under insecticide-treated nets.

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INCREASE OF MALARIA TRANSMISSION IN MILITARY CAMPS IN TANZANIA

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Malaria remains a public health problem for many Tanzanians. Parasitemia prevalence in the population has declined markedly from 78.4% (2003) to 13.0% (2008). Recent studies have reported high prevalence in Kigoma (26%), Mara(25%) and Kagera(37%). We investigated malaria burden in eight military camps in seven regions of Tanzania, Coastal, Tanga, Kigoma, Morogoro, Mara, Tabora and Kagera regions. Malaria surveillance was conducted using the Deki mRDT Reader for 2 years and malaria prevalence survey of asymptomatic military recruits at four military camps. Finger pricks were used to conduct malaria mRDT and blood smear microscopy. Studies to determine the malaria attack rate for recruits from endemic regions and nonendemic areas were performed. Consented recruits were screened negative enrolled and followed-up biweekly for six months. Malaria case detection with the Deki mRDT Reader showed increased positivity rates (PRs) over time. At Ruvu, PRs increased from 11.8 % (2013) to 15.7% (2014) to 30.9% (2015). PRs increased from 25.2%(2013) to 37.5%(2014) and to 46.2%(2015) for Mgambo and from 36.4%(2014) to 37.0%(2015) for Rwamkoma Bulombora site showed a decreasing PRs, other military camp sites showed an increasing PRs. malaria attack rates in the Ruvu and Mgambo camps were 13% and 43.3% respectively. Malaria prevalence rates by mRDT for asymptomatic recruits were 2.8% (Bulombora), 5% (Ruvu), 47.5% (Mgambo) and 39.4% (Rwamkoma). Malaria prevalence rates by microscopy were 3.4%, 5.2%, 49% and 38.5% for Ruvu, Mgambo and Rwamkoma respectively. Generally, there is an increasing trend of malaria prevalence in these areas which could be an indicator of similar trends countrywide. Further studies and an intervention plan are clearly recommended to address this public health problem.

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RISK FACTORS INVOLVED IN THE EPIDEMIOLOGY OF MALARIA IN MILITARY CAMPS IN TANZANIA

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A total of 1380 patients were interviewed from Rwamkoma and Maramba camps. More than one quarter (29%; 400/1380) of respondents had never used insecticide treated nets (ITNs) as 31.8% did not slept always under an ITN 30 days prior to interview. Respondents who reported not using ITNs had a statistically significant probability to be diagnosed with laboratory confirmed malaria ($p<0.0001$). Factors such as male gender (35.7%; 280/784) or age <17 years (49.4%; 44/89) were found to be statistically significant contributing factors to developing malaria. Although the results were not statistically significant, patients coming from malaria non-endemic districts of origin contributed to higher rate(35.2%; 25/71) of laboratory confirmed cases than patients from malaria endemic districts of origin (31.2%; 380/1218). Patients diagnosed clinically to have malaria were statistically more likely to have laboratory confirmed malaria than those diagnosed clinically free from malaria ($p<0.0001$). Patients that presented at health facility with fever of greater than 3 days were statistically more likely to have confirmed malaria than patients presenting with a fever of less than 3 days ($p<0.0001$). Although statistically not significant, patients with clinical symptoms > 5 of malaria (≥5) were more likely to have confirmed malaria than those with <5 symptoms of malaria (35.5%; 39/110). Patients who reported to have not completed a full

regimen of antimalarial treatment in the two months prior to the study were more likely to test positive for malaria than patients who completed a full course of treatment (60%; 3/5). In summary, factors associated with the epidemiology of malaria included prior experience using ITNs, gender, age, delay in seeking treatment and clinical symptom presentation.

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ESTIMATING THE MALARIA ATTACK RATE IN TANZANIAN MILITARY CAMPS. MALARIA EPIDEMIOLOGY IN SELECTED MILITARY CAMPS IN TANZANIA

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In Tanzania, malaria ranks number one cause of morbidity and mortality, accounts for over 32% of the National disease burden. There is high heterogeneity of malaria transmission depending on topographical features and climatic conditions. The aim of this study was to look at malaria attack rate among recruits entering training camps in highly endemic areas. Recruits eligible to study in Mgambo camp -Tanga, were randomly selected by multistage sampling; consented and followed for six months. Fortnightly malaria smear was collected. Blood samples for serological tests were collected. Microscopy was the gold standard method for malaria diagnosis. Data was subjected to univariate and multivariate analysis, logistic regression model was used to identify the risk factors. Among 549 recruits who were involved in this study, 31.7% (174) were malaria positive. Among positive cases, those who didn't sleep under treated net were found to have significantly increased odds of being malaria positive [OR: 7.71; 95% CI: 1.01-58.61; P=0.048]. Travelling outside the camps increased odds of being malaria positive [OR: 1.25; 95% CI: 0.87-1.79; P=0.232]. There was significant difference between malaria positivity and place of travel [X^2=40.1; P=0.015]. Female recruits had 54% significantly reduced chances of being malaria positive [OR: 0.46; 95% CI: 0.28 - 0.77; P=0.003]. This study revealed failure to use bed nets and travel are major drivers of malaria infection. Identification of gaps in net use, knowledge, relevant types of human movement and development of strategies addressing travel is highly recommended. Mgambo camp in high malarial endemic area is an ideal site for malaria drug prophylaxis or vaccine studies, where TPDF recruits from various transmission intensity areas trained under similar environment.

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CHALLENGES OF MEASURING ITN EFFICACY IN HIGH TRANSMISSION SETTINGS: AGE, SEASON AND DETECTION METHODS

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After universal distribution of insecticide treated nets (ITNs), malaria prevalence remains high in Malawi. Understanding reasons for the limited impact of ITNs on malaria control is critical for identifying methods to decrease the malaria burden. Previous studies focus on the impact of ITN use among children under 5. However, prevalence of infection is highest in school aged children (SAC), ages 5 to 15. We examined the effect of age, season, and detection method on estimates of ITN impact in Malawi. Six cross-sectional surveys using cluster random sampling were conducted in the rainy and dry seasons in southern Malawi from 2012 to 2014. Data were collected on household ITN usage and demographic variables. Blood samples for detection of *P. falciparum* infection were obtained from all household members who were present and over six months of age. Statistical analyses used generalized linear mixed models to account for clustering at the household and neighborhood level. We conducted microscopy and qPCR on ~17,500 individuals. Prevalence was higher in SAC compared to children under 5 and adults using both detection methods (15% vs. 8% and 5% by microscopy and 23% vs. 11% and 11% by qPCR respectively). The association between ITN use and infection was modified by season in SAC, but not in others. Using microscopy, ITN use was associated with protection in all age groups and all seasons (SAC in the rainy season: OR = 0.67 (95%CI: 0.46, 0.97), SAC in the dry season: OR = 0.57 (0.37, 0.88), and non-SAC: OR = 0.73 (0.56, 0.94)), but there was no protective association among SAC in the rainy season when using qPCR results (OR = 0.78 (0.56, 1.10)). Sensitive detection methods reveal a lack of uniformity in the impact of ITN use, with seasonal variation among SAC when using qPCR results. This may be due to inadequate protection of ITNs when mosquito density is high or the persistence of sub-microscopic infections over time. Single time-point cross-sectional surveillance of children under five using microscopy alone may fail to capture the community impact of ITN use on prevalence. SAC may represent persistent reservoirs of transmission and may require targeted interventions.

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DECLINING MALARIA BURDEN IN UGANDA BETWEEN 2009 AND 2014: EVIDENCE FROM THE MALARIA INDICATOR SURVEYS

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Malaria remains a major public health problem in Uganda. Although there is still on-going transmission and the entire population remains at risk of malaria infection, results from the 2014 national malaria indicator survey showed improvement in key malaria indicators. In this study, we present evidence of declining malaria burden in Uganda by comparison of key population-based malaria indicators from the 2009 and 2014 national malaria indicator surveys. We extracted and compared data on malaria biomarkers between the 2009 and 2014 national malaria indicator surveys for population-based estimates of parasitemia and anaemia as key indicators. Both surveys used a comparable sample of children aged 0-59 months who were all tested for malaria and anemia as the major outcomes. Improvement in key indicators was determined by analyzing the differences in proportions of parasitemia and anemia between the 2009 and 2014 surveys. There was a reduction in parasitemia from 42% in 2009 to 19% in 2014 (difference= 23% CI: 21.1-24.9, p < 0.001). Severe anemia (hemoglobin <8 g/dl) decreased from 9.7% in 2009 to 4.6% in 2014 (difference=5.1% CI: 3.9-6.1, p <0.001). In both surveys, parasitemia was significantly higher in older children 48-59 months 25.9%, p<0.001 and 53.2%, p<0.01 in 2009 and 2014 respectively. Although *Plasmodium falciparum* mono-infection and *P. falciparum* combined with other species constituted 97.1% of the malaria speciated in the 2014 survey (down from 99.1% in 2009), there was general increase in prevalence of non-falciparum species: *P. malariae* from 2% to 6%, *P. ovale* from 0.02% to 1.3%, *P. vivax* from 2% to less than 1% in 2009 and 2014 respectively. These results provide strong evidence of the declining malaria burden in Uganda between 2009 and 2014.

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PROXIMITY TO ENVIRONMENTAL RISK FACTORS INFLUENCES SPATIAL PATTERNING OF *PLASMODIUM* INFECTION PREVALENCE IN DANGASSA, MALI

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Malaria is highly seasonal throughout Mali, with large-scale differences in transmission across the broad range of Saharan to Sahelian to Sudano-Guinean habitats. In the tropical savanna climate of Sudano-Guinean southern Mali, transmission is seasonally intense and spatially heterogeneous. This study sought to characterize community-level spatial patterns of malaria and examine fine-scale environmental factors that may influence transmission. A cross-sectional study of 1,063 people in 190 households of Dangassa, Mali was conducted during September 2012 (end of rainy season). *Plasmodium* (primarily *P. falciparum*) infection was determined by standard microscopy, and peri-domestic land cover (crops, trees, etc.) around each dwelling was observed/GPS-located in the field (2015) and through satellite images (2013). Distances to environmental features and health-relevant locations were calculated through GIS. Household-level, multivariate linear and geographically-weighted analyses and individual-level logistic regression was performed to evaluate demographic and environmental associations with malaria, as were spatial cluster analyses. Overall, 431/1063 (40.5%) of community members were *Plasmodium*-positive, with 5-10 year-olds exhibiting the highest prevalence of infection (64.8%). Household-level prevalence was positively associated (increased) with distances to the health center and to paths, but negatively associated (decreased) with distance to forest and breeding sites. Curiously, nearby crop cover reduced household and individual infection risk. Household-level clustering of infection was demonstrated, with geographically-weighted regression producing a better model fit than linear regression. Environmental risk factors contribute to variance in malaria risk at fine spatial resolutions. Geographically-weighted regression may be useful in determining areas of increased malaria prevalence.

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EXPLORING ENVIRONMENTAL FACTORS MEDIATING SPATIOTEMPORAL VARIATION IN VECTOR CONTROL IMPACT IN SUB-SAHARAN AFRICA 2000-2015

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Previous work by the Malaria Atlas Project (MAP) has led to a detailed spatiotemporal reconstruction of the changing landscape of *Plasmodium falciparum* risk in sub-Saharan Africa since the year 2000, and an understanding of the overall contributions of vector control (primarily Insecticide treated bednets, ITNs, and indoor residual spraying, IRS) in driving these changes. However, the patterns of declining transmission are not uniform, and the likely impact of existing vector control varies substantially from place to place. Understanding what influences these variations in impact can help inform thinking around novel vector control approaches that may be needed to address this 'residual' transmission. Here, we develop a suite of relevant environmental covariates and a geospatial modelling framework to explore factors influencing observed trends in transmission. Of particular interest are aspects of the biophysical or human environment that have either: (i) mediated the impact of existing vector control interventions, particularly insecticide treated bednets (ITNs) and indoor residual spraying (IRS); or (ii) led to declines in transmission independent of vector control or other intervention efforts.

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RISK FACTORS FOR DEATH DUE TO SEVERE MALARIA IN CHILDREN UNDER FIVE YEARS, KALEMBE-LEMBE PEDIATRIC HOSPITAL OF KINSHASA, DEMOCRATIC REPUBLIC OF CONGO, 2012-2014

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Malaria remains a major public health problem in the DRC. Its prevalence in children 6-59 months old is 31% and up to 30% of pediatric deaths are due to malaria. The aim of this study is to determine the risk factors of death in severe malaria in children under 5 years old. A case-control study is conducted in 2015 at the Kalembe-Lembe pediatric hospital (Kinshasa). Cases (n = 71) were children hospitalized for severe malaria and whose outcome was fatal. Controls (n = 142) were children hospitalized for severe malaria and whose outcome was favorable. A questionnaire was used to collect the indicators (age, sex, weight, hyperthermia, nutritional status, delay of treatment at the hospital ≥ or < 24 hours, socioeconomic level…) from children's mothers or guardians. The delay of treatment is the time between the first symptoms and the treatment at the hospital. The socioeconomic level is about 5 variables. Each variable has a value of 1 in the affirmative. The socio-economic level was good if the score was ≥ 4 and low if it was < 4. Data were analyzed using Epi-Info7 software. The risk of death was estimated through the OR 95%. Both cases and controls were separately 14 months of median age with a maximum of 59 months. The median weight was 9kg (3-22Kg) for cases and 10kg (5-24kg) for controls. Concerning cases, 32.4% of children (23/71) had a poor nutritional status (Z-score < -2 SD) and 80.3% (57/71) of their households had a low socioeconomic level. In 59.2% of cases (42/71), the delay of treatment was ≥ 24 hours. Risk factors associated with the occurrence of death to cases included poor nutritional status [adj OR = 2.24 (1.16-4.33)], low socioeconomic level [adj OR = 2.17 (1.08-4.35)] and delay of treatment ≥ 24 hours [adj OR = 2.05 (1.04-4.03)]. However, no association was found between death from severe malaria and rural residence, self-medication, hyperthermia and the knowledge of the mother/guardian on malaria. In the fight against malaria, malnutrition, delay of treatment ≥ 24 hours and the low socioeconomic level are risk factors considered as being associated with this disease. It is therefore imperative to intensify the awareness of these risk factors.

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MALARIA RESURGENCE IN WESTERN KENYA HIGHLAND

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In the past decade malaria-induced morbidity and mortality were significantly reduced through the deployment of insecticide-treated nets (ITNs), indoor residual spraying (IRS) and artemisinin combination therapy (ACT). Despite very high coverage of ITNs, here we reported rapid malaria resurgence in the highlands of western Kenya. Longitudinal cross-sectional surveys of malaria prevalence in school-aged children were conducted monthly in Kisii from 2003 to 2015. Monthly clinical malaria incidence was obtained from a sub-district hospital. Indoor-resting malaria vector densities were determined and cross-sectional household surveys of ITN ownership were carried out. Malaria infection rate in school-aged children was reduced from monthly average of 15.9% in 2002 before

the introduction of SP+AQ, to 3.0% before the introduction of ACT in early 2006. It remained very low from 2006 to 2013 (monthly average of 0.6%), but it increased sharply to 5.9% in 2014 and 10.2% in 2015. Indoor resting vector density was 1.5 females/house/night (f/h/n) during high season in 2003, it remained very low from 2004 to 2008 (0.03 f/h/n), however, it gradually increased since 2009 and reached 1.8 f/h/n in 2015. Malaria vectors were shifted to from An. gambiae to An. funestus as the dominant vector. Household ITN ownership increased gradually from 11.7% in 2003 to 87.4% in 2015. Despite consistently high coverage of ITNs, malaria infections and indoor resting vector density rebounded dramatically in the past few years in western Kenya highland. There is a renewed fear of malaria epidemic in western Kenya highland, calling for urgent and improved malaria interventions being placed in these epidemic-prone highland areas.

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RESULTS AND RECOMMENDATIONS FROM THE 2015 MALARIA INDICATORS SURVEY (MIS) IN ZAMBIA

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National, population-based cross-sectional surveys such as Demographic and Health Surveys (DHS) and Malaria Indicator Surveys (MIS) provide current and historic estimates for comparing malaria infection and intervention coverage to measure progress toward national and international targets. In Zambia, surveys were conducted in 2001-2002 (DHS), 2006 (MIS), 2007 (DHS), 2008 (MIS), 2010 (MIS), 2012 (MIS), 2013-2014 (DHS), and 2015 (MIS). We reviewed information from the 2015 MIS and its cluster randomized 3,750 households to evaluate recent progress toward elimination and compared findings with prior surveys to examine lessons from 15 years of malaria control in Zambia. In 2015, 77.0% of households had at least one insecticide-treated mosquito net (ITN) and 63.9% of households reported sufficient ITNs to cover all sleeping spaces; 55.1% of individuals reported sleeping under an ITN the night before the survey. Indoor residual spraying reportedly occurred in 28.9% of households in the previous twelve months. The overall malaria prevalence in children was 20.3% and varied by province from 0.5% to 32.5%. Despite high coverage and use of key interventions, malaria parasite prevalence increased overall to a prevalence of 20.3% in 2015 compared to 14.9% in 2012. The increase in malaria prevalence despite high sustained coverage suggests that additional strategies will be needed to move Zambia towards elimination. The 2015 MIS also collected information relevant for monitoring the progress of the roll out of integrated community case management (iCCM), intermittent preventive treatment during pregnancy (IPTp), case management, and treatment-seeking behavior. Information on these indicators will also be reported. Overall, the 2015 MIS provides the most comprehensive assessment of Zambia's current malaria state, demonstrates that tremendous progress has been made in Zambia, and is helping to inform the planning process for future elimination efforts.

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MALARIA EPIDEMIOLOGY IN LOW ENDEMICITY AREAS OF THE NORTHERN COAST OF ECUADOR

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The recent scale up in malaria control measures in Latin America has resulted in an impressive decrease in the number of reported cases in several countries including Ecuador, with a very low malaria incidence in recent years (544 reported cases in 2012 and 377 cases in 2013) and occasional outbreaks of both *Plasmodium falciparum* and *P. vivax* in the coastal and Amazonian regions. This success in malaria control in recent years has led Ecuador to transition its malaria policy from control to elimination. Nevertheless, it is unlikely that current interventions will lead to malaria elimination in the country unless asymptomatic parasite carriers are identified and treated. This study reports the general knowledge, attitude and practices (KAP) about malaria, as well as its prevalence in four communities of an endemic area in northwest Ecuador. A total of 258 interviews to assess KAP in the community were evaluated showing that most people in the study area have a basic knowledge about the disease. Six hundred and forty-eight blood samples were collected and analyzed by thick blood smear (TBS) and real-time PCR, as well as by serology using ELISA and immunofluorescence. In addition, the distribution of the infections was mapped in the communities. The total malaria prevalence by PCR was 7.5%, comparable to that reported in endemic areas of neighboring countries with higher malaria transmission, indicating a much higher prevalence than expected. Results suggest that the transition from control to elimination strategies in a country like Ecuador would demand an improvement in malaria diagnostics to detect parasite asymptomatic carriers, as well as studies on the bionomy of *Anopheles* mosquitoes with potential vectorial capacity.

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MALARIA INDICATORS IN MALANGA, KIMPESE HEALTH ZONE IN DEMOCRATIC REPUBLIC OF THE CONGO

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Background Malaria remains a public health problem in the DRC with a morbidity and mortality highest in the world. Knowledge of different indicators to better undertake the fight against this epidemic Methodology We conducted a community survey in 60 households in the villages Malanga, Nkumba, Zamba and Malanga Station. We also conducted human landing catches.Finally we conducted *Anopheles* susceptibility testing according to the WHO protocol Results The overall prevalence of infection by *Plasmodium* to Malanga was 41.4% with 43.4% for the age group of 0-5 years, 69% for the portion of 6 to 10 years and 59.1% for range 11 to 15 years. The capture of *Anopheles* returned 186 specimens with 5 species *A. gambiae* (83.8%), *A. funestus* (11.3%), *A. nili* 3.2%; *A. moucheti* 0.5% and 1.5% *A. tenebrosus*. The evaluation of the sensitivity of the *Anopheles* to insecticides found broad a total susceptibility to bendiocarb; Susceptibility to deltamethrin and permethrin was 53.4% and 23.7% respectively conclusions: Malaria Indicators to Malanga not allow to glimpse an effective control of malaria.

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UNDERSTANDING VULNERABILITY AND RESILIENCE OF INDIVIDUALS TO *PLASMODIUM FALCIPARUM* INFECTION IN A STABLE MALARIA TRANSMISSION AREA OF DANGASSA, MALI

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The goal of this study was to examine the factors associated with the absence or presence of persistent *Plasmodium falciparum* infection (parasitemia) among subjects living in the stable high transmission area of Dangassa in Mali. In a cohort of 750 children and adults examined during 4 consecutive surveys, we identified 89 subjects with positive thick smears at all visits (persistent positives) and 91 subjects with negative thick blood smears at all visits (persistent negatives) between August 2012 and June 2014. A household survey was also performed to define the level of exposure to malaria control interventions and tools and characterize sociodemographic conditions. During each survey, clinical and laboratory examinations were performed in all subjects to estimate the frequencies of symptomatic and asymptomatic parasitemia. In addition, passive case detection was performed at the health center to estimate the incidence of uncomplicated and severe malaria in the community. Most persistently-negative subjects were adults (62.6%). In contrast, most persistently-positive subjects were children less than 17 years of age (98.9%). Persistently-negative subjects were more likely to seek care for malaria at the health clinic (72.5%) than persistently-positive subjects (34%) and the use of insecticide-treated nets was greater among persistently-negative (61.5%) than persistently-positive subjects (38.2%). Finally, persistently-positive subjects had 64% less risk of severe malaria than persistently-negative subjects (Relative Risk 0.36; 95% CI = [0.16, 0.8]. In conclusion, most persistently-positive subjects were asymptomatic and sought malaria treatment at the health center clinic less frequently than persistently-negative subjects. However, the substantial numbers of persistently-positive subjects in Dangassa provide a reservoir for the continued transmission of malaria in the community, particularly during the dry season.

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MALARIA PARASITE POPULATION STRUCTURE AND HUMAN MOBILITY IN NORTHWEST THAILAND

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Southeast Asia is the epicenter of antimalarial drug resistance. Its disposition potentially relates to regional factors, including malaria parasite population structure. Differences in parasite population structure have been associated with resistance, as well as population decline following elimination efforts; human mobility is also thought to play a role. To investigate the relationship between parasite population structure and human mobility, we aim to model the genetic relatedness of parasites sampled from four sites in Northwest Thailand under models excluding and including human connectivity. We estimate genetic relatedness between sites using published data from over 1000 *Plasmodium falciparum* positive samples genotyped at 96 single nucleotide polymorphisms (SNPs). For a pair of samples from different sites, the percentage of the parasite genome that is identical by descent (IBD) is inferred under a hidden Markov model. To account for the relatively small number of SNPs, the IBD estimates are calibrated using a regression model trained on results derived from distinct samples sequenced across the entire genome. Preliminary estimates of IBD suggest the parasite populations are partially clonal, and that a subset of parasites at different sites is highly related, implying a network of interconnected populations. Discordance between the genetic relatedness of sites and their spatial distribution suggests human mobility could play a role. We thus hypothesize that the inclusion of human connectivity in the proposed model of genetic relatedness will increase its explanatory power. Model selection will provide a quantitative assessment of our hypothesis, yielding valuable insight into the host-parasite relationship at the population level, which can be leveraged to improve the design and maintenance of critical malaria elimination strategies.

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IMPACT OF ALL-CAUSE ANEMIA ON THE RISK OF FALCIPARUM MALARIA IN MALIAN CHILDREN

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Anemia and falciparum malaria co-exist throughout much of sub-Saharan Africa. Recurrent malaria can cause anemia, and some forms of anemia, such as iron-deficiency, can reduce the risk of severe malaria. The relationship between all-cause anemia and uncomplicated malaria is less well understood. We investigated the relationship between hemoglobin concentrations and subsequent risk of malaria in a large cohort of children in Southern Mali. The KIDS-Malaria cohort comprised over 1500 children who were followed over 4 successive malaria transmission seasons. Hemoglobin levels were measured at the start of 2 transmission seasons, and with each malaria episode at diagnosis and during day 4 of treatment. We estimated the risk of subsequent episodes of malaria using inverse probability weighting in models adjusted for age, ethnicity, and alpha-thalassemia. Overall, we recorded over 4000 episodes of malaria during over 2500 child years of follow-up. Mean hemoglobin values were 11.0 g/dL in the two baseline surveys, 10.6 at the time of malaria diagnosis, and 9.5 on day 4 of treatment during these episodes. Using hemoglobin values measured either at baseline surveys or at day 4 during their previous malaria episode, we categorized malaria episodes as occurring in a child with pre-existing severe anemia (<8 g/dL, n=646), mild anemia (8-11 g/dL, n=2379), or normal (>11 g/dL, n=1179). Compared to episodes occurring in children with normal hemoglobin levels, the risk of malaria was reduced in those with severe anemia (relative risk [RR] 0.74; 95% confidence interval [CI] 0.604-0.906) and unchanged in those with mild anemia (RR 0.939; 95% CI 0.845-1.044). This effect was most pronounced in the final year of observation, when, relative to episodes occurring in children with normal hemoglobin levels, the risk of malaria was reduced in those with both severe (RR 0.255, 95% CI 0.16-0.4) and mild anemia (RR 0.60, 95% CI 0.5-0.73). These data suggest that all-cause anemia reduces the risk of uncomplicated malaria in this cohort of Malian children.

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CROSS-BORDER MALARIA: THE CONTRIBUTION OF POPULATION MOVEMENT TO SUSTAINED MALARIA TRANSMISSION IN MUTASA DISTRICT, ZIMBABWE

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Malaria persists as a public health problem in Zimbabwe despite continued vector control efforts with long-lasting insecticide treated nets and indoor residual spraying. Population movement between Mozambique and Zimbabwe may play a role in sustaining malaria transmission in Mutasa District, Zimbabwe. The aim of this study is to assess cross-border malaria transmission between Mozambique and Zimbabwe. Between 2012 and 2016, passive case detection of malaria cases, determined by rapid diagnostic test (RDT), in 43 clinics in Mutasa District were collected using the country's established health management information system. In 2015, six clinics in Mutasa District began reporting weekly data on confirmed malaria in patients from Mozambique seeking care in Zimbabwe. Preliminary data show that approximately 18.2% of all confirmed malaria cases in border clinics are patients residing in Mozambique. This study will address three hypotheses as to why higher numbers of incident cases of malaria are reported on the border of Mozambique. First, these areas may have a higher incidence of malaria because of ecological factors favoring vector breeding sites. Second, higher case numbers at clinics near the border may reflect the health seeking behaviors of symptomatic individuals residing in Mozambique. Third, movement of parasites and vectors across the border from Mozambique may promote malaria transmission in eastern Zimbabwe. Using a malaria risk map and controlling for ecological factors, we will quantify the increased risk due to ecological factors and health seeking behaviors on the Mozambique border in Mutasa District, Zimbabwe, providing a deeper understanding of cross-border malaria transmission.

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DEFINING THE MICRO-EPIDEMIOLOGY OF MALARIA

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Malaria risk varies considerably over fine spatial scales but this 'micro-epidemiology' is not well understood. A systematic review and meta-analysis was conducted to identify factors that explain micro-epidemiological variation in malaria risk and define the scope, theory and methods for malaria micro-epidemiology. PuBMed, ISI Web of Knowledge and LILACS databases were searched for studies assessing variation in malaria risk between individuals or households within villages or between neighbouring villages. We included 51 of 738 studies screened that investigate demographic, social, environmental and epidemiological risk factors. Most studies investigated environmental risk factors for malaria, of which proximity to breeding sites and housing structure most frequently explained variation in risk. Social characteristics beyond bed net use were not widely considered, though mobility patterns and access to health care were frequently associated with malaria risk. There is limited evidence that crude estimates of the effects of environmental factors are confounded by social and epidemiological characteristics, including village population size and clinical and genetic characteristics, yet these variables are not included in most studies. There was substantial heterogeneity in effect estimates, including by study design, transmission context, exposure classification and analysis level, as there was only partial overlap between factors associated with malaria risk at individual compared to cluster or village level. Pooled estimates could therefore not be produced. Instead, a causal framework for the relationships between different malaria risk factors associated at micro-epidemiological scales was developed. In conclusion, micro-epidemiological studies should measure social, epidemiological and environmental factors consistently associated with fine scale variation in malaria risk. Control of confounding and multilevel analysis could be improved through use of causal frameworks for design and analysis of micro-epidemiological studies of malaria.

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CHARACTERIZING THE ASYMPTOMATIC AND SUBMICROSCOPIC MALARIA RESERVOIR IN SOUTHERN ZAMBIA: ASSOCIATED RISK FACTORS AND GAMETOCYTE PREVALENCE

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To achieve and sustain malaria elimination, identification and treatment of the chronically infected, asymptomatic reservoir is critical. Such individuals are not likely to seek medical care yet can harbor gametocytes and serve as reservoirs for transmission. Characteristics of asymptomatic individuals infected with *Plasmodium falciparum* were evaluated in Choma District, southern Zambia where malaria transmission has declined dramatically over the past decade. Households were randomly selected for participation in community-based, cross-sectional surveys between 2008 and 2013. Questionnaires were administered to collect information on age, sex, recent history of malaria symptoms and recent anti-malarial medication use. Asymptomatic malaria was defined as the absence of fever (tympanic temperature ≥38°C) on the visit day or no self-reported fever with chills during the last 48 hours. Blood samples were collected by finger prick for Pfhrp2-based rapid diagnostic tests (RDT), blood smears and dried blood spots (DBS). DNA was extracted from the DBS and a cytb-targeted nested PCR (nPCR) and a Pfs25 RT-nPCR were performed to detect malaria parasites and gametocytes, respectively. Of 4,101 participants with complete data, 99 (2.4%) were positive by RDT or nPCR and 98% (n=97) of them lacked visible parasites by microscopy. Seventy-four % of these malaria cases (n=73) were classified as asymptomatic and 52% (n=38) of the asymptomatic cases were RDT negative but nPCR positive. Compared to RDT and nPCR negative individuals, asymptomatic, RDT negative and nPCR positive individuals were more likely to be male (p=0.004) and all were above 5 years of age. The prevalence of gametocytemia by RT-nPCR was higher among participants who were RDT and nPCR positive (33%) or RDT negative but nPCR positive (24%) than those who were RDT and nPCR negative (1.1%; p<0.0005). In areas of declining malaria transmission where the majority of infected individuals are RDT negative, more sensitive screening tools or focal drug administration strategies are needed for further reduce malaria transmission and achieve malaria elimination.

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RETURNING TO THE PROBLEM OF MALARIA IN CHILDREN UNDER FIVE IN LIBERIA

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Prior to the Ebola virus (EVD) epidemic, Liberia was recovering from a prolonged civil war that had decimated the health care system. Malaria was reported to be the leading cause of inpatient admission and death in children under 5 years of age. Determining the cause of acute disease in endemic regions is often complicated by the presence of premonition - 32% of children in Liberia test positive for malaria and are asymptomatic.

Thus, other causes of acute febrile illness may mimic malaria and remain untreated. We conducted a prospective, hospital-based study of children under 5 years who presented to JFK Medical Center in Monrovia with presumed malaria from June 2013-May 2014. Clinical data was obtained on admission and discharge. Malaria diagnosis was confirmed by microscopy and/or malaria rapid diagnostic test. Children were treated for malaria using national treatment guidelines. 351 children who were admitted to JFK and treated for severe malaria agreed to participate in the study. Of this cohort, 34% were previously admitted to the hospital from 1-4 times for treatment of malaria. 49% of this cohort met the case definition of severe malaria (confirmed malaria infection with resolution of symptoms and parasitemia by day 3). For these patients, the most common presenting symptoms included fever (100%) for an average of 3.8 days prior to presentation, headache (95%), prostration (80%), cough (68%), seizures (33%), diarrhea (30%) and respiratory distress (25%).. 44% of patients had anemia on admission with an average hemoglobin level of 9.8 mg/dl. 26% of these patients hospitalized with presumed severe malaria tested negative for malaria. The most common presenting complaints in this group were; cough (47%), headache (27%), prostration (20%), diarrhea (16%) and respiratory distress (4%). In conclusion, this pilot study clearly demonstrates that children in Liberia suffer from more than malaria and should be clinically assessed and treated for other febrile illnesses. Future studies will include defining the landscape of febrile illness in children in Liberia.

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POPULATION-BASED MALARIA SURVEILLANCE BY HEALTHCARE WORKERS IN THE PROVINCES OF HAUT KATANGA AND LUALABA IN THE DRC

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In the Democratic Republic of the Congo (DRC), malaria is reported to be the primary cause of morbidity and mortality, estimated to account for >40% of outpatient visits and attributable mortality of 40% in children under 5. However, health information systems are not widely available and consequently the accuracy of field data is limited. A public-private partnership was established to implement a malaria case management and surveillance system in Haut Katanga and Lualaba provinces. Over 250 public primary healthcare workers and managers are being equipped with Fionet, an integrated system with point-of-care devices to guide case management and analyze RDT test results combined with remote oversight. The implementation of Fionet commenced in January 2016 and will be fully implemented by November 2016. As of March 2016, there are 150 healthcare workers covering >20% of public health facilities in the two provinces that are operational with Fionet. 6,780 malaria RDTs have been processed with Fionet, which ensures quality processing and automated interpretation, and uploaded to Fionet cloud for monitoring, quality control, analysis and reporting. In febrile children under 5 years of age, we observe an 80.6% positivity rate, which is one the highest levels reported globally. Additionally, 62% of patients over 5 years of age and 56% of pregnant women tested positive for malaria. Patient information is also routinely collected on Fionet, for example, only half of the patients' report having a bed net in their household. Fionet has demonstrated feasibility and usability in the hands of healthcare workers in the field. The implementation at scale will provide accurate, population-based, real-time data on malaria prevalence with the ability to allocate resources and monitor outbreaks. Field data collected using the system can be integrated with databases (e.g. DHIS2) and utilized for public health decision-making and research.

1587

EVALUATION OF SEROLOGICAL BIOMARKERS OF *PLASMODIUM VIVAX* AND *P. FALCIPARUM* TRANSMISSION IN THE SOLOMON ISLANDS

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In the Solomon Islands, malaria transmission is low, highly heterogeneous, and dominated by *Plasmodium vivax* (*Pv*), but has pockets of persistent *P. falciparum* (*Pf*). This study utilized a cross-sectional serological survey of 2000 individuals of all ages from five geographical regions of Ngella Province of varying transmission levels, analyzing antibody responses to 13 *Pf* and 8 *Pv* antigens using a bead-array immunoassay. Methods were developed to assess optimal cutoffs for serological positivity, cross-reactivity between and Pv and Pf antibodies, seroconversion rates, and correlations between seropositivity and infection rates in the different transmission zones to identify the most promising antigens as biomarkers of transmission. Significant correlations were found for four antigens: *Pf* CelTOS (p=0.036, r=0.90), *Pf* MSP2 (p=0.026, r=0.92), *Pv* DBP-AH (p=0.045, r=0.89), and *Pv* DBP-P (p=0.019, r=0.94). The results show that antibody responses to these four antigens are promising biomarkers of malaria transmission levels and suggest that variants of the *Pv* Duffy Binding Protein are particularly indicative of malaria exposure. Additional analysis is underway to confirm these initial findings and to identify additional optimal serological biomarkers of malaria transmission.

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EPIDEMIOLOGY OF CHRONIC ASYMPTOMATIC *PLASMODIUM FALCIPARUM* INFECTIONS AMONG ALL AGES IN AN AREA WITH SEASONAL MALARIA TRANSMISSION IN BONGO DISTRICT, GHANA

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Despite efforts to control and eliminate *Plasmodium falciparum*, malaria still remains a major public health concern. Exposure to antigenically diverse *P. falciparum* isolates at a young age leads to the acquisition of protective immunity and the development of chronic asymptomatic malaria infections in endemic areas. Understanding the role asymptomatic infections play in sustaining the reservoir of infection needs to be examined so that countries can shift towards malaria elimination. This research describes a longitudinal cohort (N=2,000) study designed to evaluate the reservoir of asymptomatic *P. falciparum* infections among all ages in an area with seasonal transmission in Bongo District, Ghana. Using different methods for parasite detection we evaluated how age, seasonality, spatial location and other factors affect the epidemiology of asymptomatic malaria at the end of the 2012/13 wet and dry seasons. Asymptomatic *P. falciparum* prevalence by microscopy decreased significantly from 42.5% at the end of the wet to 27.5% at the end of the dry season ($p < 0.001$). Using the 18S rRNA nPCR, all microscopy negative samples were further screened for submicroscopic infections. Resulting prevalence of submicroscopic infections also decreased significantly, with 55.4% and 20.7% at the end of the wet and dry seasons respectively ($p < 0.001$). Combining detection methods, 74.4% of the population in the wet and 42.5% in the dry season had evidence of an active *P. falciparum*

infection. Interestingly in those >20 years of age, we found evidence of infection in 64.3% of the population in the wet and 27.0% in the dry season. Using the combination of microscopy and PCR we have shown that the asymptomatic reservoir peaks at the end of the wet season and that infections in all age groups contribute to maintaining the reservoir of malaria infection. These results suggest that if elimination is to succeed, interventions will need to target not just children but all asymptomatic *P. falciparum* infections and be implemented towards the end of the dry season in this area of West Africa.

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DEMOGRAPHIC AND CLINICAL PROFILES OF *PLASMODIUM FALCIPARUM AND P. VIVAX* PATIENTS AT A TERTIARY CARE CENTER IN SOUTHWESTERN INDIA

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India is a highly heterogeneous country, comprising more than 1.2 billion people, 2000 ethnic groups and 22 official languages. On the Indian subcontinent, there are more than 500 million people at risk for malaria, with reports of up to two million cases and 50,000 deaths per year. In contrast with Africa, malaria transmission is more limited, severe malaria disease is more frequently detected in adolescents and adults, and a substantial proportion of cases are infected with *P. vivax* rather than the traditionally more-virulent *P. falciparum* in India. Expansive studies on determinants of severe disease and mortality among malaria-positive patients are fewer and smaller in scope than in Africa or SE Asia. The present study describes the population served by Goa Medical College (GMC) and the demographic, diagnostic and clinical characteristics of malaria-positive study participants enrolled to-date at the Centre's principal research site. A total of 74,571 febrile individuals presented to GMC between January 2012 and December 2015 and were tested for malaria. Of those, 6,277 (8.4 %) were determined to be positive for malaria infection. Over four years of passive surveillance, the number of malaria-positive cases presenting to GMC steadily and significantly increased, from 873 cases in 2012 to 2,263 cases in 2015. While, a critical component of modern improvements in patient care are to meld clinical care, research, and treatment activities with the powerful potential of basic science to untangle variables that may contribute to susceptibility, pathogenesis, and resistance in malaria, this is not always possible on a large scale. Based on extensive statistical analysis of our patient group, the present study reveals three potentially valuable, simple prognostic indicators of disease severity in India among malaria-positive patients: increasing age, high fever and anaemia (others were ruled out). The predictive indicators may be employed by clinicians at GMC and in similar resource-limited settings when making hospital admissions decisions.

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CONSERVED SEQUENCE MOTIFS IN PLACENTAL MALARIA VACCINE CANDIDATE VAR2CSA DESPITE LARGE OVERALL SEQUENCE DIVERSITY

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VAR2CSA, a member of the *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1) family, mediates the binding of *P. falciparum*-infected erythrocytes to chondroitin sulfate A, and is a key protein in the pathogenesis of placental malaria. VAR2CSA is a leading vaccine candidate against placental malaria, as it is a target of naturally acquired immunity to the disease. However, antigenic diversity presents a significant challenge to the development of a VAR2CSA-based vaccine. A broadly effective vaccine that overcomes strain specificity will likely require more than one allele of var2csa. To evaluate the possibility of regional differences in vaccine efficacy, we investigated whether sequence similarity among var2csa alleles is related to their geographic origin. We analyzed 90 var2csa allelic sequences from 12, 43, and 7 samples from West Africa, East Africa, and a published dataset reflecting global diversity, respectively. The sequences from East Africa were generated using Pacific Biosciences (PacBio) amplicon sequencing, whereas those from West Africa were assembled from a combination of PacBio and Illumina whole genome sequence data. We analyzed patterns of similarity, based both on overall and partial sequences, as well as on k-mer composition. Our preliminary results show that VAR2CSA proteins are highly diverse (mean amino acid sequence similarity of 75%) and sequences did not cluster by geographic origin. However, we identified some conserved motifs in DBLepam4 and DBLepam5 among parasites collected from distant geographic regions. These findings support the possibility of developing a broadly protective VAR2CSA-based vaccine from a limited number of strains. We are extending our analysis with additional sequences from Southeast Asian isolates.

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INDEPENDENT ORIGIN AND GLOBAL DISTRIBUTION OF DISTINCT *PLASMODIUM VIVAX* DUFFY-BINDING PROTEIN GENE DUPLICATIONS

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Plasmodium vivax causes the majority of malaria episodes outside Africa, but remains a relatively understudied pathogen. The pathology of *P. vivax* infection depends critically on the parasite's ability to recognize and invade human erythrocytes. This invasion process involves an interaction between

P. vivax Duffy-binding protein (PvDBP) in merozoites and the Duffy antigen receptor for chemokines (DARC) on the erythrocyte surface. Whole-genome sequencing of clinical isolates recently established that some *P. vivax* genomes contain two copies of the PvDBP gene. The frequency of this duplication is particularly high in Madagascar, where there is also evidence for *P. vivax* infection in DARC-negative individuals. The functional significance and global prevalence of this duplication, and whether there are other copy number variants at the PvDBP locus, is unknown. Using whole-genome sequencing and PCR to study the PvDBP locus in *P. vivax* clinical isolates, we found that PvDBP duplication is widespread in Cambodia. The boundaries of the Cambodian PvDBP duplication differ from those previously identified in Madagascar, meaning that current molecular assays were unable to detect it. The Cambodian PvDBP duplication did not associate with parasite density or DARC genotype, and ranged in prevalence from 20% to 38% over four annual transmission seasons in Cambodia. This duplication was also present in *P. vivax* isolates from Brazil and Ethiopia, but not India. PvDBP duplications are much more widespread and complex than previously thought, and at least two distinct duplications are circulating globally. The same duplication boundaries were identified in parasites from three continents, and were found at high prevalence in human populations where DARC-negativity is essentially absent. It is therefore unlikely that PvDBP duplication is associated with infection of DARC-negative individuals, but functional tests will be required to confirm this hypothesis.

1592

INTERACTIONS AND COMPETITIVE GROWTH WITHIN MIXED INFECTIONS OF *PLASMODIUM FALCIPARUM*

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Infections by multiple genetically distinct *Plasmodium falciparum* parasites are common in cases of human malaria. The dynamic interactions of parasites in mixed infections include competition between co-infecting strains and potential for selection of parasites with fitness advantages. With emerging artemisinin resistance, it is essential to understand the relative fitness of parasites exhibiting the delayed clearance phenotype that could influence their spread in populations. Although artemisinin resistance has been associated with the *pfkelch13* gene, the *in vivo* delayed clearance phenotype does not have a corresponding *in vitro* IC_{50} shift; furthermore, resistant parasites that lack *pfkelch13* mutations have not been well characterized. The relative fitness of parasites displaying delayed clearance to artemisinin, both with and without *pfkelch13* mutations, is unknown, emphasizing the need for analyses of fitness costs and benefits of resistance mutations. In this research, pair wise competition assays were used to ascertain fitness of mutations associated with delayed clearance to artemisinin treatment. Slow clearance parasite isolates from Southeast Asia, with and without *pfkelch13* mutations, were evaluated. Results indicate a range of relative fitness phenotypes associated with different mutations. Further experiments are in progress to implement competitions of these isolates in the presence of low level artemisinin drugs to elucidate which resistance associated mutations provide the most fitness for parasites to proliferate under drug pressure within mixed infections.

1593

MULTIPLEX BARCODED NEXT-GENERATION SEQUENCING OF MULTICLONAL *PLASMODIUM FALCIPARUM* GENOTYPES

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Large-scale molecular epidemiologic studies of *Plasmodium falciparum* parasites can investigate parasite biology and transmission, identify and predict the spread of drug resistance, and assist in the evaluation of vaccine candidates. The polyclonal nature of most infections in high transmission settings undermines many traditional genotyping approaches. Next-generation sequencing approaches to parasite genotyping may allow sensitive detection of minority variants, disaggregation of complex parasite mixtures and scalable processing of large samples sets. Therefore, we designed, validated, and applied to field parasites a new approach to parasite genotyping that leverages next-generation sequencing of individually barcoded samples in a highly scalable and multiplex manner. We utilize variant barcodes, invariant linker sequences and modular template specific primers in such a way as to allow for the simultaneous generation of high-dimensional sequencing data of multiple gene targets. The modularity of this approach permits a cost-effective and easily reproducible way to query many genes without experimental redesign. In practice, this approach generates large numbers of high quality reads in a manner that is robust to different sequencing technologies including both Ion Torrent and Illumina MiSeq. In mixtures of reference parasite genomes, we qualitatively and quantitatively detected unique haplotypes comprising 0.1% of polyclonal infection. We demonstrate concordance of the outcomes of this method compared to traditional Sanger sequencing and pooled next-generation sequencing. Finally, we applied this genotyping approach to fresh parasites collected in Western Kenya in order to rapidly obtain parasites genotypes at three unlinked loci. In summary, we present a rapid, scalable and flexible method for genotyping individual *P. falciparum* parasites that further enable molecular epidemiologic studies of parasite evolution, population structure and transmission.

1594

THE DIVERSITY OF RNAS EXPRESSED IN *PLASMODIUM VIVAX*

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The biology of the widespread human malaria parasite, *Plasmodium vivax*, remains largely unknown due to a lack of a robust *in vitro* culture system. Here, we used stranded RNA-sequencing technologies paired with depletion of highly expressed transcripts from the host (i.e. globin mRNAs and rRNAs) for assessing global transcriptome changes in the host and parasite during infection and treatment. From 100uL of patient blood, we isolated RNAs and sequenced >50 million paired end reads from three patients. 15-25% of the reads aligned to the *P. vivax* genome sequence, as a result of >80% reduction in human rRNAs and globin mRNAs. Using this large amount of sequence data, we *de novo* assembled all RNA transcripts expressed by intraerythrocytic *P. vivax* parasites. Many of our transcripts coincide with annotated protein-coding genes, though a very high number of genes have misannotated 5' and 3'UTRs that often include unannotated introns likely involved in gene regulation. Additionally, we identified 1,388 genes with multiple isoforms as a result of alternative splicing, intron retention, and alternative transcriptional start and stop sites. 5,207 assembled transcripts had no coding potential and are likely noncoding RNAs. Because our data can separate reads that originate from different strands of RNA, we can assess that a large number of these noncoding reads are antisense RNAs for coding genes, while the rest are a mixture of small RNAs and intergenic long noncoding RNAs. Finally, as a result of high read coverage, we were able to additionally find >4,800 polymorphisms in each patient sample, data that can help us to determine the complexity of infection and how different polymorphisms affect transcription. Together, our study reveals the diversity and complexity of RNAs expressed by intraerythrocytic *P. vivax* parasites and show that stranded RNA-seq is a robust method to study host/parasite interactions using patient samples.

1595

EXCEPTIONALLY LONG-RANGE HAPLOTYPES IN *PLASMODIUM FALCIPARUM* CHROMOSOME 6 MAINTAINED IN *AN* ENDEMIC AFRICAN POPULATION

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Previous genome-wide analyses of single nucleotide variation in *Plasmodium falciparum* identified evidence of an extended haplotype region on chromosome 6 in West Africa, suggesting recent positive selection. Such a pattern is not seen in samples from East Africa or South East Asia, so it could be marking a selective process particular to West Africa. Analyses of the haplotype structure in samples taken at different times could give clues to possible causes of selection. This study investigates chromosome 6 extended haplotypes in the Gambia by analysing alleles at multiple microsatellite loci using genome sequence data previously obtained from clinical isolates collected in 2008, followed by genotyping 13 loci in 405 isolates from 1991, 2008 and 2014. Multiple long haplotypes were evident in the population sample, and a region of high linkage disequilibrium was shown to span ~200 kilobases (Kb), with a core region of ~70 Kb having the most intact haplotype structure. Two of the haplotypes were detected in samples from 1991, which predates the time when chloroquine and antifolate resistance alleles became common locally, and these haplotypes were still present in 2014. The occurrence of several long haplotypes at intermediate frequencies suggests an unusual mode of selection in chromosome 6, possibly combined with recombination suppression on specific haplotypes. Such selection apparently occurred before the emergence of known antimalarial drug resistance alleles, and could be due to effects of other drugs or unknown processes that have long been operating in this endemic region.

1596

DE NOVO VARIANT CALLING TO RESOLVE TRANSMISSION DYNAMICS WITHIN CLONAL *PLASMODIUM FALCIPARUM* SAMPLES: A CRUCIAL TOOL FOR THE MALARIA 'ENDGAME'

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Genetic studies have provided an increasingly clear view of malaria transmission; genotyping a small number of loci can allow us to study changes in transmission dynamics and relatedness in response to malaria control. Yet the genetic epidemiology of malaria lags far behind other pathogens. In bacterial or viral pathogens the higher mutation rates allow de novo variation to be used. With the *Plasmodium falciparum* genome, however, SNPs do not show sufficiently high mutation rates to allow us to distinguish between 100% related parasites. Conversely short tandem repeat (STR) loci, which mutate many orders of magnitude faster, are difficult to genotype from short-read sequence. Recent work at the Broad Institute - improved library preparation, read lengths, and methods of genotyping - now enable this kind of study to be undertaken. Applying these methods to *P. falciparum*, we show that INDEL and STR variation can now be called with greatly increased accuracy even in low-complexity regions of the *P. falciparum* genome, affording newfound access to significant amounts of de novo variation. We have employed these approaches to examine a set of parasites from Thies, Senegal that have not outbred and indistinguishable using current genotyping approaches. Using de novo variants only we have derived phylogenies and transmission networks for these parasites. As we move towards low-transmission or 'pre-elimination' settings, in which highly-related parasites are the norm, we propose a framework to examine de novo mutation as a crucial tool for the 'endgame' of malaria eradication. By using derived mutation we add a valuable temporal dimension to genomic epidemiology; we will show how examining substitution rates in clinical sample sets can distinguish between different modes of transmission - potentially allowing us to identify superspreaders within transmission chains.

1597

ELUCIDATION OF THE DIVERGENT APICOPLAST GENOMES OF *PLASMODIUM OVALE CURTISI* AND *P. OVALE WALLIKERI*

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Whole genome sequencing was attempted on parasite DNA extracted from the peripheral blood of malaria patients infected with *Plasmodium ovale wallikeri*. A full-length contig of the apicoplast genome was successfully assembled and this was compared to published sequences from the *P. ovale curtisi* apicoplast genome. Observed dimorphic regions are being confirmed in archived DNA from multiple isolates of both species. The potential for an apicoplast-mitochondrial barcode of defined polymorphisms that perfectly discriminates these two closely related parasite species will be considered.

1598

WHOLE-GENOME PROFILING OF DIFFERENTIALLY EXPRESSED GENES IN CHILDREN WITH MALARIAL ANEMIA

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Application of whole genome expression profiling is a useful approach for identifying important gene pathways associated with disease outcomes, especially in a multifocal disease such as falciparum malaria. In high transmission regions, malaria commonly manifests as severe malarial anemia [SMA, hemoglobin (Hb)<5.0 g/dL], primarily in infants and young children. Currently, the molecular mechanisms that condition the development of SMA are largely undefined. Therefore, use of unbiased transcriptomics is suitable in young children with SMA where sample volume is limited to perform individual gene quantification. Malarious children (n=1218, aged 3-36 mos) were stratified based on disease severity into 'polarized' extremes of non-SMA (Hb, 8.0-10.9g/dL, n=532) and SMA (n=228) groups after exluding children's with co-infections (bacteremia and HIV-1) and hemoglobinopathies (SSD, G6PD deficient and α-thalassemia). RNA was isolated from leukocytes collected on first hospital enrollment to the study prior to treatment interventions. Based on RNA quality checks, 72 samples (non-SMA, n=51; SMA, n=21) were selected for transcriptomics analysis. Gene expression analysis was performed using the Illumina® HumanHT-12 beadchip covering 47,231 transcripts specific to 19,185 genes. Data were analyzed through step-wise procedures to exclude transcripts identified as having an "absent" expression. A second quality control filter removed transcripts with low signal values, resulting in 3,981 genes. Transcripts with ≥1.5-fold change in SMA relative to non-SMA group (P<0.05) were 629 genes; 597 upregulated and 32 downregulated. To infer biological significance, we generated relational pathways, resulting in 30 networks. The networks that showed highest significance (P=3.53x10-19) were enriched for immune response, signal transduction and hematopoiesis genes. Additionally, validation of selected genes [HSPA1A (n=89), IL-18 (n=77) and COX2 (n=23)] showed an

identical trend. In summary, transcriptomic arrays identified both novel and known genes/gene pathways that are important for the host immune response to malaria infection.

1599

ANALYSIS OF MULTIPLICITY OF ETHIOPIAN *PLASMODIUM VIVAX* INFECTIONS AND RELAPSE PATTERNS USING PVMSP1 AMPLICON DEEP SEQUENCING

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Parasite genetic diversity and multiplicity of infection (MOI) affect clinical outcomes, response to drug treatment and naturally acquired or vaccine-induced immunity. Based on microsatellite and merozoite surface protein (MSP) markers, a number of studies have reported on MOI and the frequency of multiclonal infections in Plasmodium parasites. However, traditional methods often underestimate the MOI and the frequency of multiclonal infections due to technical sensitivity and specificity. Next-generation sequencing techniques provide a novel opportunity to study parasite diversity. In this study, we conduct amplicon deep sequencing of PvMSP1 to determine MOI and detect the relapse pattern of *Plasmodium vivax* from Southwestern Ethiopia. A total of 139 *P. vivax* dry blood samples were pyro-sequenced on a 422 bp fragment of PvMSP1 amplicon, yielding a total of 231 haplotypes. The average of MOI was 4.68, ranging from 2 to 14 clones in a single individual. However, using 3 microsatellite markers, an average of MOI=2.64 was detected with only 1-5 clones in a single subject. Four (80%) out of 5 subjects with recurrent vivax malaria were found to be relapse 44-65 days after chloroquine treatment. Significantly different MOIs were found among age groups, locations, and transmission seasons as well as between symptomatic and asymptomatic samples. Significantly higher MOI was found in clinic samples. Young children and old adults showed a higher MOI than that of older children. These results suggest that *P. vivax* multiclonal infections were common together with high proportions of relapse in Ethiopia. This study has important implication for the provision of primaquine to prevent relapse, anti-relapsing interventions and eliminating malaria in the low transmission areas.

1600

IMPROVED, HIGH-RESOLUTION SINGLE-CELL GENOMIC PROFILING OF HUMAN MALARIA PARASITES

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In many cases, malaria patients suffer from complex infections caused by multiple parasite lineages. The prevalence of, and interactions between, unique parasite genotypes within such multiple genotype infections are wholly unknown, complicating efforts to understand the spread of drug resistance, genetic recombination, and transmission to mosquitoes. For a first look at component genotypes within complex infections, we previously developed a single-cell genomics platform where individual parasites were briefly cultured *ex vivo*, captured by flow cytometry and whole-genome amplified prior to deep sequencing. Our analyses showed that coverage of the genome was highly variable, limiting the construction of complete haplotypes. We have since further optimized our protocol, focusing our analysis on late-stage parasites, which contain a higher amount of DNA template, by increasing the time of culture and utilizing restrictive flow cytometry gating. Analysis of these replicating parasites yielded superior genomic data in terms of the rate of successful amplification, the fraction of reads mapped to the parasite genome (purity), and genome-wide coverage. With broad haplotype blocks in hand, it is now feasible to obtain a comprehensive portrait of genetic variation in each cell. Here, we report the analysis of several dozen individual *Plasmodium falciparum* genomes collected from patients in a region of high malaria transmission. These improvements enable characterization of the genetic diversity in malaria infections at unprecedented resolution and scale.

1601

DISTINCT ARCHITECTURE OF *PLASMODIUM FALCIPARUM* POPULATIONS FROM SOUTH ASIA, WITH COUNTRY-LEVEL CLUSTERING OF GENOMIC RELATIONSHIPS

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India records about two to ten million cases of malaria every year, with about 50,000 deaths per year. Compared to the heavy emphasis of malaria research in Africa and in Southeast Asia, much less is known about genomic and phenotypic properties of parasites from the Indian subcontinent and such omissions need attention since *Plasmodium falciparum* isolates differ genetically as well as by phenotypes relative to their geographic origins. Previous genome-wide comparisons have analyzed stratification in global parasite populations across different continents. However, these studies have not included Indian isolates in the past. Here, we examine whole genome sequences from 23 Indian parasite isolates and their relationships with hundreds of other isolates from around the globe. Our analysis provides a rich collection of over 360,000 high quality variants. The entire collection of these variants was used to calculate a nucleotide distance estimate between each pair of global isolates. Principle coordinate analysis showed that parasite isolates segregate based on geographic locations, where an entire cluster can be classified as originating from a single continent. Removing the highly variable var genes from all the genomes prior to estimating the pair-wise distances eliminates many of the sequencing and alignment errors and reveals an even higher resolution geographic segregation. Surprisingly, Indian isolates segregate into a unique cluster widely separated even from other South Asian isolates. Nearest neighbors to Indian isolates are Bangladesh followed by other South-East Asian countries. This, therefore, reveals a unique place for India in the world malaria map. Monitoring of global malaria elimination strategies as well as global parasite evolution will have to include India due to the unique history of these parasites and their strategic position between Southeast Asia and Africa.

1602

EVALUATING THE INFORMATION VALUE OF PARASITE GENOMICS FOR MALARIA ELIMINATION

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As countries push towards malaria elimination, there is an increasing need to understand the disease transmission network and particularly the dynamics of residual foci. Sequencing parasite genomes has the potential to distinguish between local and imported cases by source, to assist in categorizing areas with varying capacities for transmission and their relative connectivity, and to validate the relevant time and space scales that define effectively disconnected regions. Using a dynamical model that tracks full parasite genomes of indiviudal *Plasmodium*

falciparum infections, we demonstrate the potential information value of different sequencing technologies and sampling frames to address these operationally critical questions.

1603

ABSENCE OF *IN VIVO* SELECTION OF K13 POLYMORPHISMS AFTER ARTEMETHER LUMEFANTRINE TREATMENT IN UGANDA

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In Southeast Asia, the *Plasmodium falciparum (Pf) kelch13* (PF3D7_1343700) gene constitutes a useful molecular marker for artemisinin resistance surveillance. Mutations in the *Pfkelch13* are known to be involved in the development of delay of parasite clearance after artemisinin treatment. This delayed clearance has also been significantly associated with the following six particular SNPs in *Pf; ferredoxin (fd), apicoplast ribosomal protein S10 (arps10), multiple resistance protein 2+(mdr2), chloroquine resistance transporter (crt), phosphoinositide-binding protein (pibp)* and *protein phosphatase* (*pph)* genes in South East Asia. Individual treatment would select resistant parasites in the human body, namely *in vivo* selection. Currently, there's a paucity of data about in-vivo selection of the above mentioned mutations. We conducted an artemether-lumefantrine (AL) follow-up study in Uganda, in which genotypes in *Pfkelch13* and six SNPs were compared before drug administration and in all recurrent parasites during a follow-up period of 28 days. We found that AL treatment was very effective with PCR adjusted efficacy of 95.1%. Only three cases showed late clinical failures. Among a total of 161 isolates before AL treatment, almost all (96.8%) had wild type alleles in *Pfkelch13*. Similarly, only wild type alleles were observed in *fd, arps10, mdr2, pibp* and *pph* genes. Mixed alleles (wild and mutant) were observed in 2.3% of isolates in *crt*. In all follow-up cases, presence of parasites was molecularly confirmed and 21 positive results were obtained. All these isolates harbored wild type alleles in *Pfkelch13* and the six genes. These results suggest that very few isolates were observed after AL treatment in Gulu Northern Uganda, but this may not be the case because of the potential selection of mutant alleles in the genes that are associated with artemisinin resistance in Southeast Asia.

1604

EUPATHDB: A POWERFUL EUKARYOTIC PATHOGEN GENOMIC AND FUNCTIONAL GENOMIC DATA MINING RESOURCE

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The Eukaryotic Pathogen Database (EuPathDB, http://eupathdb.org) is a free, online data mining resource that facilitates the discovery of meaningful biological relationships from large volumes of data by integrating pre-analyzed omics data with advanced search capabilities, data visualization and analysis tools. EuPathDB supports over 170 organisms within Amoebazoa, Apicomplexa, Chromerida, Diplomadida, Trichomonadida, Kinetoplastida and numerous phyla of oomycetes and fungi. For these organisms, EuPathDB integrates a wide range of data including genome sequence and annotation, transcriptomics, proteomics, epigenomics, metabolomics, population resequencing clinical and field isolates, and data that inform host-pathogen interactions. Data are analyzed using standard workflows and an in-house analysis pipeline generates data including domain predictions, orthology profiles across all genomes and GO term associations. Our unique strategies system offers over 100 structured searches that query the pre-computed data. Individual search results can be combined into strategies that easily merge evidence from diverse data types and across organisms. Easily accessible tools enhance the search strategy system and include dynamic data visualization, comparative genome analysis, population genetics tools, and functional or pathway enrichment. Forthcoming new tools and functionalities include a private user work-space for primary data analysis, functional analysis tools for result summarization, genome browser and query improvements. This comprehensive resource places the power of bioinformatics with the entire scientific community in support of hypothesis driven research. EuPathDB's active user support offers an email help desk (help@eupathdb.org), social media, a You Tube channel with tutorials and a worldwide program of workshops.

1605

THE BIOLOGICAL FUNCTION OF ANTIBODIES INDUCED BY THE RTS,S/AS01 MALARIA VACCINE CANDIDATE IS DETERMINED BY THEIR FINE SPECIFICITY

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Recent vaccine studies suggest that the magnitude of an antibody response is often insufficient to explain efficacy, suggesting that characteristics regarding the quality of the antibody response, such as its fine-specificity and functional activity, may play a major role in protection. Previous studies of the lead malaria vaccine candidate, RTS,S, have shown that circumsporozoite protein (CSP)-specific antibodies and $CD4^+$ T cell responses are associated with protection, however the role of fine specificity and biological function of CSP-specific antibodies remains to be elucidated. Here, we addressed the relationship between fine specificity, opsonization-dependent phagocytic activity, and protection in RTS,S-induced antibodies. We developed a new method for measuring the phagocytic activity mediated by CSP-specific antibodies and applied it to samples from a completed phase 2 RTS,S/AS01 clinical trial. We also assessed the fine-specificity of the antibody response using ELISA against three antigen constructs of CSP: the central repeat region, the C-terminal domain, and the full-length protein. We carried out multi-parameter analysis of phagocytic activity and fine-specificity data across to identify potential correlates of protection in RTS,S. We found that phagocytic activity was correlated with full-length CSP and C-terminal specific antibody titers, but not to repeat region antibody titers. When expressing the phagocytic activity as 'opsonization index', a relative measure that normalizes phagocytic activity with CS antibody titers, we found, surprisingly, that protected subjects had a significantly lower opsonization index than non-protected subjects. The data suggest that the opsonization is a surrogate marker of protection induced by the RTS,S/AS01 vaccine and determined how antibody fine-specificity is linked to opsonization activity. Our findings suggest that the role of opsonization in protection

in the RTS,S vaccine may be more complex than previously thought, and demonstrate how integrating multiple immune measures can provide insight into underlying mechanisms of immunity and protection.

1606

PLACENTAL MALARIA IS ASSOCIATED WITH ALTERED FETAL CYTOKINE PROFILES

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Malaria during pregnancy threatens the health of mothers and newborns and may have long-lasting consequences on infant health. Our previous work shows that placental malaria is associated with increased risk of malaria in the infant. We hypothesize that this is due to priming of the fetal immune system toward immunoregulatory responses as a consequence of maternal malaria infection. We collected cord blood serum from children born to mothers with detailed antenatal histories and followed a subset of these children through the first year of life, collecting serum at 12 months of age. We used multiplexed electrochemiluminescent immunoassays (Meso Scale Discovery) to measure 11 cytokines (IL-1β, IL-2, IL-4, IL-6, IL-10, IL-12p70, IL-13, IFNγ, TNFα, TGFβ and CRP). We analyzed cord serum from 26 infants born to mothers with no malaria during pregnancy, 26 born to mothers with peripheral malaria, 87 born to mothers with placental malaria, and 14 North American control infants never exposed to malaria. We observed that children born to mothers with chronic placental malaria had significantly elevated levels of TNFα (a pro-inflammatory cytokine), IL-10 (an immunoregulatory cytokine) and CRP (a marker of inflammation) at the time of birth as compared to children born to mothers with peripheral malaria during pregnancy ($p=0.003$, $p=0.001$, $p=0.014$, respectively), no malaria during pregnancy ($p=0.003$, $p=0.037$, $p=0.006$, respectively) or North American controls ($p=0.002$, $p<0.001$, $p=0.045$, respectively). Cytokine levels normalized by one year of age. We propose a model in which placental malaria causes chronic *in utero* inflammation with compensatory production of IL-10 and induction of T regulatory cells (Tregs). After birth, cytokine levels normalize, but Tregs are maintained preventing effective immune responses to malaria and resulting in increased risk of malaria during infancy. We are currently conducting flow cytometric studies on cord blood to further explore this hypothesis. Our results will inform the design and implementation of prenatal interventions to protect the health of pregnant women, newborns and infants from malaria.

1607

PLASMODIUM FALCIPARUM INFECTION AND VACCINE RESPONSES: SHOULD WE TREAT PRESUMPTIVELY?

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Individuals with malaria may have blunted immune responses to some vaccines, suggesting that there is an active immune suppression or immunomodulation during infection. How and which vaccines are impacted by clinical malaria or asymptomatic parasitemia is not completely clear, nor is whether the impacts are sufficient to recommend delaying or presumptively treating individuals prior to routine vaccinations or in malaria vaccine trials. In a series of studies, we are examining the impact of malaria on immune cell function and on vaccine responses in cohorts of adults at various study sites in Mali, West Africa. These studies have examined whether antimalarial treatment, or episodes of parasitemia, alter antibody responses, T cell markers, and/or protective efficacy/activity following vaccination with approved routine vaccines (N=45; Euvax® or TWINRIX® and Menactra®), a whole organism malaria vaccine (N=30, PfSPZ Vaccine) and a transmission blocking vaccine (N=120; Pfs25H-EPA/Alhydrogel®). Data from all three studies will be presented examining the impact of antimalarial treatment or of incidental malaria episodes on T cell exhaustion and regulation, as well as on vaccine responses.

1608

PATTERNS OF ANTIBODY RESPONSES TO *PLASMODIUM FALCIPARUM* INVASION LIGANDS ACROSS DIFFERENT ENDEMIC POPULATIONS IN WEST AFRICA

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Plasmodium falciparum uses a large repertoire of parasite proteins for invasion of erythrocytes, which appears to serve as an immune evasion mechanism, making it difficult to identify targets of invasion inhibitory responses. It is possible that endemicity influences gene expression of invasion ligands and receptor preferences of *P. falciparum* clinical isolates. Therefore, we hypothesized that antibody responses to parasite invasion ligands in individuals living in endemic areas would differ and also correlate with parasite ligand gene expression. To examine this hypothesis, plasma samples from 528 children (2-14 yrs) with malaria across four endemic areas in Ghana (Accra, Kintampo, Navrongo and Hohoe) and one endemic site in Nioro du Sahel in Mali were tested by ELISA for antibodies to *P. falciparum* invasion ligands, including EBA 175, EBA140, EBA181, Rh2, Rh4 and Rh5. The seroprevalence of antibodies to the different antigens ranged from 8% to 70% among the clinical cases tested in this study. Consistent with previous reports, seroprevalence to all the antigens

increased in an age-dependent manner and antibody responses to all antigens were negatively correlated with parasite density. When expressed relative to total antibodies detected, anti-Rh2 levels were significantly higher while anti-Rh4 levels were lower in the Kintampo, Navrongo and Hohoe compared to Nioro du Sahel. Altogether, our data reveals patterns of antibody responses to specific invasion ligands that may be influenced by multiple host and parasite factors including parasite biology, age and endemicity, and deeper understanding of how these factors interplay may be important in identification of potential blood stage vaccine targets.

1609

ISOLATION AND CHARACTERIZATION OF HUMAN MONOCLONAL ANTIBODIES TO *PLASMODIUM VIVAX* DUFFY BINDING PROTEIN FROM MALARIA EXPOSED INDIVIDUALS FROM BRAZILIAN

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Plasmodium vivax merozoites recognize specific receptors on the host cell surface to selectively invade reticulocytes. A critical parasite ligand is the Duffy binding protein (PvDBP), expressed in micronemes, which binds specifically to an erythrocyte membrane glycoprotein known as Duffy blood group antigen/receptor for chemokines (DARC). Antibodies to the cysteine-rich domain II of PvDBP can inhibit binding of this parasite ligand to DARC and inhibit *P. vivax* invasion of reticulocytes *in vitro*. In our previous study with an Amazonians population, we found individuals with high-level BIAb responses (> 80% binding inhibition) developed in 26.6% of subjects under conditions of low malaria endemicity that prevail in Amazonia. Once acquired, high-level BIAb responses were predominantly PvDBP variant-transcending and that subjects with the strongest BIAb response had a >40% decrease in the risk of clinical vivax malaria during the follow-up, compared to those with the weakest BIAb response. We obtained PBMCs from 7 Amazonians with high levels of BIAbs, and sorted single PvDBPII-specific IgG+ memory B cells from two individuals, PCR amplified their IgG heavy and light chain variable regions, and cloned them into a human IgG expression vector to generate a panel of human monoclonal antibodies (mAbs). We found one mAb which recognized PvDBPII. We are now characterizing this mAb in terms of *P. vivax* DBPII strain-specificity, the PvDBPII epitopes it recognize, affinity for PvDBPII, and ability to block *P. vivax* invasion of reticulocytes *in vitro*.

1610

INVESTIGATING A POTENTIAL ROLE FOR TH1-POLARIZED TFH CELLS IN DRIVING ATYPICAL MEMORY B CELL EXPANSION IN MALARIA

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Malaria-specific antibody responses are short lived in children, leaving them susceptible to repeated bouts of clinical malaria. The B and T cell biology underlying short-lived antibody responses to malaria remains unclear. We recently found that chronic malaria exposure is associated with a large increase in atypical memory B cells (MBCs) that express inhibitory receptors and exhibit stunted BCR signaling and impaired B cell proliferation, cytokine production and antibody secretion. T follicular helper (Tfh) cells are known to play a critical role in helping B cells and generating long-lived antibody responses. In other recent work we demonstrated that acute febrile malaria in children preferentially activates Th1-polarized PD-1+CXCR5+CXCR3+ memory Tfh (Tfh-1) cells that exhibit impaired B cell help. In ongoing work we aim to understand the impact of malaria-induced Tfh-1 activation on the B cell response to malaria. Our preliminary findings suggest that Tfh-1 cells contribute to the expansion of T-bet+ B cells that phenotypically resemble atypical memory B cells—providing a potential link between the quality of Tfh cell responses to malaria and atypical MBC expansion.

1611

HUMAN ANTIBODIES IN MALARIA: STRUCTURE, FUNCTION, MECHANISM AND NEUTRALIZATION

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The design of effective malaria vaccines will require harnessing the human antibody response to produce broadly-neutralizing antibodies against *Plasmodium* antigens, which contain both protective and non-neutralizing epitopes. Highly-immunogenic non-neutralizing epitopes produce high-titers of non-protective antibodies and limit the production of neutralizing antibodies against protective epitopes. Therefore, accurate human B cell epitope maps of *Plasmodium* antigens are necessary to identify and retain protective epitopes, while eliminating highly-immunogenic non-protective epitopes for vaccine designs. We will present the first structural and functional data on naturally-acquired human antibodies that target *Plasmodium* antigens. Human monoclonal antibodies were isolated, cloned, expressed, and purified from individuals exposed to malaria. X-ray structures of antibodies in complex with *Plasmodium* antigens provided high-resolution definition of the epitopes and of the mechanisms of neutralization. In addition to crystallography, epitopes were identified by mutational, computational, and biophysical methods in a combinatorial approach. Within a given antigen, the most effective inhibitory human antibodies share a protective epitope, prevent the function of the antigen, and appear to be strain-transcending. Strikingly, the epitopes recognized by human monoclonal antibodies are distinct from neutralizing epitopes defined by mouse vaccinations, emphasizing the need to study human antibody responses as results derived from murine studies may not translate to human immunology and likely confound the design of human vaccines. These studies provide comprehensive explanations of human antibody neutralization mechanisms and expand our understanding of the function of *Plasmodium* antigens. These data, in combination with other data on epitopes known to be broadly-neutralizing, will improve the development of next-generation protective vaccines.

1612

ELUCIDATING NATURAL KILLER CELL-MEDIATED ANTIBODY-DEPENDENT CELLULAR CYTOTOXICITY TOWARDS RED BLOOD CELLS INFECTED BY *PLASMODIUM FALCIPARUM*

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In malaria endemic areas, humans develop clinical immunity only after years of recurrent exposure. This naturally acquired immunity depends primarily on antibodies specific for parasite antigens. The underlying basis of this protective response remains unclear. In particular, the contribution of antibody-dependent cellular cytotoxicity (ADCC) to malaria immunity remains unclear. Primary human natural killer (NK) cells from peripheral blood exhibit potent ADCC through $Fc_{\gamma}RIII$ (CD16) binding to IgG-coated target cells. Without specific antibodies, NK cell-mediated natural cytotoxicity towards both uninfected and infected RBCs was undetectable. Addition of serum from rabbits immunized with human RBC, however, resulted in NK cell-dependent lysis of both uninfected and infected

RBC. Rabbit serum antibodies specific for PfEMP1, an immunodominant *P. falciparum* variant antigen at the surface of infected RBC, induced selective lysis of infected RBC by NK cells. Plasma from malaria-immune individuals also triggered selective NK-mediated ADCC of infected RBCs. Granzyme B, a serine protease released by cytotoxic lymphocytes, is a key effector of target cell death. Granzyme B activity was detected in infected RBCs during NK-mediated ADCC using a granzyme B reporter in live cells. Furthermore, the general serine protease inhibitor DCI blocked RBC lysis. These results suggest that granzymes contribute to NK-mediated cytotoxicity towards infected RBCs. Using time-lapse imaging, dynamics of granzyme B delivery into infected RBC and subsequent outcome will be analyzed. Furthermore, NK-mediated ADCC triggered by antibodies from malaria-immune individuals inhibited *Plasmodium falciparum* growth, as determined by the re-invasion of fresh, uninfected RBC. Therefore, primary human NK cells have the potential to limit the growth of blood-stage *P. falciparum* through specific ADCC-mediated lysis of infected RBC.

1613

TRANSPLACENTAL TRANSFER OF MATERNAL MALARIA-SPECIFIC IGG3 IS ALTERED BY A POLYMORPHISM IN THE BINDING DOMAIN TO FCRN

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Transfer of protective maternal IgG to the fetus occurs by active transport via the Fc Receptor (FcRN) on the syncytiotrophoblast cells of the placenta, contributing to antibody-mediated protection against malaria in early infancy. IgG1 and IgG3 subclasses are the most efficiently transported antibodies. Although IgG1 subclasses dominate the immune response to many pathogens, robust malaria-specific IgG3 often occurs to many malaria proteins and have been strongly correlated with protection against clinical malaria as it is the case for the Merozoite Surface Protein 2. Interestingly IgG3 subclass is the only IgG subclass to contain a single amino acid polymorphism R435H, localized on the Fc fragment of the heavy chain, that affects the *in vitro* IgG3 binding of IgG3 to FcRN *in vitro*. Indeed, the R435 proteoform is associated with a reduced binding). Based on a cohort of mother-newborn pairs from a malaria endemic area of Benin (N=527), where the R435 allele frequency is 80%), we show for several asexual blood stage antigens a reduced efficiency of the transplacental transfer of malaria-specific IgG3 relative to IgG1 to the same blood stage antigen (MSP1, MSP2-3D7, MSP2-FC27, MSP3, Apical Membrane Antigen 1, Glutamate-Rich Protein region R0 and R2). This impaired transport is associated with the R435H polymorphism (p=0.01) after adjustment on for malaria exposure, and *P. falciparum* placental malaria infection. Thus transplacental transfer of malaria-specific IgG3 can be impaired by an individual polymorphism in the binding domain of the Fc fragment to the FcRN and may affect the efficacy by which of newborns acquire sufficient levels of protective antibodies to combat malaria.

1614

EVALUATING THE ANTIMALARIAL ANTIBODY RESPONSE TO SEVERE *PLASMODIUM FALCIPARUM* MALARIA IN UGANDAN CHILDREN: A CASE-CONTROL STUDY

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Severe malaria remains a leading cause of morbidity and mortality worldwide. The brunt of the disease is borne by children in the sub-Saharan region where *Plasmodium falciparum* is endemic. This sub-population is at a higher risk of developing severe disease because it is only beginning to develop immunity to the disease. Epidemiologic studies have established that individuals in endemic areas acquire immunity through repeated exposure over many years, but this is not fully protective. We conducted a prospective study in Uganda, a malaria endemic area, to evaluate the antibody responses to several well characterized sporozoite and merozoite *P. falciparum* antigens. The study enrolled 711 children and followed them for a year with data collection completed every 6 months. We compared antibody responses in 498 children diagnosed with severe malaria, in the form cerebral malaria or severe anemia due to malaria, to that in 213 healthy controls matched for age and place of residence. Preliminary data indicate that, at enrollment, children with severe malaria had significantly higher antibody levels to CSP, EBA-140, EBA-175, EBA-181, MSP-2, MSP-3 and SERA5 antigens (all $p < 0.0001$) with no diminution of statistical significance following Bonferroni adjustment for multiple comparisons. Data analysis, including evaluation of response to 5 unique PfEMP1 antigens, is ongoing.

1615

TESTING ANTIGEN INTERFERENCE ON A MULTIPLEX PLATFORM FOR MALARIA VACCINE RESEARCH

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The enzyme-linked immunosorbent assay (ELISA) is a technique, commonly used to measure antibody responses in serum or plasma samples. A traditional limitation of this technique is that, individual testing is required for every antigen against which samples are evaluated. With the introduction of multiplex detection assays, such as the one developed by the Luminex Corporation (Austin, Texas), more than one analyte can now be measured simultaneously. Such assays use fluorescent coded microspheres to which individual antigens or antibodies are covalently linked. The luminescence generated from each microsphere is used to quantify the amount of antigen/antibody present in a given test sample. This technique is currently being used for the multiplex detection of malaria antibodies in serum/plasma samples.The expanded capabilities of multiplex assays bring their own inherent challenges secondary to the potential for unintended protein- protein interactions. Such interactions may alter the measurable antigen concentrations or the antibody binding affinity, leading to antibody interference, higher background signals and decreased assay sensitivity. It is thus crucial to identify any interferences that may occur in assays, in order to provide acceptable ranges for each of the multiplexed antigens in the given test serum.We tested for the interference of different malarial antigens on a Luminex multiplex platform. Recombinant full length CSP, peptides of (NANP)6 antigen and Pf16, three antigens known to impact immunity in malaria, were used. These antigens were tested in various combinations of up to four antigens

per assay, on a standard platform with blank microspheres as controls. Data were presented in Median Fluorescent Intensity (MFI). The results will be discussed.

1616

DEFINING MOLECULAR ADJUVANT EFFECTS ON HUMAN B CELL SUBSETS

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The development of malaria blood stage antigen vaccine has been difficult and there has been only limited success to date in eliciting a potent and protective immune response in humans given these vaccines. The overall goal of this project is to identify and characterize new molecular adjuvants and adjuvant combinations that may be used in vaccines, including a malaria vaccine, to induce human B cell development and activation and to promote T follicular (Tfh) helper cell differentiation. To this end our lab examined the stimulation properties of six PRR ligands: R848 (TLR7/8), GLA (TLR4), iE-DAP (Nod1), poly(I:C) (TLR3), TDB (Mincle), and CpG (TLR9), on three human B cell subsets at different developmental stages, the immature transitional B cells, the mature marginal zone and the follicular B cells. These B cell subsets are part of normal B cell development and response to infection or vaccination via PRR ligand recognition and may affect the differentiation and activation of these B cell populations, as well as downstream Tfh cell interactions. Extensive research in mice has shown that PRR ligands affect B cell differentiation and activation, however the interaction of these molecules with these B cell populations has yet to be fully investigated in humans. Transitional B cells in human peripheral and cord blood were stimulated with various PRR ligands to determine their ability to mature transitional B cells to either a marginal zone or follicular B cell phenotype. Following stimulation, the majority of immature transitional B cells differentiated into a mature follicular B cell phenotype. Marginal zone and follicular B cells were isolated from human tonsils and stimulated to determine their ability to up-regulate activation markers like CD86. Following stimulation, tonsil-derived marginal zone and follicular B cell CD86 expression increased in response to TLR7/8 and TLR9 ligands. Understanding how PRR ligands affect human B cell subset differentiation and activation will give insight into their ability as vaccine adjuvants to drive the human adaptive immune response.

1617

IDENTIFICATION OF PROTECTIVE B-CELL EPITOPES WITHIN PFSEA-1, A NOVEL VACCINE CANDIDATE FOR *PLASMODIUM FALCIPARUM* MALARIA

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We discovered *Plasmodium falciparum* Schizont Egress Antigen-1 (PfSEA-1) by whole proteome differential screening using plasma from resistant and susceptible children living in a holoendemic region of Tanzania. Naturally occurring antibodies to the immunorelevant region of PfSEA-1 (aa 810-1023; PfSEA-1A) protect young children from severe malaria and vaccination of mice with PbSEA-1A protect against *P. berghei* ANKA challenge. To identify protective B-cell epitopes in PfSEA-1A, we vaccinated non-human primates (*Aotus sp*; n=7) with rPfSEA-1A. We performed linear, B-cell epitope mapping of PfSEA-1A using anti-sera collected from vaccinated animals and screened microarrays containing 15mer overlapping peptides spanning PfSEA-1A. These serum samples recognized 5 unique, linear B-cell epitopes within PfSEA-1A. We next determined if antibodies to these 5 epitopes were associated with protection from parasitemia following treatment in a cohort (males; age 7-30) from a holoendemic region of western Kenya. Volunteers were enrolled and drug cured of malaria infections at the start of a high transmission season, and followed with weekly blood films (18 wks) to assess reinfection. Blood was collected for serologic assays 2 weeks post treatment, prior to reinfection. We synthesized 5 peptides (~25aa) each containing one of the identified epitopes, coupled them to Luminex microspheres, and measured anti-peptide IgG antibody levels in the 2wk post treatment sera collected (n=141). When analyzed as continuous antibody levels in GEE models, IgG responses to epitopes 1, 4, and 5 predicted significantly decreased parasitemia over 18 weeks of follow-up (P=0.005-0.015). When analyzed dichotomously, individuals with high antibody levels (≥median) to these epitopes had 25-26% decreased parasitemia (P=0.009-0.012) over the 18 wks of follow-up compared to individuals with low antibody levels (<median). To advance the development of PfSEA-1 as a vaccine candidate, we are now designing immunogens targeting antibody responses to these three protective epitopes and have begun work to identify the Tfh-cell epitopes driving these responses.

1618

DISTINCT EXPRESSION PATTERN OF INHIBITORY MOLECULES ON CD4+ T CELLS IS ASSOCIATED WITH UNCOMPLICATED VERSUS COMPLICATED MALARIA

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Infections with *Plasmodium falciparum* (Pf) can lead to a wide clinical spectrum, ranging from life-threatening malaria to asymptomatic infections. The immune response of the infected host is one of the main factors influencing the clinical picture of a Pf infection. In endemic areas, regularly exposed children over years develop a "clinical immunity" which protects from severe malaria and is associated with mild or asymptomatic Pf infections. The immunological mechanisms involved remain poorly understood but immune tolerance has been proposed to contribute to "clinical immunity". We therefore examined the CD4+ T cell response of Ghanaian children with 1) complicated malaria, requiring inpatient treatment, 2) uncomplicated malaria, treated as outpatients; 3) asymptomatic Pf infection and 4) uninfected children. Using flow cytometric analysis, we characterized the expression of inhibitory molecules on CD4+ T cells such as CTLA4, PD1, TIM3, LAG3 and CD39, which play important roles in the T cell regulation in acute and chronic infections. Both groups of children with acute malaria showed high expression of PD1 and CTLA4. But children with uncomplicated malaria showed a significantly higher expression of inhibitory molecules such as TIM3 and CD39 compared to children with complicated malaria. In contrast, asymptomatically infected children expressed only low levels of inhibitory molecules. A stronger expression of inhibitory molecules is associated with a clinically milder course of acute malaria. The identification of an "optimal" CD4+ T cell response could contribute to the development of new treatment and vaccination strategies for complicated malaria.

1619

USING DEEP POPULATION SEQUENCING TO INVESTIGATE IMMUNE-BASED SELECTION ON ANTIGENIC *LOCI* IN *PLASMODIUM FALCIPARUM*

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It is commonly presumed that the human immune response interacts with *Plasmodium* antigens in an allele-specific manner. Until recently, however, we lacked the large-scale data to rigorously address these questions in naturally acquired infections. As part of the recent RTS,S/AS01 phase 3 trial, we obtained genetic data from over 5,000 infections at 11 study sites across Africa. This deep population sampling provides extremely high genetic resolution for three polymorphic *P. falciparum* proteins: CSP, SERA2, and TRAP. Previous analysis of these data compared vaccinated and control individuals to find that the RTS,S vaccine shows allele-specific efficacy at CSP. Here, we focus on the unvaccinated control individuals to investigate whether naturally acquired immunity exerts selective pressures and shapes patterns of polymorphism at these three loci. At CSP, but not SERA2, we found significant linkage disequilibrium both within and between putative T cell epitopes. Using a combination of haplotype-based analysis and in silico population genetic modeling, we showed that the linkage at CSP is most consistent with a model of long-term balancing selection. Further, the dataset's unprecedented size provides the power to investigate intrahost selection at all three proteins. We examined polymorphism within each infection and found regions of reduced intrahost diversity in older children compared to younger children in both CSP and SERA2. Analysis of TRAP is ongoing. This pattern is consistent with a model of immune-mediated, allele-specific selection. Combining the signals of population-level and intra-host selection, we pinpointed specific amino acids that appear to drive the evolutionary dynamics at each protein, furthering our understanding of how the human immune system may interact with, and shape the diversity of, the parasite.

1620

ANTIGENICITY AND TRANSMISSION-BLOCKING EFFICACY OF *PLASMODIUM VIVAX* PVS48/45 PROTEIN

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Plasmodium P48/45 are gametocyte antigens involved in parasite fertilization which induce immune responses that lead to blockage of parasite transmission to mosquito and are therefore considered candidates to develop a malaria transmission-blocking (TB) vaccines. In the process of developing *P. vivax* 48/45 as potential vaccine we recently expressed as a full length a recombinant product (rPvs48/45) and 5 internal fragments covering the entire protein. Immunogenicity studies in mice and Aotus monkeys indicate that anti-Pvs48/45 antibodies prevent the formation of oocyst in the mosquito midgut. Antigenicity studies were carried out in 235 plasma of individuals from malaria endemic areas of Colombia. Samples were assessed using the Pvs48/45 full-length protein and reactivity in 160 samples using five sub-fragments recombinant products by ELISA. Overall response indicated that 75.3% (177/235) of the sera recognized the full-length protein, whereas the N-term fragment encompassing the sequence between a.a.14 and a.a.186 was the most frequently recognized, 35.6% (57/160). Although all other fragments were recognized, their reactivity ranged between 15.5 - 21.7%. Transmission blocking assays in 152 samples tested showed that ~10% of these sera contains specific *P. vivax* antibodies with high TB activity (90-100 %), 51% showed an intermediate (50-90%) TB activity and 38% presented low (0-50%) TB activity. Furthermore, affinity purified anti-Pvs48/45 cross-reacted with *P. falciparum* gametocytes by IFAT. These results confirm the high antigenicity of Pvs48/45 and identify the N-term fragment as the most antigenic. The functional activity of affinity purified specific anti-Pvs48/46 N-term fragment is being tested.

1621

HUMAN CORD BLOOD CXCR5+ CD4 T CELLS: ASSOCIATION WITH *IN UTERO* EXPOSURE AND ANTIBODY RESPONSES TO *PLASMODIUM FALCIPARUM*

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Fetuses exposed to *Plasmodium falciparum* (Pf) from infected mothers can make anti-malarial antibodies but it is unclear if long-lived plasma cells (LLPC) and memory B cells (mBC) contribute to the antibody production *in utero*. T-follicular helper cells (TFH) in adult germinal centers provide help for primed B cells to differentiate into LLPC and mBC and CXCR5-expressing CD4 T cells in peripheral blood are circulating counterparts of TFH. The present study investigated whether CXCR5+ CD4 T cells are present in umbilical cord blood and if they are associated with *in utero* exposure and antibody responses to Pf antigens. Expression of CXCR5 messenger RNA (mRNA) was quantified by real-time PCR in CD4+ T cells isolated from peripheral blood mononuclear cells (PBMC) of 30 Cameroonian neonates and 6 Cameroonian adult controls. Day 5 supernatants of neonatal PBMC cultures were tested for IgM and IgG to a panel of blood-stage Pf antigens using the MagPix. Placental malaria (PM) was assessed by microscopic examination of placental impression smears. A total of 20 out of 30 neonates (66.7%) had detectable CXCR5 mRNA with 20% having higher CXCR5 expression than the least expressing adult sample and 3.3% having expression levels comparable to the median adult expression. The presence of PM significantly increased CXCR5 expression ($p=0.016$) and in 55% of neonates, *in vitro* treatment of PBMC with MSP-1 antigen also increased CXCR5 expression. Also, 0% and 16.7% of culture supernatants tested positive for Pf IgM and IgG respectively but CXCR5 expression levels did not correlate with IgG levels. Collectively, the data show that circulating TFH-like cells can be produced *in utero* and their frequency increases in response to fetal Pf exposure. Supplemental studies are on thee way to determine if Pf-specific mBC and LLPC are generated *in utero*.

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PLASMODIUM FALCIPARUM WHOLE PROTEOME ANTIBODY PROFILES OF EUROPEAN VOLUNTEERS IMMUNIZED WITH SPOROZOITES UNDER CHLOROQUINE CHEMOPROPHYLAXIS

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Malaria remains a major disease burden in developing countries, killing over 438,000 people in 2015. Controlled human malaria infection (CHMI) has allowed for rational vaccine discovery and development.

Immunizations with both sporozoites from mosquito bites (CPS) and live, metabolically active cryopreserved sporozoites (CVac) under chloroquine Chemoprophylaxis in naïve healthy individuals induces dose-dependent sterile protection against sporozoites, but no immunity against blood stage infection. Understanding the antibody immune response in this immunization strategy is an important step in elucidating the mechanism of protection and designing next generation vaccine strategies. To understand antibody responses associated with protection, whole proteome microarrays covering 91% of the *P. falciparum* proteome were developed. We probed and analyzed serum samples collected from 39 Dutch individuals in three clinical trials who had undergone CPS immunization and 27 German volunteers in a trial of CVac at time points before and after immunization and CHMI. Protected CPS-immunized individuals showed a dichotomous antibody profile: low and high responders. High dose recipients had a broader repertoire of antibody reactivity compared to medium and lower dose recipients. Unprotected individuals showed boosting of antibody levels after CHMI. Only antibodies against CSP were boosted in protected individuals. In CVac recipients, liver stage proteins had the highest seropositive rate. The high dose group (9/9 protected) had a larger network or immunoreactive proteins than the low and medium dose groups (3/9 and 6/9 protected, respectively) in bipartite network analysis. Dichotomous antibody profiles suggest two mechanisms of protection: 1) early protection that prevents increased parasitemia and limits antigen exposure, and 2) delayed protection at the late liver stage with greater antigen exposure. Correlates of susceptibility likely illustrate higher levels of antigen exposure due to more frequent and greater blood stage parasitemia.

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RAPID ASSESSMENT OF A NATIONWIDE LONG LASTING INSECTICIDAL (MOSQUITO) NETS DISTRIBUTION CAMPAIGN IN BENIN

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In 2014 Benin's National Malaria Control Program (NMCP) distributed more than 6 million long-lasting insecticidal nets (LLINs) through a national mass distribution campaign. The NMCP assessed post-campaign household (HH) coverage to help inform decisions about future campaigns and distributions. Random cluster sampling of villages and city neighborhoods was conducted in Benin's 12 geographic departments. The final sample included all of Benin's 34 health zones. Each health zone contained 30 clusters containing 10 HH each. Data were collected through interviews and direct observation of LLIN ownership and use. Population based estimates of universal coverage rate (one net per two persons), LLIN use and respondents' knowledge of malaria were generated. Of 10,002 HHs surveyed, 88% (95% CI: 87.2, 88.5) received a campaign coupon to claim a free net. Of these HHs, 89,857 (89%) provided information on the number of LLIN received and 8,528 (85.%, (95% CI: 84.5, 85.9) reported receiving at least one LLIN. Less than 3% of HHs received a coupon, but did not receive at least one LLIN. Fifty-six percent (95% CI: 55.4, 57.5) of HH receiving an LLIN during the campaign received 2-3 LLINs. Approximately 76% (95% CI: 75.3, 77.2) of HHs receiving LLIN during the campaign reported adequate coverage (at least one LLIN for two persons). The gap in "adequate" coverage during the mass distribution campaign was in part due to an underestimate of the population in need of LLINs and a gap in the number of LLINs available for distribution during the campaign. The survey also found that 77.2% (95% CI: 76.4, 78.0) of HHs used an LLIN the previous night, and 89% (95% CI: 88.5, 89.7) of HHs demonstrated a good knowledge of the benefits of LLIN use for malaria prevention. Although this initial post-campaign assessment found that a high proportion of HHs received at least one net and the majority of HHs received an adequate number of nets during the national campaign, additional investments in campaign planning and logistics are needed to improve national universal coverage in Benin.

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DATA QUALITY ASSURANCE AND DATA MANAGEMENT IN A LARGE SYSTEMS BIOLOGY PROJECT: MAHPIC

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Large systems biology projects, such as the NIH/NIAID supported Malaria Host Pathogen Interaction Center - MaHPIC involve several collaborating research centers. Often, each center is specialized in different aspects of the system and each generates different data types such as genomics, transcriptomics, proteomics, metabolomics, lipidomics, immune profiling, clinical data, interaction models etc. Often, each center is accustomed to their own protocols, standards, and research practices, as dictated by their respective fields of specialization. However, together, they generate thousands of files that occupy terabytes of storage space. These data include raw and intermediate data (processed/normalized), result files, metadata, SOPs and other supporting documents. Data integration and mathematical modeling are the cornerstones and preferred approaches for systems biology research. However, for data integration and modeling to be possible, high-quality data in defined formats are necessary to make them computationally tractable. In the face of the high volume, high variety and high velocity of data generation, ensuring quality, accuracy, and accessibility to modelers, the team and the research community is a huge challenge. Solutions require rigorous standards, and willing participation by all involved. The Informatics Core of the MaHPIC works closely with all data producers and consumers to implement standards (when they exist) and to develop and/or implement rigorous protocols for data collection, validation, transformation, and dissemination. The solutions we have developed include metadata and result templates designed for each data type, data transfer protocols that include a pre-transfer review of data, validation scripts and procedures, a dedicated file repository, a relational database for rapid data access by mathematical modelers, ontological mark-up of experimental processes and results, and several Web-based resources managed under a single project Portal. This high level of quality control for rich, well-curated data creates a valuable resource that should permit discoveries for years to come.

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FLUORESCENT LABELLING OF WILD TYPE *PLASMODIUM* SPECIES WITHIN THE MOSQUITO HOST: A NOVEL METHOD TO TARGET SPOROZOITES

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Mutant *Plasmodium* parasites expressing fluorescent proteins allow preclinical imaging of malaria development and distribution in both cell lines and animal models. However, the widespread application of genetically altered organisms is limited, due to regulatory constraints and the inability to culture some *Plasmodium* species, such as *P. vivax*. As such, reporter lines are not available for all *Plasmodium* species. This calls for more generic approaches that allow for the targeting and (molecular) imaging of sporozoites. Here we present a novel method to fluorescently label the sporozoite stage of wild-type *Plasmodium* species *in vivo* and without the need for genetic modification or the extraction of the parasite from its mosquito host. *In vitro* studies demonstrated a tailored fluorescent

cyanine-5 (Cy5) dye could efficiently stain sporozoites *in vitro*. By membrane-feeding infected *Anopheles* mosquitoes on glucose using the exact same dye we were even able to specifically label sporozoites within the mosquito's salivary glands *in vivo*. The Cy5-dye was preferentially taken up by the mitochondrion of sporozoites and the uptake therein was higher compared to native mosquito tissue such as salivary gland cells or cells of the midgut. This specificity indicates that the mitochondrial activity of sporozoites provides a valuable (*in vivo*) targeting mechanism. To demonstrate cross-species utility of this technology, it was successfully applied in *Plasmodium yoelii*, *berghei* as well as *falciparum*. Viability of the fluorescently labelled sporozoites was confirmed in a hepatocyte cell line. Targeting plasmodium sporozoites through the feed removes the need for genetic modification with imaging vectors, thereby allowing more detailed studies for species such as *Plasmodium vivax*. In addition, the specificity of the mitochondrial uptake that we observed, suggests this to be a possible molecular targeting route for sporozoites residing in the mosquito host.

1626

THE IMPACT OF REVISED HEALTH MANAGEMENT INFORMATION SYSTEM (HMIS) REPORTING FORMS ON THE QUALITY OF MALARIA SURVEILLANCE DATA IN UGANDA: AN INTERRUPTED TIME SERIES ANALYSIS

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Malaria control programs need accurate data to implement and evaluate malaria interventions. In July 2015, Uganda introduced revised HMIS reporting forms to health facilities (HFs) to improve data quality. To evaluate this intervention, we assessed data completeness and accuracy in five HFs in Kayunga district. We abstracted data from 7,523 records in outpatient (OPD) registers and surveillance summary reports for 12 months before and four months after the intervention. Monthly completeness was measured as the proportion of malaria patient records with: 1) all data fields completed, and 2) clinically-relevant fields completed. Accuracy was the relative difference between numbers reported in the OPD register and surveillance reports for total patients, malaria patients, malaria tests performed, and positive malaria tests. Data were analyzed as interrupted time series with segmented linear regression. The current analysis is limited to one HF with complete time series available; data collection for other HFs is ongoing. Completeness for all data fields ranged from 0-14% over time, with no effect of the intervention (*P*-value of instantaneous change [*P*(i)]=.94, *P*-value of slope change [*P*(s)]=.66). Completeness of clinically-relevant fields, which averaged 30% at baseline, showed an improvement of 38 percentage-points immediately following the intervention (95% CI: 0.28-0.49; *P*(i)<.0001). This increase was driven by improvement in recording patients' weight. Discrepancies between surveillance reports and registers ranged from 0-15% for all patients, 1-9% for malaria patients, 24-71% for tests performed, and 0-20% for positive tests, with no significant intervention effect. In conclusion, revised reporting forms improved completeness for clinically-relevant data but had no effect on data accuracy. Analysis of additional HFs will assess intervention effectiveness in a broader setting.

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IMPACT OF THE APPLICATION OF THE NEW GUIDELINES OF MALARIA CASE MANAGEMENT IN SENEGAL

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Since 2013 The NMCP has made a review of its management policy and malaria prevention with the introduction of the new guidelines as recommended by the WHO. . So in February 2014, we updated the training book, the facilitator and participant's guide with the support of all stakeholders . With the mobilization of 15 trainers from the central level, between February and April 2014, the agents of medical regions were oriented and 312 out of 328 were trained that makes a total participation rate of 95% with a sex ratio of 54% of men and 46% women. At the operational level, among the 2991 agents which were provided for, 2951 were trained that is to say a participation rate of 98% covering all the districts in the country with a sex ratio of 42% women and 58% men. At the community level, 50 community health workers were trained with 36 from health huts and 16 from ICCM sites with a focus on pre-referral treatment of severe cases. Thus between 2013 and 2015, there has been a positive improvement of some indicators of malaria case management in health structures resulting in a remarkable rise of the screening rate from 87.49% to 99.3%, the rate of ACT distribution from 98.3% to 99.5%, the rate of the 2nd of IPTp coverage from 65.93% to 70.18% with an effective implementation of the 3rd dose of IPTp amounting to 42.72% in 2015. In the same year, 7684 pregnant women with simple cases of malaria were effectively managed with ACT according to the new guidelines. . The management of severe cases by the injectable Artesunate became effective in 2015 at 07 pilot health units with a cure rate of 90% among the enrolled cases. All these positive results have shown that the implementation of the new guidelines can considerably improve the management of malaria at the level of prestation places and reduce the impact of morbidity and mortality at the same time. The NMCP is prospecting to continue the training of new providers but also to ensure a steady supervision of the trained staff to maintain the achievements in terms of capacity building in order to fully pre-eliminate malaria in Senegal.

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THE "DYNAMIC EPIDEMIOLOGY" OF MALARIA ELIMINATION IN EL SALVADOR: THE ROLE OF PROGRAM DECENTRALIZATION, STRATIFICATION, AND TIMELY TREATMENT IN THE RAPID AND DURABLE DECLINE IN MALARIA INCIDENCE SINCE THE EARLY 1980S

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Resurgence of malaria cases in the 1970s following the end of the Global Malaria Eradication Program led El Salvador to re-evaluate and alter its national malaria control strategy. By the early 1980s, El Salvador had the highest burden of malaria in Mesoamerica (95,835 cases in 1980, ~20% being *Plasmodium falciparum*). In 1995 El Salvador had its last autochthonous *P. falciparum* case. Today, it is on the verge of malaria elimination with fewer than 20 *P. vivax* cases per year since 2011 while its immediate neighbors continue to have the highest malaria incidences in the region. We reviewed and evaluated the policies and interventions implemented by the Salvadoran national malaria control program that likely contributed to this progress toward malaria elimination. Decentralization of the program, early regional stratification by risk, and

stratum-specific program actions resulted in the timely and targeted allocation of resources toward vector control, surveillance, and infection detection and treatment. The presumptive treatment regimen of combined chloroquine + primaquine was also shortened to five days, which greatly improved compliance. Importantly, weekly reporting by health workers and volunteer collaborators distributed throughout the country by strata, and informed via a reliable digital information system, enabled local malaria teams to provide rapid, adaptive, data-based responses in a locally focused manner leading to the description of the program in El Salvador in the 1980s and 1990s as "dynamic epidemiology". Data-based adaptation of the program continues to yield favorable results to maintain pre-elimination levels, with most of the current cases being imported from neighboring countries. Additional support for systematic elimination efforts in neighboring countries, potentially learning and adapting from the El Salvador experience, will undoubtedly benefit each of these countries and may be required for El Salvador to fully achieve malaria elimination. El Salvador provides a relevant country case study and learnings can guide application of similar strategies in other countries approaching malaria elimination.

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MOVING TOWARD IMPROVED MEASUREMENT OF MALARIA MORTALITY AT THE POPULATION LEVEL

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Measuring malaria-specific mortality at the population level is challenging due to the difficulty in assessing malaria and the fact that most malaria deaths occur outside of the formal health system. To address this gap, the verbal autopsy (VA) method was developed to ascertain cause of death at the population level, yet there are limitations with current VA tools and approaches for measuring malaria-specific mortality. Given the emphasis in the new Global Technical Strategy for Malaria on monitoring malaria-specific mortality, there is a strong need for the malaria community to develop improved methods for measuring malaria-specific mortality. To help inform a strategy for improving current methods, we carried out a systematic literature review to assess how VA tools and approaches have been used to measure malaria-specific mortality and the key challenges and limitations of existing tools and methods. A key limitation we found was the varying and overall low levels of sensitivity and specificity of VA tools for measuring malaria mortality, due to the non-specific symptoms of malaria and the differing malaria epidemiological contexts in which studies are conducted which result in misclassification bias (either over- or under-estimating the burden). Further, most VA validation studies use hospital records as the gold standard to compare VA results, yet these are not a true gold standard since it is a reflection of a different population and are often incomplete. Comparability of malaria mortality results across study sites was also a challenge, due to a lack of standardization in the application of VA tools and methods and the limited details provided overall in many published VA studies on the methods and tool used, including how malaria cause of death is determined. Given these limitations, we propose using community estimates of mortality measured through VA and complementing them with in-patient mortality data from health facilities that incorporate malaria parasitological confirmation to produce more robust population-level estimates of malaria mortality.

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MEASURING THE IMPACT OF MALARIA ON HEALTH-RELATED QUALITY OF LIFE OF CHILDREN IN RURAL WESTERN KENYA

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The aim of our study was to assess the impact of malaria on health-related quality of life (HRQoL) using subjective patient reported measures. We used purposive sampling at outpatient and inpatient hospital wards to select our sample from those families seeking care at a private facility in rural western Kenya, an area with high baseline malaria parasitaemia. Our subjects met the following criteria: i. Child under age 15 years; ii. Laboratory-confirmed malaria. We collected information from parents or caregivers of 64 children, age 4 months - 13 years via audio recorded interviews at the time of receiving medical care and then in person or by phone 5-7 days after the health facility visit. Using mixed methods, we gathered narrative and survey information on the child's baseline health prior to the current illness and data on quality of life from the time of illness onset through recovery, such as the ability to play, go to school, or participate in normal activities. Overall, 42% of our sample was hospitalized children. Mean HRQoL values were 0.7312 SE 0.0161. HRQoL values were inversely correlated with high fever (pwcorr -0.8830, $p < 0.001$; Bonferroni adjustment), with the lowest rating of HRQoL occurring between 1 and 4.5 days after the onset of symptoms. We converted HRQoL to a summary outcome measure, Quality Adjusted Life Years (QALYs). The mean QALYs lost per child during a treated episode of malaria in our sample were 0.005 (SE 0.0006, 95% CI 0.0044-0.0056). Generalizability of our results was limited due to the high proportion of hospitalized children in our sample, reflecting greater severity of illness than would be experienced by children who were not hospitalized. Nevertheless, our study offers the first time that HRQOL values have been mapped, and QALYs calculated for pediatric malaria.

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THE CATASTROPHIC IMPACT OF MALARIA ON HOUSEHOLDS IN RURAL WESTERN KENYA

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We measured the impact of pediatric malaria on the total value of out of pocket payments and opportunity costs to households in rural western Kenya. We used purposive sampling at outpatient and inpatient hospital wards to select our sample from those families seeking care at a private facility in rural western Kenya, an area with high baseline malaria parasitaemia. Our subjects were children less than 15 years of age with laboratory-confirmed malaria. We collected information from parents or caregivers of 64 children, age 4 months - 13 years via audio recorded interviews at the time of receiving medical care and then in person or by phone 5-7 days after the health facility visit. We gathered information on illness-associated costs from travel, treatments, medical care, food, lost time from work, school, and the status of children left at home while a parent or caregiver was attending to the ill child. Overall, 42% of the sample was hospitalized children. A total of 11% of all families used herbs prior to bringing the child to the hospital, paying a

median cost of 250 Ksh, over 83% of the cost of a full pediatric course of artemether/lumefantrine. Use of herbs was significantly associated with a longer interval between symptom onset and presenting for diagnosis and treatment (Pearson $\chi 2$ 136.5, $P < 0.000$) but a lower chance of hospitalization. Even when there were no charges for direct medical care, study families incurred high costs due to lost work time and out of pocket costs for transport. We found that total costs for a single treated episode of pediatric malaria in our sample represented Ksh 1750 or USD 20.59, which is 34% (sd 0.24) of the estimated median monthly household consumption of Ksh 5000 or USD 58.82 in rural Kenya. We found that significant components of costs to households during an episode of malaria were pre-hospital payment for medicinal herbs, and the gap in time from the first symptoms of illness until arrival at the health facility. In many cases, pre and post treatment costs alone imposed a catastrophic burden on families, forcing parents to sell assets, borrow money, or reallocate existing funds for school fees to cover the costs of care.

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IMPROVING PRIVATE SECTOR MALARIA CASE SURVEILLANCE AND QUALITY ASSURANCE USING DHIS2: LESSONS LEARNED

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Ensuring private providers report on all malaria cases accurately is critical to transform malaria surveillance into a core intervention as recommended in WHO's Global Technical Strategy for Malaria (2016-2030). Equally important is ensuring the quality of fever case management (FCM) by private sector providers. However, in many malaria endemic countries, health management information systems (HMIS) for the private sector are parallel to the government or non-existent, and do not include quality of care data. In response, PSI in collaboration with partners and funded by UNITAID, developed a routine monitoring system for private providers, combining case surveillance data (provider reported) with quality of care data (supervisor reported). Data was managed through DHIS2 to allow for integration with national HMIS. Piloted in Kenya, Tanzania, and Madagascar from 2013-2016, this approach has resulted in valuable lessons learned: Provider reporting rates varied across provider type and country, and were difficult to track due to limitations in managing a dynamic network of outlets in DHIS2. Quality of care data was successfully integrated into DHIS2 and managed alongside case surveillance data to prioritize scheduling of follow-up supervision visits. Quality of surveillance data, measured through Data Quality Audits highlighted challenges in the reliability of aggregated case surveillance data; while the quality of supervision data could not be assessed as there is no universally accepted gold-standard checklist. These lessons highlight actionable conclusions. 1) Routine monitoring systems and analytics tools (such as DHIS2) need to be as dynamic as the network of providers being monitored; 2) a globally validated tool to measure quality of FCM will allow countries to validate country-specific tools; and 3) provider level reporting should be simplified and streamlined, while considering provider incentives to improve data quality. The implementation of these lessons will help strengthen malaria surveillance and quality assurance in the private health sector and therefore accelerate progress towards malaria elimination.

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METHOD FOR THE SIMULTANEOUS MEASURE OF THE LEVEL OF NINE ANTIMALARIAL DRUGS IN DRIED BLOOD SPOT SAMPLES USING LC-TANDEM MASS SPECTROMETRY AND RELATIONSHIP OF LUMEFANTRINE CONCENTRATIONS IN DRIED BLOOD SPOT SAMPLES AND IN PLASMA

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Storage and transportation of blood samples are common problems for studies in areas with a high prevalence of malaria. The dried blood spot (DBS) sampling technique is promising for that use, enabling easier storage and transportation requirements. We present a method for the analysis of antimalarials in DBS. We also show the relationship between the concentrations of lumefantrine in DBS and with the usual method of plasma sampling. We added known concentrations of amodiaquine, desethylamodiaquine, quinine, chloroquine, mefloquine, sulfadoxine, pyrimethamine, lumefantrine and desbutyl-lumefantrine in whole human blood. A 10µl aliquot of this blood was applied on a filter paper card and allowed to dry for three hours at room temperature. We took a 3 mm punch out of each dried blood spot and extracted it with 100 µl of methanol containing the stable isotopically labeled Internal Standards for all the antimalarials. We used a multiplex chromatography coupled to tandem mass spectrometry (LC-MS/MS) method for the simultaneous measure of the 9 antimalarials. We measured the concentrations of lumefantrine both in DBS and in plasma obtained in 16 healthy volunteers after they had received a single dose of artemether-lumefantrine. Lower limits of quantification were 2 ng/ml for pyrimethamine, 6 ng/ml for desthylamodiaquine, and 20 ng/ml for the other antimalarials. The inter-day variation coefficient was 2.1-15.2%. Lumefantrine concentrations measured in plasma were twice as high as those measured in DBS and were highly correlated (r=0.99). Our technique enables both precise and sensitive measurement of antimalarials in DBS, despite the low volume of blood sampled. The correlation between lumefantrine concentrations in DBS and in plasma is almost perfect. This relationship could thus contribute to defining the therapeutic ranges of lumefantrine concentrations measured in DBS. The ratio between the concentration in DBS and in plasma could reflect the distribution of lumefantrine in the different blood compartments. The DBS sampling method is suitable for antimalarials level measurements and could be convenient for epidemiological studies.

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MALARIA AND HELMINTH COINFECTION-INDUCED OXIDATIVE STRESS AND CHANGES IN ANTIOXIDANT STATUS AMONG AFEBRILE SCHOOL CHILDREN IN IBADAN, SOUTHWEST NIGERIA

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Malaria and helminth infections are common tropical diseases in Sub-Saharan Africa. Little is known about the effect of co-infection of the

two diseases on the antioxidant defense system. This study determined the effect of malaria and helminth co-infection on antioxidant status in afebrile school children. A total of 99 afebrile school children were chosen comprising 25 with helminth infection, 25 with malaria-helminth co-infection and 24 negative for both malaria and helminth infections. Malaria parasite was determined by microscopy while helminth infection was confirmed by Kato-Katz method. Plasma hydrogen peroxide (H_2O_2), malondialdehyde (MDA), protein carbonyl (PC), xanthine oxidase (XO), NADPH oxidase (NOX), myeloperoxidase (MPX), reduced glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidise (GPX), glutathione S-transferase (GST), ascorbic acid (AA) and α-tocopherol (TOC) were determined. Plasma levels of H_2O_2, MDA, PC as well as activities of XO, NOX and MPX were significantly higher in children with co-infection of malaria and helminth followed by helminth only and malaria only relative to uninfected children ($p<0.05$). GST activity, GSH and AA levels were significantly reduced while SOD and GPX activities were significantly higher in co-infected children followed by malaria only and helminth only relative to uninfected children ($p<0.05$). CAT activity was significantly higher in malaria only followed by co-infection and helminth only infected children relative to uninfected children ($p<0.05$). TOC level was significantly lower in helminth only followed by co-infection and malaria only relative to uninfected children ($p<0.05$). Malaria and helminth co-infection in afebrile school children caused a reduction in plasma antioxidant status as evident from significant increases in oxidative stress markers (H_2O_2, MDA, PC levels and activities of XO, NOX and MPX) and consequent depletion of the thiol GSH, AA, TOC and GST activity.

1635

HIGH THROUGHPUT IDENTIFICATION OF *ANOPHELES GAMBIAE* MIDGUT GENES INVOLVED THE INVASION OF *PLASMODIUM FALCIPARUM*

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An anopheline mosquito midgut is an important organ for malaria transmission. However, the interaction between a mosquito midgut and *Plasmodium* parasites is not well elucidated. This study aims to investigate the molecular mechanisms of *Plasmodium* invasion in midguts. First, we developed a computational algorithm to predict candidate mosquito midgut proteins based on sequences and oligo-array data. Ninety-four candidate genes were predicted in *Anopheles gambiae* mosquito midguts and expect to involve in A. gambiae and *Plasmodium falciparum* interaction. More than 90% of these candidates are novel. Next, we cloned these genes and successfully expressed 68 in insect cells. ELISA binding assay revealed that 28 recombinant proteins bound to *P. falciparum*-infected cells. Furthermore, we determined functional effects of 28 candidate genes on *P. falciparum* infection in mosquito midguts using dsRNA-mediated gene expression silencing assays. The results indicated that three genes facilitated the infection of *P. falciparum* parasites and five genes inhibited the infection of *P. falciparum* parasites in mosquito midguts. Together, these results support our hypothesis that mosquito midgut proteins play critical roles in regulating *P. falciparum* parasite transmission. Notably, the results from this project lay a solid foundation to develop novel approaches to block malaria transmission.

1636

RELATIONSHIPS BETWEEN TRAVEL AND RTS,S MALARIA VACCINE EFFICACY IN LILONGWE, MALAWI

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The RTS,S/AS01 vaccine was recently approved by the European Medicines Agency Phase III after clinical trials showed moderate levels of efficacy that varied between the 11 clinical trial sites throughout Africa. This study assesses the relationship between travel and vaccine efficacy in a seasonal-transmission region of sub-Saharan Africa in one of the trial sites, Lilongwe, Malawi. Travel and mobility have been shown to be significant risk factors for malaria incidence but the relationship between travel and malaria vaccine efficacy has not been studied. We followed children (5-17 months of age) and infants (6-12 weeks of age) who had been randomly assigned to either a vaccine group, vaccine with booster group, or control group. Primary efficacy was defined as development of clinical malaria (fever ≥37.5°C and *Plasmodium falciparum* parasitemia >5,000 per microliter). A travel history was collected for the 1552 trial participants at 6-month intervals throughout the 3-year study period, as well as the spatial location of each participant's household and destination of travel. During the study, 30.34% of participants who received the placebo and 32.20% of participants who received the vaccine with or without the booster traveled outside the study catchment area at least once, with travel defined as at least one night spent outside Lilongwe. Overall vaccine efficacy was 34.5% among participants who traveled and 20.0% among those who did not. Travel was significantly associated with increased vaccine efficacy when controlling for socioeconomic status, participant age, seasonality of travel, and destination of travel ($p<0.001$). The reason the efficacy is higher for participants who travel is not well understood and further study is necessary. One potential explanation is that *Plasmodium falciparum* strains outside of Lilongwe are more sensitive to the RTS,S vaccine.

1637

ASSESSMENT OF A TRANSGENIC *PLASMODIUM BERGHEI* PARASITE EXPRESSING *P. FALCIPARUM* CELL-TRAVERSAL PROTEIN FOR OOKINETES AND SPOROZOITES (PFCELTOS) FOR USE AS A HOMOLOGOUS RODENT CHALLENGE MODEL TO TEST VACCINE EFFICACY

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The use of animal models to simulate human malaria infection is critical for malaria vaccine development and down-selection. While nonhuman primate models may approximate clinical immunology, they do not allow testing of protection against *Plasmodium falciparum* infection. Thus, the majority of malaria vaccine development utilizes rodent models to assess immunogenicity and vaccine efficacy. Studies have shown that the Cell-Traversal protein for Ookinetes and Sporozoites (CelTOS), highly conserved among Plasmodium species, plays a major role in parasite invasion of both mosquitos and vertebrates. A unique feature of CelTOS is that cross-species protection is achievable, as evidenced by our ability to attain 60% heterologous protection against *P. berghei* malaria following vaccination with *P. falciparum* CelTOS (PfCelTOS)/ISA 720. Although protection is routinely observed with the wild type *P. berghei* challenge model, a heterologous challenge for PfCelTOS-specific responses is needed for assessing homologous responses and establishing immune correlates of protection. To address the homologous challenge issue, a

chimeric *P. berghei* parasite, where the gene encoding PbCelTOS has been replaced with coding sequence for PfCelTOS (PbPfCelTOS(r)PbCelTOS) was developed as a readout and preclinical analysis method for homologous protection in mice. Experimental data will be presented on the molecular and cellular characterization of the PbPfCelTOS(r)PbCelTOS parasite with respect to: mosquito- and mouse-stage development, antibody recognition of PfCelTOS on sporozoites using anti-PfCelTOS (monoclonal and polyclonal) antibodies , *in vivo* infectivity using our standard immunogenicity testing regimens and use as a murine parasite challenge model.

1638

LACK OF GEOGRAPHIC SIGNAL IN THE PATTERN OF ALLELE AND EPITOPE FREQUENCIES IN FOUR MALARIA LIVER STAGE CANDIDATE VACCINE ANTIGENS

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Both blood stage and pre-erythrocytic malaria vaccines, including the most advanced malaria vaccine, RTS,S, have shown moderate and strain-specific efficacy, highlighting the need for new vaccines with potent cross-protective efficacy. The selection of antigen variants to include in future vaccines, and in particular their constituent epitopes, will be crucial to determine the extent of cross-protection as well as regional differences in efficacy. We assessed the potential of *Plasmodium falciparum* circumsporozoite surface protein (CSP), liver stage antigens 1 and 3 (LSA1 and LSA3), and sporozoite asparagine rich antigen 1 (SAP1), as whole antigens or deconstructed into B- or T-cell epitopes, to elicit strain-transcending immune responses. To evaluate the vaccine potential of these antigens, we determined allele and epitope frequencies among 30 isolates collected from Mali, Malawi, Myanmar, and Cambodia, and the pattern of geographic distribution of those variants. DNA was isolated from leukocyte-depleted blood samples, and used to generate de novo genome assemblies using Pacific Biosciences (PacBio), and Illumina HiSeq sequencing data. Custom scripts were used to identify, extract and align the genomic sequences for each of the target loci. For CSP, only two variants were identified in the N-terminal region of the gene, with prevalences 64% and 36%; in contrast, in the C-terminal region, 17 variants were found in the T-helper cell epitope 2 (Th2R) (range: 2.6% to 21%), and eight variants in the Th3R region (range: 2.6% to 34.2%). The alleles contained between 31 and 37 NANP repeats, and three or four NVDP repeats. The distribution of the haplotypes was not determined by geography. Among these 30 isolates, we identified eight conserved B-cell epitopes and 11 potential CD4+/CD8+ CSP epitopes restricted by the most frequent HLA allele in Mali. Identical analyses were conducted for sap1, lsa1, and lsa3 genes. The results from this study suggest that conserved epitopes of CSP and LSA3 present in both West and East Africa, as well as in South East Asia, may be promising candidates for inclusion in a multi-epitope malaria vaccine.

1639

A CONSENSUS PROTEOME OF *PLASMODIUM VIVAX* SPOROZOITES

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Plasmodium vivax represents the most geographically distributed species of human malaria. Basic research studying *Plasmodium vivax* infectious stages has lagged behind *P. falciparum* for two principal reasons: 1. *P. vivax* infections cause morbidity, but low mortality compared to *P. falciparum* and 2. we currently lack a culture system capable of growing *P. vivax in vitro*. As a result, several research groups have characterized the proteins of the *P. falciparum* sporozoite and merozoite infectious stages but the *P. vivax* sporozoite proteome has not yet been published. A detailed understanding of the *P. vivax* proteome is critical for drug and vaccine development efforts against genetically distinct species of malaria. We performed MudPIT mass spectrometry of tryptic peptides from *P. vivax* sporozoites dissected from *Anopheles dirus* mosquitoes. *P. vivax* peptides were distinguished from Anopheline peptides by searching tandem mass spectra from tryptic sporozoite peptides against a concatenated *P. vivax* - Anopheline protein database. We identified 421 core sporozoite proteins, many of which are abundant proteins with unknown functions. Additionally we identified orthologs of previously implicated vaccine candidates including CSP and TRAP (expressed abundantly on the sporozoite surface), a putative heat shock protein, SPECT1, GAMA and GEST.

1640

PLASMODIUM FALCIPARUM CIRCUMSPOROZOITE PROTEIN ADJUVANTED WITH LIPOSOMAL ADJUVANT INDUCES HIGHLY PROTECTIVE RESPONSES IN C57BL/6 MICE AGAINST TRANSGENIC PARASITE CHALLENGE

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Falciparum malaria continues to be a highly lethal infectious disease among children in tropical areas of the world. The most successful malaria vaccine candidate is RTS,S (GlaxoSmithKline), which targets the circumsporozoite protein (CSP) expressed by *Plasmodium falciparum* sporozoites. RTS,S contains the central repeat region and the C-terminal region of CSP expressed on a hepatitis B surface antigen particle, but it lacks the N-terminal region. Several lines of evidence suggest the N-terminal region of CSP contains residues that are critical for hepatocyte invasion. Furthermore, the cost of producing a soluble CSP vaccine may be significantly lower than particulate vaccines. We have produced a nearly full-length CSP soluble protein vaccine (FMP013) in the *E. coli* system. In order to select an adjuvant for this vaccine, FMP013 was tested with a battery of adjuvants in the C57BL/6 mouse challenge model. We report here the immunogenicity and protective efficacy of the GMP FMP013 product with the Army Liposome Formulation (ALF) adjuvant. ALF contains immuno-stimulant 3D-PHAD (Avanti Polar Lipids) formulated in liposomes composed of phospholipids and cholesterol. Mice were immunized three times with a sub-saturating dose of CSP+ALF combined with either aluminum hydroxide (ALFA) or QS-21 (ALFQ). Immunogenicity and protection of the ALFA and ALFQ adjuvants were compared to Montanide ISA 720 adjuvanted FMP013. Mice were challenged with transgenic *P. berghei* sporozoites expressing *P. falciparum* CSP two weeks after final immunization. Especially high levels of protection (up to 100%) were observed in the CSP+ALFQ groups; this correlated with significantly higher CSP and NANP-specific antibody titers. ALFQ groups also showed higher IgG2c titers and a TH1-biased IgG2c:IgG1 ratio. Enhanced signs of early

B-cell development and germinal centers were observed in the ALFQ groups compared to the Montanide group. Initial findings suggest that soluble CSP and ALFQ combination may hold promise. These data are now being confirmed in the Rhesus model, which will be the final go-no-go decision point For advancing this vaccine into humans.

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MASS DIRECT SKIN FEEDS OF *ANOPHELES COLUZZII* IN THE CONTEXT OF MALARIA TRANSMISSION BLOCKING VACCINE TRIALS IN BANCOUMANA, MALI

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As proxy measures to assess the activity of malaria transmission blocking vaccines (TBV), standard membrane feeding assay (SMFA), direct membrane feeding assay (DMFA), and direct skin feeds (DSF) were previously implemented only at small scale. Here we investigate the performance of mass DSF in Bancoumana, Mali. From September to November 2015, we prepared each week ~30,000 laboratory reared female *Anopheles coluzzii* 3-8 days of age, and conducted twice-weekly feeding assays for 6 weeks on ~200 volunteers aged 18-50 years participating in Pfs25-Pfs230 TBV trials. Thirty starved mosquitoes in each of two cups were fed on the arms of volunteers for 15 minutes. Fed mosquitoes were dissected for *Plasmodium falciparum* oocyst counts. Of 119,220 mosquitoes the feeding rate was 97% and the survival rate of fed mosquitoes was 75%. Of 87,487 dissected mosquitoes, the overall infection rate was 0.5% resulting from 58/2008 DSF assays (2.5%). During the 6 weeks of DSF assays, weekly infection rates varied from 0.1% to 1.1%. Among infected mosquitoes, 78% had loads between 1-10 oocysts per midgut, 7% had between 11-20 oocysts, and 15% had more than 20 oocysts. Of all DSF assays performed, greater than mild adverse events were only observed in one individual, and resolved with topical treatment. The results demonstrate the feasibility and safety of mass DSF for implementation in TBV trials, and provide a basis to calculate sample sizes for this community using mosquito infection as an endpoint.

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DESIGN, EXPRESSION AND SCALABLE CGMP PRODUCTION OF FMP014 - A SELF-ASSEMBLING PROTEIN NANOPARTICLE AS THE BASIS OF A VACCINE AGAINST *PLASMODIUM FALCIPARUM* MALARIA

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In spite of the best efforts of the global research community only a handful of vaccine candidates have shown promise in combating malaria and most of these vaccines have been presented as live attenuated viruses or as virus-like particles. We have developed FMP014, a vaccine against *Plasmodium falciparum* malaria, which is comprised of 60 identical protein chains that form a small icosahedral shaped self-assembling protein nanoparticle (SAPN) similar to the capsid of small viruses. Each monomer displays selected *P. falciparum* Circumsporozoite Protein (PfCSP) CD4 and CD8 epitopes, universal TH epitopes, and portions of the α-TSR domain and NANP repeats of the PfCSP. Here we describe the conditions that are required for successful scale-up and cGMP manufacturing of FMP014. Furthermore, we demonstrate that when assembled and formulated with the Army Liposomal Formulations ALFA, ALFQ or ALFQA the nanoparticle vaccine prevents infection of mice by an otherwise lethal dose of transgenic *P. berghei* sporozoites expressing the complete PfCSP. (In depth analysis of the humoral and cellular responses to FMP014 are given in accompanying posters by Kaba et al. and Storme et al.). The cGMP SAPN and ALF adjuvants are currently undergoing studies in nonhuman primates and will be further tested in human volunteers in 2017.

1643

OVERCOMING DIVERSITY OF AMA1: EVIDENCE OF POLYMORPHISM DILUTION MEDIATED REFOCUSING OF RESPONSES TOWARDS CONSERVED EPITOPES IN RHESUS MONKEYS VACCINATED WITH QUADVAX+AS01

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Vaccines against polymorphic antigens require strategies that cover the entire antigenic spectrum of prevailing strains in an endemic area. Given the extreme diversity of Apical Membrane Antigen-1 (AMA1) of *Plasmodium falciparum*, the down-selection of strains for a polyvalent vaccine remains a challenge. We propose that QuadVax (a mixture of only four AMA1 strains: 3D7+FVO+HB3+W2mef) may provide global coverage as was observed by *in vitro* GIA using rabbit antibodies to QV generated using Montanide ISA720 adjuvant (Dutta et al. PLOS Pathogens 2013). We now report results of a Rhesus trial that was conducted to determine if observations made originally in the rabbit model using Montanide adjuvant would hold up in the Rhesus model using QV formulated in a human-use adjuvant AS01 (GlaxoSmithKline). Two groups of Rhesus (n=7) received three immunizations of 20 ug (LO group) or 80 ug (HI group) monovalent 3D7 AMA1 in AS01 and two comparator groups received 20 (LO) or 80 ug (HI) QV in AS01. There was no significant difference between the immunogenicity and GIA activity of the LO vs. HI dose groups. Both the 3D7 and QV vaccinated animals induced high levels of ELISA and GIA activity against the homologous 3D7 strain parasites. In heterologous ELISA and GIA the two QV groups (HI and LO) showed significantly higher cross-reactivity and heterologous GIA compared to the two 3D7 AMA1 groups. The original observations of strain-broadened responses by QV in rabbits were reproduced whereby QV vaccination shifted the immune response towards cross-reactive epitopes on the domain-3 and conserved face of AMA1. Furthermore increased responses to the conserved regions correlated positively with higher cross-strain GIA. Our data pave way for continued development of AMA1 as a malaria vaccine. The concept of polymorphism dilution by mixing a small number of antigenically diverse strains may be applicable to developing pan-reactive vaccines against other diverse pathogens like dengue, HIV and influenza.

1644

T CELL IMMUNOGENICITY AND CORRELATES OF PROTECTION FROM A DOSE-ESCALATION SAFETY AND EFFICACY STUDY OF PFSPZ WITH CHLOROQUINE IN MALARIA-NAÏVE ADULTS

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High-level protective efficacy is induced in a dose-dependent manner by infectious PfSPZ administered under chloroquine prophylaxis (Pf-CVac) followed by controlled human malaria infection (CHMI). Here we present the T cell responses after three immunizations with 3.2x10^3, 1.28x10^4, and 5.12x10^4 PfSPZ at 4-week intervals while taking chloroquine. The memory phenotype and effector function of T cell responses were assessed by 14-16-color multi-parameter flow cytometry after the final immunization and at the time of homologous CHMI. Pf-specificity was determined by incubating PBMCs with aseptic PfSPZ, vaccine diluent (HSA), Pf-infected erythrocytes (PfRBC), or uninfected RBCs. The staining panel included CD4, CD8 and γδ T cells, chemokines, activation markers, and the cytokines IFN-γ, IL-2, TNF-α, IL-4, and IL-10. The magnitude of Pf-specific Th1 cytokine-producing CD8, CD4, and γδ T cell responses following Pf-CVac were dose-dependent. A high frequency of PfRBC-specific memory CD4 T cells (median of 1.6%) was detected after final immunization in the highest dose group of 5.12x10^4 PfSPZ. In an exploratory analysis, immune responses that correlated with protection were assessed using a stratified Wilcoxon test controlling for vaccine dose. The magnitude of PfRBC-specific memory CD4 T cells simultaneously expressing IFN-γ, IL-2, and TNF-α correlated with protection with an uncorrected P-value of 0.00043. These findings demonstrate that Pf-CVac induced high-magnitude cytokine-producing T cells in multiple effector lineages in a dose dependent manner and provide evidence that PfRBC-specific multi-functional memory CD4 T cells may be a correlate of protection. Establishing such responses as a correlate of protection will require validation in larger prospective studies.

1645

EQUATORIAL GUINEA'S FIRST EVER CLINICAL TRIAL: TOLERABILITY, SAFETY AND IMMUNOGENICITY OF PFSPZ VACCINE IN YOUNG EQUATOGUINEAN ADULTS

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PfSPZ Vaccine is a candidate pre-erythrocytic malaria vaccine composed of radiation-attenuated, aseptic, purified, cryopreserved *Plasmodium falciparum* (Pf) sporozoites (SPZ). In clinical trials in the U.S. and Africa, PfSPZ Vaccine administered by direct venous inoculation (DVI) provides durable protection against heterologous strains and heterogeneous populations of Pf. Trials in young children are underway. A robust malaria control program has substantially reduced the malaria burden on Bioko Island, Equatorial Guinea (EG). With an eye toward eventual elimination of malaria on Bioko, a malaria vaccine initiative was established to evaluate the potential utility of PfSPZ Vaccine, resulting in the first ever clinical trial in EG. This first trial was a phase 1, randomized, double blind placebo-controlled trial to assess the tolerability, safety, and immunogenicity of PfSPZ Vaccine administered by DVI in young, healthy 18-35 year olds. A sentinel group of three volunteers received an initial PfSPZ Vaccine dose of 1.35x105 PfSPZ and a second dose of 2.7x105 PfSPZ was given two weeks later. Following review by the safety monitoring committee, 30 volunteers were randomized to receive three doses of either 2.7x105 PfSPZ or normal saline placebo at 0, 8 and 16 weeks. Adverse events (AEs) were solicited for 7 days after each vaccination. There was enhanced surveillance and reporting for unsolicited AEs for 28 days after each vaccination. Monitoring for severe adverse events was done throughout the 40-week study period. Blood samples for safety monitoring of hematological, renal and hepatic functions were taken at baseline, 2, 7, 14 and 28 days after each vaccination and monthly after the last dose for six months. Blood samples for antibody and cellular immunology endpoints were taken at baseline and 1 month after the last vaccination. The results of safety, tolerability and immunogenicity will be presented, including a comparison of the immunological response to the same dose of PfSPZ in EG and Tanzania.

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PLACENTAL MALARIA VACCINES: COMPARING GLYCOSYLATED AND NON-GLYCOSYLATED N-TERMINAL DOMAINS OF *PLASMODIUM FALCIPARUM* VAR2CSA PROTEIN PREPARED AS RECOMBINANT PROTEIN OR DNA VACCINES

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Placental malaria (PM) causes poor pregnancy outcomes including severe maternal anemia and low birth weight, but adverse outcomes decrease over successive pregnancies as women acquire immunity. PM is caused by parasites that express the PfEMP1 variant antigen VAR2CSA and bind the placental receptor chondroitin sulfate A (CSA). With increasing parity, women acquire anti-VAR2CSA antibodies and serum activity that inhibits adhesion of infected erythrocytes to CSA. Naturally acquired protection suggests that a vaccine against PM is feasible. While multiple VAR2CSA domains bind CSA in *in vitro* assays, earlier work showed the N-terminal fragment binds CSA with similar kinetics as the full length protein. We expressed glycosylated and non-glycosylated versions of two Pf FCR3 VAR2CSA N-terminal regions NTS–DBL1X–ID1–DBL2X and ID1–DBL2X–ID2a in insect cells as secreted proteins. We purified the histidine-tagged recombinant proteins from culture media using Ni Sepharose excel medium. In parallel, we generated DNA vaccine constructs using the same boundaries. Immunization studies using these proteins and DNA vaccine constructs have been initiated in rats. We will report the effect of DNA versus protein vaccinations as well as glycosylation status on the CSA binding inhibition activity of the resulting antibodies.

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CHANGING THE PARADIGM OF VACCINE DEVELOPMENT: FROM A WESTERN-LED TO AN INTERNATIONAL, MULTI-PARTNER, PARTIAL AFRICAN-FUNDED CONSORTIUM APPROACH

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Publication of the first PfSPZ Vaccine trial (Epstein et al., Science. 2011 334:475-80) offered clear scientific rationale to continue clinical development of *Plasmodium falciparum* (Pf) sporozoite (SPZ) products. In the absence of major continued funding, the International PfSPZ Consortium (I-PfSPZ-C) was established to design and execute a series of independently funded clinical studies of PfSPZ Vaccine and PfSPZ Challenge (infectious PfSPZ) by partner institutions. I-PfSPZ-C members have finalized the dose and route of administration of PfSPZ Challenge and used PfSPZ Challenge as a unique clinical research tool to understand malaria biology in 18 controlled human malaria infection (CHMI) studies in the US, and Europe and Africa. Members have completed clinical trials of PfSPZ Vaccine and PfSPZ-CVac in the US (N=4), Europe (N=3) and Africa (N=3), are currently or imminently conducting 12 new studies, and are developing new partnerships in Asia. Intravenous immunization with PfSPZ can repeatedly induce high level protection against CHMI and natural malaria exposure. Studies developed under a Clinical Development Plan are reviewed by the I-PfSPZ-C, their cross-site adaptive design being used to inform ongoing and future trials. The I-PfSPZ-C has remained inclusive - individuals, groups, institutions and funding organizations participate in reviewing all published and unpublished data, and planning new trials and strategies. The I-PfSPZ-C meeting at ASTMH Philadelphia 2015 included 104 participants from 38 organizations in 18 countries, all with independent funding. Collaborating I-PfSPZ-C members provide essential planning and governance for PfSPZ Vaccine and PfSPZ-CVac licensure. Uniquely, there has been significant investment by African governments; first by the Tanzanian Commission on Science Technology, and the second and largest ($48.5M) by the Government of Equatorial Guinea (75%) and 3 private energy partners (25%), plus $10M from the Government of Gabon. The I-PfSPZ-C has changed the paradigm of vaccine development from a western driven effort, to one funded and led by all partners, and uniquely those in Africa.

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ADVANCING PARASITOLOGY, ENTOMOLOGY AND VACCINOLOGY BY MANUFACTURING WHOLE PARASITE (EUKARYOTIC CELL) ASEPTIC, PURIFIED VACCINES PRODUCED IN ARTHROPODS: CHALLENGES, SUCCESSES AND TRAJECTORY FOR PFSPZ VACCINES

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There are no 1) vaccines for parasitic diseases, 2) preventative vaccines composed of eukaryotic cells, or 3) vaccines produced in mosquitoes that are licensed by the FDA. Thus, when Sanaria proposed producing *Plasmodium falciparum* (Pf) whole parasite sporozoite (SPZ) (eukaryotic cell) vaccines in aseptic mosquitoes to prevent malaria, most thought this would be impossible. When we began there were no methods to, 1) produce aseptic mosquitoes or aseptic PfSPZ in these mosquitoes, 2) aseptically harvest the parasites from mosquitoes, 3) adequately purify PfSPZ from the mosquito material, 4) stabilize these eukaryotic cells, 5) do this in compliance with regulatory standards (GMPs), or 6) at a scale adequate to support commercial launch in a facility that cost less than $4M. During the last decade we have accomplished all of this, and demonstrated that the PfSPZ are potent: 3 doses of a PfSPZ-based vaccine provides the highest durable protection ever demonstrated against controlled human malaria infection and exposure to malaria in the field. In double blind, placebo-controlled trials there have been no differences in adverse events between vaccinees and controls validating that the PfSPZ are pure. Trials to finalize immunization regimens for phase 3 trials and licensure are underway. We now produce in the same facility and with the same staff, >7 fold more PfSPZ in a single day than we did during the manufacturing campaigns that supported our first 18 clinical trials conducted from 2009 to 2014. Creating this disruptive technology has required, 1) ignoring conventional approaches to manufacturing and storing vaccines, 2) enormous attention to establishing highly efficient manufacturing teams and transparent communication with regulatory authorities, and 3) never forgetting that licensure and deployment of a highly effective vaccine for those most in need in Africa is our raison d'etre. We believe the principles and techniques Sanaria has followed/established leading to this success may be of technical and inspirational value to those working to produce vaccines against other parasites and arthropod diseases, and these will be presented.

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DISTRIBUTION LOGISTICS TO SUPPLY CRYOPRESERVED PFSPZ VACCINE TO TRAVEL CLINICS AND MALARIA MASS IMMUNIZATION PROGRAMS

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Sanaria's *Plasmodium falciparum* (Pf) sporozoite (SPZ)-based PfSPZ Vaccine, PfSPZ Challenge and PfSPZ-CVac, are live whole organism products thermostabilized by cryopreservation, stored and distributed in liquid nitrogen vapor phase (LNVP) using LNVP dry shippers. LNVP storage imparts extreme stability compared to conventional vaccines' fridge or freezer storage, and provides many additional advantages. The LNVP cold chain has supplied 18 PfSPZ clinical trials to date and an additional 11 trials in progress (or commencing during 2016) in the USA, Europe and Africa. Vaccine is packaged in cryovials: until now, closure of these cryovials has been by standard cryovial screw caps necessitating vaccine thawing/reconstitution and syringe preparation in a biological safety cabinet (BSC) at the clinic. A revolutionary new design of cryovial with tamper-evident closure and integral co-molded septum allows for immunizations anywhere without need of a BSC. A new custom dry thawing device obviates the need for a water bath for cryovial thawing. A new high density packaging system delivers 1,300 cryovials per standard dry shipper (or 200 per backpack dry shipper for last mile) to immunization clinics. The highly efficient hub-and-spoke (H&S) distribution system is used for distributing PfSPZ directly from Sanaria to immunization sites without intermediate stops; dry shippers also provide the local on-site storage. For travel medicine clinics in the USA, Europe and Japan H&S distribution will incorporate reverse logistics to rotate dry shippers on a 2-4 week schedule of restocking and resupply, augmented where appropriate, with extended local LNVP or Stirling freezer storage at immunization clinics and be coordinated by a third party logistics company. In malaria endemic regions, scale up first to Phase 3 trials and then to implementation in mass immunization programs for malaria elimination, distribution will utilize the H&S system operating from major regional storage hubs. In its scope and scale, this application of LNVP cold chain logistics will become a new operational standard.

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EVALUATION OF RPFMSP2-BASED VACCINES FOR INCLUSION IN A MULTI-COMPONENT MALARIA VACCINE FORMULATION

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Naturally-acquired antibody responses to Merozoite Surface Protein 2 (MSP2) in individuals living in *Plasmodium falciparum* endemic areas are associated with resistance to malaria. Earlier vaccine trials showed that

immunization-induced *Pf*MSP2 specific antibodies correlated with partial protection against *P. falciparum* malaria. These naturally-acquired and vaccine-induced anti-*Pf*MSP2 antibodies are primarily directed against the central variable region of two major allelic variants of *Pf*MSP2. These data suggest that *Pf*MSP2 has significant potential as a vaccine candidate, likely as a component of a multiantigen formulation. In prior studies with multivalent blood-stage antigen formulations, we showed that genetic fusion of *Pf*MSP1$_{19}$ to the N-terminus of MSP8 facilitated vaccine production, minimized antigenic competition and markedly enhanced induction of functional antibodies. To further improve protective efficacy, we are working to formulate *Pf*MSP2 in combination with the *Pf*MSP1/8 candidate. A synthetic *Pf*MSP2 (3D7) gene, codon-harmonized for expression in *E. coli*, was used to produced r*Pf*MSP2 as an unfused full-length protein or as a chimeric antigen linked to the N-terminus of *Pf*MSP8. Purification of r*Pf*MSP2 and chimeric r*Pf*MSP2/8 yielded 29 mg/L and 54 mg/L of bacterial culture respectively. Vaccine-induced anti-*Pf*MSP2-specific antibodies were quantitated following immunization of CB6F1/J mice with unfused *Pf*MSP2, chimeric *Pf*MSP2/8, or an admixture of r*Pf*MSP2 and r*Pf*MSP8, each formulated with Alhydrogel as adjuvant. High and comparable anti-MSP2 antibody titers were elicited by immunization with the expected predominance of Th2-biased antibody isotypes. Of significance, no evidence of competition between component antigens was noted. Sera from immunized animals strongly recognized native MSP2 in both immunoblot and indirect IFA of *P. falciparum* blood-stage parasites. Comparative assessment of vaccine-induced B and CD4+ T cell responses and antibody-mediated merozoite neutralization will inform selection of a highly immunogenic *Pf*MSP2 antigen to be formulated in combination with *Pf*MSP1/8.

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USE OF A *PLASMODIUM*-SPECIFIC CARRIER PROTEIN TO ENHANCE PRODUCTION OF RECOMBINANT PFS25, A LEADING TRANSMISSION-BLOCKING VACCINE CANDIDATE

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Despite reductions in morbidity and mortality worldwide, eradication of *Plasmodium falciparum* malaria will most likely require a multi-stage, multi-antigen vaccine that incorporates a target of transmission-blocking antibodies. *Pfs*25 is one such transmission-blocking vaccine candidate. Antibodies directed against conformational epitopes within the highly constrained EGF-like domains of *Pfs*25 block sexual stage development in mosquitoes. However, the large-scale production of properly folded recombinant *Pfs*25 has been challenging. We have previously shown that use of *Pf*MSP8 as a fusion partner for *Pf*MSP1, facilitated production of properly folded *Pf*MSP1$_{19}$ and promoted induction of high titers of growth inhibitory antibodies upon immunization. Here, we applied a similar strategy for the production of a *Pfs*25-based vaccine. The gene for *Pfs*25 was codon-harmonized for expression in *E. coli* SHuffle^{Tm} T7 Express *lysY* cells. Recombinant *Pfs*25 was produced as a single, unfused antigen (u*Pfs*25) or chimeric *Pfs*25-*Pf*MSP8 fusion protein (c*Pfs*25/8). uPfs25 was purified under denaturing conditions with subsequent refolding with a yield of ~8.4 mg/L of bacterial culture. The chimeric *Pfs*25/8 was successfully purified under non-denaturing conditions with a yield of 43 mg/L, highlighting value of the *Pf*MSP8 carrier. Proper folding of these antigens was verified by SDS-PAGE under reducing vs non-reducing conditions and by immunoblot analysis with mAb 4B7 which recognizes a conformational epitope of *Pfs*25. Antisera against both antigens were produced in NZW rabbits and assessed for titer and functionality. Both antigens induced strong and comparable titers against *Pfs*25 which potently inhibited parasite transmission to mosquitoes in a standard membrane feeding assay. Although *Pf*MSP8 is also expressed in gametocytes, rabbit anti-*Pf*MSP8 antibodies did not block transmission. Comparative immunogenicity studies are currently underway to further assess the humoral and cellular response to these antigens, to aid in selection of a highly efficacious *Pfs*25 based vaccine to include in a multi-antigen, multi-stage vaccine formulation.

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ANTIBODY PROFILES TO WHEAT GERM CELL-FREE SYSTEM SYNTHESIZED *PLASMODIUM FALCIPARUM* PROTEINS CORRELATE WITH PROTECTION FROM SYMPTOMATIC MALARIA IN UGANDA

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The key targets of protective antibodies against *Plasmodium falciparum* remain largely unknown. The aim of this study was to identify proteins whose antibodies are correlates of malaria acquired immunity that could be relevant for development as malaria vaccine candidates. We profiled immune responses to 1827 wheat germ cell-free system (WGCFS) expressed proteins derived from 1586 genes representing ~30% of the *P. falciparum* entire genome. We previously reported that several WGCFS expressed *P. falciparum* proteins could elicit antibodies in immunized animals that exhibited growth inhibition activity *in vitro*, suggesting that the recombinant proteins, at least in part, retain their natural conformations. Serum samples were obtained from individuals aged 6-20 years who are indigenous residents of a malaria holoendemic community in Northern Uganda. They were enrolled at the start of the rainy season and prospectively monitored for clinical malaria episodes for one year. Protein immunoreactivity to serum samples was determined by AlphaScreen; a homogeneous high-throughput system to detect protein interactions. More than 51% (936/1827) of the proteins reacted with the sera. Subsequently, antibody levels to 9 proteins, encoded by 8 genes significantly associated with time to the first symptomatic malaria episode in children. The 9 proteins comprised both previously characterized vaccine candidates and novel uncharacterized proteins. WGCFS combined with AlphaScreen offer an alternative approach to genome-wide screening of malaria antigens associated with acquired immunity.

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IDENTIFICATION OF PFRIPR, AN RH5-INTERACTING PROTEIN, AS A HIGHLY CONSERVED BLOOD-STAGE MALARIA VACCINE CANDIDATE AGAINST *PLASMODIUM FALCIPARUM*

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Blood-stage malaria vaccine (BSV) candidates of high efficacy against *Plasmodium falciparum* (Pf) remain elusive, mainly because of genetic diversity and allele-specific immunity in endemic regions. Here, we hypothesize that in order to improve efficacy, BSV candidate antigens should contain conserved targets of immunity. Hence, identification of suitable candidates for downstream clinical studies in the quest for next generation vaccines requires understanding of the extent of genetic polymorphisms in the antigen targets and the roles played in naturally acquired immunity. Using field isolates from Uganda, we carried out genetic analyses on genes encoding four recently reported novel BSV candidate proteins; RH5 interacting protein (PfRipr), GPI anchored

micronemal antigen (PfGAMA), rhoptry-associated leucine zipper-like protein 1 (PfRALP1) and Duffy binding-like merozoite surface protein 1 (PfMSPDBL1). In addition, we expressed recombinant proteins of these candidates based on Pf laboratory clone 3D7 sequences using wheat germ cell-free system, immunized rabbits to obtain specific antibodies (Abs) and performed functional studies (Growth inhibition Assay, GIA). The GIA activity of the raised Abs in blocking erythrocyte invasion was determined using both the homologous 3D7 and heterologous FVO strains *in vitro*. *Pfgama* and *pfmspdbl1* are relatively polymorphic and Abs against the 3D7 recombinant PfGAMA and PfMSPDBL1 inhibited merozoite invasion of 3D7 but not FVO. Although both *pfripr* and *pfralp1* are conserved and Abs against their 3D7 recombinant proteins potently inhibited merozoite invasion of both 3D7 and FVO, the GIA activity of anti-PfRipr was much higher than that of anti-PfRALP1 on both 3D7 and FVO. Furthermore, *pfralp1* is comparatively diverse, with varied number of regions having insertions and deletions, asparagine and 6-mer peptide repeats in the sequences that are lacking in *pfripr*. Therefore, PfRipr is a highly conserved promising BSV candidate in the design of next-generation vaccines against *P. falciparum*.

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MULTIPLE INSECTICIDE RESISTANCE IN *AN* HIGHLY INFECTED POPULATION OF THE MALARIA VECTOR *ANOPHELES FUNESTUS* IN BENIN

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Knowledge on the spread and distribution of insecticide resistance in major malaria vectors such as *Anopheles funestus* is key to implement successful resistance management strategies across Africa. Here, by assessing the susceptibility status of an inland population of *An. funestus* (Kpome) and investigating molecular basis of resistance, we show that multiple resistance in this species now extends beyond the original coastal region of Benin and is associated with high infection rate. The TaqMan analysis of plasmodial infections revealed an unusually high infection rate (18.2%) of *An. funestus* in this locality. The WHO bioassays revealed a multiple phenotypic resistance profile for *An. funestus* in Kpome. This population is highly resistant to pyrethroids (permethrin and deltamethrin), organochlorines (DDT), and carbamates (bendiocarb). A reduced susceptibility was observed with dieldrin. Mortalities did not vary after pre-exposure to PBO for DDT indicating that cytochrome P450s play little role in DDT resistance in Kpome. In contrast, we noticed, a significant increase in mortalities when PBO was combined to permethrin suggesting the implication direct involvement of P450s in pyrethroid resistance. A high frequency of the L119F-GSTe2 DDT resistance marker was observed in this highly DDT resistant population (9%RS and 91%RR) whereas the A296S mutation was detected at a low frequency (1%RS and 99%SS). In conclusion, the extension to the inland locality of the multiple resistance in *An. funestus* populations suggests resistance could be widespread in Benin and this highlights the need for further studies to assess the geographical distribution of insecticide resistance across Benin and neighboring countries as well as a more comprehensive analysis of the resistance mechanisms involved. Keywords: Malaria, Benin, *An. funestus*, insecticide resistance, resistance mechanisms, malaria control.

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COVERAGE OF SEASONAL MALARIA CHEMOPREVENTION DELIVERED BY MOBILE TEAMS AT FIXED POINTS IN 14 DISTRICTS IN MALI, THROUGH ACCESS-SMC

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Mali was one of the first countries to introduce Seasonal Malaria Chemoprevention (SMC), starting in one district in 2012, and reaching 48 of the 63 districts in the country in 2015. We evaluated the effectiveness of SMC delivery in 14 districts where implementation was supported by the ACCESS-SMC project funded by UNITAID. SMC was administered four times at monthly intervals starting late August. Delivery was at fixed points by mobile teams who checked children for fever, administered SMC to those who were well, and tested those with fever with a malaria Rapid Diagnostic Test (RDT). RDT positive children were treated with artemether-lumefantrine, negative children received SMC drugs and if appropriate, an antibiotic. Most children could therefore be treated by the team rather than having to be referred to a health facility. Each child was issued with a three-year SMC record card on which the date of attendance, and whether the child was treated with SMC, or tested and treated for malaria, or excluded, was recorded. A survey was undertaken in December to assess coverage. 50 clusters (villages or quarters of urban areas) were selected with probability proportional to size in 5 districts. In each cluster about 20 children aged between 4 months and 7 years were surveyed. 1037 children were surveyed of whom 740 were eligible for all 4 SMC cycles because they were aged between 3 months and 5 years at the time of the first cycle. 85% of these children had received an SMC card. Based on the card, and on caregiver's recall if the card was not seen, 38% of eligible children who had received 4 SMC cycles. The main reasons for not receiving SMC were being away when the team came (50%); being unaware of when SMC teams would come (35%); and the caregiver being too busy (17%). Reported adherence to the two amodiaquine doses administered by the caregiver was 99% for each dose. A high proportion of children were reached at least once but, in this first year of national scale-up, less than half received the full number of cycles. Emphasis on sensitization of the community, and adoption of door-to-door distribution, may be needed to maximize the number of children protected.

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DESIGN, MONITORING, AND IMPLEMENTATION OF THE THIRD AND FOURTH ROUNDS OF SCHOOL NET DISTRIBUTION TO MAINTAIN UNIVERSAL ACCESS TO LONG-LASTING INSECTICIDAL NETS IN SOUTHERN TANZANIA

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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. This strategy was piloted in the Southern Zone beginning 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. By August 2016 Tanzania will have implemented four annual rounds of school-based distribution in three Southern regions. 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. The 4th round in August 2016 will continue in the three regions in the south and expand to four regions in the Lake Zone; 1,310,000 ITNs will be delivered to 5,054 schools in a total of seven regions. Working with a multi-sectoral task force including Ministry of Health, Ministry of Education, and local officials, enrolment data was gathered from each school, verified, and used to quantify deliveries for each school. After training and delivery of ITNs to schools, teachers distributed ITNs to the eligible students in the targeted classes, and provided behavior change messages on net use, care, and malaria prevention. We will discuss the design, implementation and outcomes of SNP3 and SNP4, including the process of quantifying the ITN needs, training and sensitization activities, data management, and logistics considerations for an ongoing, mass yearly distribution of nets to schoolchildren. We will also discuss changes from SNP1 to SNP4 in the operations management, in particular, decisions to adjust the number of classes targeted each year based on evaluation data, and implications for future national scale-up.

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PLANT-MEDIATED EFFECTS ON MOSQUITO CAPACITY TO TRANSMIT HUMAN MALARIA

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The ecological context in which mosquitoes and malaria parasites interacts has received little attention, compared to the genetic and molecular aspects of malaria transmission. Plant nectar and fruits are important for the nutritional ecology of malaria vectors, but how the natural diversity of plant-derived sugar sources affects mosquito competence for malaria parasites is unclear. To test this, we infected *Anopheles* coluzzi, an important African malaria vector, with sympatric field isolates of *Plasmodium falciparum*, using direct membrane feeding assays. Through a series of experiments, we then examined the effects of nectar from Thevetia neriifolia and Lannea microcarpa, and fruit from Barleria lupilina and Mangifera indica on parasite and mosquito traits that are key for determining the intensity of malaria transmission. We found that the source of plant sugar differentially affected infection prevalence and intensity, the development duration of the parasites, as well as the survival and fecundity of the vector. These effects are likely the result of complex interactions between toxic secondary metabolites and nutritional quality of the plant sugar source, as well as of host resource availability and parasite growth. Using an epidemiological model, we show that plant sugar source can be a significant driver of malaria transmission dynamics, with some plant species exhibiting either transmission-reducing or -enhancing activities.

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PROCESS EVALUATION OF CONTINUOUS ITN DISTRIBUTION IN ZANZIBAR

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In 2013, Zanzibar designed a continuous ITN distribution strategy to maintain high levels of ITN ownership and use. ITNs are given to pregnant women and caretakers of young children through free distribution at 1st ANC visit and 9 month measles vaccination, respectively. At the community level, households request a coupon from the sheha to redeem for a LLIN and then exchange the coupon at a health facility for a new ITN. Coupons are then returned to ZaMEP. In addition, coupons can be issued by district malaria surveillance officers during case investigations if LLIN need is identified. From June 2014 to January 2016, the Zanzibar Malaria Elimination Program (ZaMEP) reported that 289,661 ITNs were distributed through continuous distribution: 65,325 to pregnant women at ANC, 60,507 to caretakers at EPI clinics, and 163,829 through the community channel. A total of 40 semi-structured interviews took place in early April in a convenience sample of 8 shehias and 16 distribution points on Unguja and Pemba Islands, Central, West B, Chake Chake, and Mkoani districts. Interviews took place at distribution points for coupons and for LLINs, with central level stakeholders, and with health facility staff and shehas at community level to identify implementation bottlenecks and best practices. Record reviews of LLIN distribution, stock data, and costs were done at central, district, shehia, and health facility level. Ten observations of coupon/ITN redemption were made. Preliminary findings indicate that overall, the CD channels are functioning, with challenges in stockouts of both ITNs and coupon-books at certain health facilities and shehias, and in adequate storage facilities for ITNs at health facilities. Stakeholders at all levels felt the program has made a positive contribution to malaria control, but there is a need for clarity in reporting systems and increased supportive supervision and refresher training, particularly at the shehia level. Additional findings, including estimates of ITN ownership and access resulting from the continuous distribution, and cost per-ITN-distributed, will be presented after the data is fully processed and analyzed.

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ASSESSMENT OF MALARIA TRANSMISSION FROM HUMAN TO MOSQUITOES IN SEASONAL MALARIA CHEMOPREVENTION IN THE WESTERN REGION OF BURKINA FASO

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Seasonal malaria chemoprevention (SMC) can reduce malaria cases up to 80% in sahelian region. However the impact of SMC on human to mosquito malaria transmission is currently unkown. Here, we evaluated the infectiousness to mosquitoes of volunteers receiving SMC by membrane feeding assays. Children over the age of 2 years, participants of a SMC clinical trial were randomly selected. They were invited to participate after

a clinical examination and irrespective of their parasite carriage status. Blood sample were collected in 5 sites (4 under SMC treatment and 1 control) over a period of 4 months from August to November 2015. In total 301 children were involved, 204 children in SMC group and 77 in control group. For each blood sample, 80 female *Anopheles* mosquitoes were provided a blood meal through a parafilm membrane. On day 7 after feeding, mosquitoes were dissected and midguts were screened for the presence of oocysts. Generalized linear mixed models were used to compare mosquito infection in treatment and control group and to estimate intervention efficacy. Results showed that gametocytemia was lower in the SMC groups with respect to the control: month 1 (X22=6.14, p=0.046), month 2, 3, and 4 (X21=57.3, p<0.0001). There was a strong impact of SMC on both oocyst prevalence with a 93 % reduction in mosquitoes that received blood from SMC patients (X22=182, p<0.0001), and oocyst density with a 86% reduction (X22=70.6, p<0.0001). In conclusion, in an area of seasonal malaria transmission, chemoprevention highly reduces human to mosquitoes malaria transmission.

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COMMUNITY ENGAGEMENT AROUND THE IMPLEMENTATION OF TRIAL OF INSECTICIDE-TREATED WALL LINING FOR MALARIA CONTROL IN RURAL TANZANIA

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Community engagement (CE) during community trials is a complex social phenomenon that defies simple explanation or mechanization. We present findings from an assessment of the sensitization process, experiences, and challenges in improving understanding and subsequent acceptance of an insecticide-treated wall lining (ITWL) program. The initial project sensitization plan relied on the traditional approach of inviting villagers to meetings with researchers. However, meeting schedules coincided with farming activities and Tanzania's presidential elections, resulting on poor attendance. Sensitization was re-strategized to add door-to-door sensitization using local advocates, announcements using a megaphone, and designing and distributing brochures detailing the study objectives and consenting process. The process continued during the ITWL installation phase. Following re-strategizing of sensitization, the ITWL acceptance rose to 86.4%. However, some clusters still had some refusals. Reasons included gender and consent, for example, in some houses the head of house (generally a man) refused installation after the wife had accepted. Old rumors resurfaced that ITWL contributed to male impotence. Some installers, initially unprotected, developed skin rashes. In one case, one resident's skin rashes spread fear to a whole hamlet. Households with better socio-economic status cited personal ability to control malaria and feared damage to their walls by the installation process. Directives that children should not touch the wall liners and confusion from installation delays all fed into refusal rates. Rumors of side effects from the ITWL contributed much on project challenges including refusals. Re-strategizing sensitization plus continuous sensitization throughout and after the official installation period increased ITWL acceptance. Future projects should incorporate continuous sensitization and consider using specialized village research committees for improved CE.

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SMALL SOLAR POWERED 'BɔKɔ' FANS IMPROVE COMFORT INSIDE MOSQUITO NETS IN SOUTHERN GHANA

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In rural Greater Accra, in 2014, 49% of people didn't use mosquito nets despite having access to a space under one. Discomfort due to heat is the most stated reason, but this problem is largely unaddressed. With advancing electrification and dropping price of solar power, 'Bɔkɔ' 0.8 W net fans equipped with a 0.1 W LED could improve comfort inside nets and be affordable to populations in malaria endemic areas. Ninety-two households (HHs) from rural communities in Greater Accra, divided into three groups, participated in a 10-month randomized cross-over trial, where fan systems (one fan per HH member in Group 1) were crossed over with water filters between Groups 1 and 2, while Group 3 served as control. Intervention HHs participated in fortnightly surveys on HH's practices related to mosquito nets, fans and water filters, while control HHs were questioned only at start, mid-point and study end. Further, key-informant interviews were held before mid-point (cross-over), and willingness to pay for fans was assessed in individual auctions at study end. Baseline net use conditional on access in the study communities was unexpectedly high at 92, 93, and 87% for Groups 1, 2 and 3, respectively, and increased to 99 and 99% at cross-over and 97 and 90% at end-point in intervention Groups 1 and 2, respectively, while it reduced to 81 and 84% in the control Group 3 at cross-over and end-point, respectively, indicating a Hawthorne / study effect. Stated fan use was 88-100% depending on the fortnight of survey. The main reason for using fans was heat, but it was also mentioned that they drove mosquitoes away. Key informants suggested they slept less exposed outside due to the fan during part of the night during the dry season. Despite the low power rating, nine out of 13 key informants stated that they placed the fan outside the bed net explaining that the air produced by the fan was enough to reach them through the net. The average bid price per fan was GH₵ 55 (~US$ 13.5), and in total 98 Bɔkɔ fans were sold to participating HHs. Small electric fans were accepted and desired in the study community and may be an affordable innovation to improve comfort inside mosquito nets in hot climates.

1662

DIHYDROARTEMISININ-PIPERAQUINE AS INTERMITTENT PREVENTIVE TREATMENT FOR MALARIA IN *A* REFUGEE CAMP, ADJUMANI, UGANDA

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An intermittent preventive treatment (IPT) program using dihydroartemisinin-piperaquine (DP) was implemented between March and July 2015 in a refugee camp in Adjumani District, Uganda. To our knowledge, this is the first implementation of IPT in the setting of a humanitarian emergency. Weight-dosed DP was offered to all children aged 6 months-14 years in the camp in March, May, and July 2015, at eight-week intervals. On average, 13 537 children received each distribution. To evaluate malaria incidence, reported cases were compared to the same 6-month period from 2014 taking into account population changes. To evaluate malaria prevalence, in the week prior to each

distribution and 8 weeks following the final distribution, malaria surveys were conducted in the camp. Thick and thin smears were collected from a target sample size of 250 persons in each of three age groups: <5 years; 5-14 years; ≥15 years. Direct microscopy was performed. In 2014, among children <5, malaria incidence was 0.71 cases/person over the 6 month-period running from March-August; in 2015 it was 0.52 (IRR 0.73, 95%CI 0.69-0.77) over the same period during IPT implementation. In children aged 5-14 years, the incidence was 0.96 cases/person in 2014 and 0.67 in 2015 (IRR 0.70, 95%CI 0.67-0.72). For those >15 years, the incidence was 0.37 cases/person in 2014 and 0.55 in 2015 (IRR 1.49, 95%CI 1.42-1.56). Among children <5, the prevalence of parasitemia by microscopy was 5.1% (95%CI 3.0-8.5) at baseline and 15.1% (95%CI 12.1-18.7) two months following the final distribution of DP. Among children aged 5-14 years, these figures were 8.7% (95%CI5.8-12.9) and 26.7% (95%CI 20.9-33.6), respectively. Among those over 15, the prevalences were 6.1% (95%CI 3.9-9.7) and 18.7% (13.7-25.0), respectively. In the setting of a humanitarian emergency, IPT reduced the incidence of malaria among its target population. Its impact may appear mitigated because of strong transmission seen at the end of the program in 2015, after the protective effect of DP had ended, as evidenced by the high incidence and prevalence seen in the untreated population.

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LLINS ASSESSMENT OF HOUSEHOLD COVERAGE IN DEMOCRATIC REPUBLIC OF CONGO BETWEEN 2004 AND 2014

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In DRC, malaria remains a major public health problem as the first cause of morbidity and mortality. To remedy this, the control strategies and approaches have been developed including support cases and vector control. The insecticide-treated net (LLIN) is a component of the vector control implementation in the country for over a decade. To obtain data that could reorganize malaria control, a critical analysis of the coverage level spaced ten years was performed. A community survey targeting the effective coverage of LLINs in households through the distribution and use of this material treated by communities (9 sentinel sites of NMCP). Cluster sampling was used for data collection. The statistical analysis took into account the 5% significance level. Between 2004 and 2014, the ownership of LLINs increased between 51.3% and 92%. As to the use, it was between 51% and 85% depending on the sites. The proportion of pregnant women sleeping under LLINs was between 61.7% and 89%. The protection of children under 5 years by the LLIN was between 57% and 91.6%. The sleeping places hedging average was 9.9% (3.4 to 16.4%, I.C 95%) in 2004, while coverage was 61.9% in 2014 (34.9 to 85%, I.C 95%). In conclusion, the universal coverage threshold was not achieved globally in the country. Efforts should be made to allow universal coverage nationally.

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ANOPHELES SUBPICTUS, A NEW DOMINANT MALARIA VECTOR IN AN URBAN AREA OF WESTERN INDIA

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Plasmodium and *Anopheles* in South Asia are under constant selection pressure due to ecological changes and parasite/vector control strategies of the Government of India. Most of the vector-parasite compatibility studies done in South Asia have centered around 6 major malaria vectors (*Anopheles culicifacies*, *An. fluviatilis, An. stephensi, An. minimus, An. dirus* and *An. sundaicus*), and these vectors are estimated to contribute to 95 - 98% of malaria cases. Much less is known about the role of other Anophelines in *Plasmodium* transmission in South Asia. There is a consistent increase in *P. falciparum* (Pf) cases in the last 40 years. As of December, 2015, Pf contributes to 67.5% of malaria cases in the Indian subcontinent. We hypothesize that, under intense drug and insecticide pressures, new parasite-vector associations could emerge based on the right physiological and phenotypic matches. In our US NIH International Centers of Excellence for Malaria Research (ICEMR) study site in Goa, Western India, a 2-year longitudinal study identified the mosquitoes that are naturally transmitting malaria in this region. *An. subpictus*, a previously overlooked minor vector, has emerged as a dominant malaria vector overtaking the primary vector, *An. stephensi*, and is transmitting malaria throughout the year. While in nature there are two sibling species of *An. subpictus* (A and B) in our study site, salivary gland infections were seen only in the sibling species, B. To facilitate larger surveys involving sibling species A and B of *An. subpictus*, a multiplex PCR assay has been developed. We have also colonized *An. stephensi* and have successfully performed laboratory infection experiments with patient blood containing *P. vivax* and *P. falciparum* infected patient blood. We describe efforts to colonize *An. subpictus* and compare the vector competence of *An. subpictus* and *An. stephensi* through controlled feeding experiments.

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COST-EFFECTIVENESS OF INSECTICIDE-TREATED WALL LINER AND INDOOR RESIDUAL SPRAYING TO PREVENT MALARIA IN KENYA AND TANZANIA

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Despite widespread distribution of long lasting insecticide bed nets (LLINs), malaria continues to be a major cause of morbidity and mortality globally. Indoor residual spraying (IRS) has proved efficacious, but is expensive, logistically complex due to needed reapplication every 6-12 months, and challenging due to insecticide resistance. A new technology, the non-pyrethroid insecticide treated wall liner (ITWL), may provide 3-4 years of protection from a single installation. Its two insecticides with different modes of action should curb insecticide resistance. We computed the incremental cost-effectiveness ratios (ICERs) of ITWL and IRS as supplements to LLINs in Kenya and Tanzania. One cluster randomized trial of the previous pyrethroid ITWL as a supplement to LLINs was conducted in Kenya. A similar trial (with the non-pyrethroid ITWL) is underway in Tanzania. We obtained acceptance rates, financial costs to the health

system, and effectiveness of IRS and ITWL through original data collection and available literature. Costs covered outreach, projected procurement and installation. We incorporated savings in medical costs from fewer malarial episodes. Estimated ITWL acceptance rates averaged 98% in Kenya (where extensive prior meetings with village leaders occurred) and 68% in Tanzania (where outreach began later). Average inpatient and ambulatory malaria episodes cost $59.80 and $8.06 in Kenya and $212.12 and $23.17 in Tanzania, respectively. In Kenya, one-time ITWL costs were $64.23 per person in 2010 while IRS cost $3.16 per person annually. In Tanzania, annual IRS costs were $15.59 per household in the target areas. Good communications proved critical to acceptance of ITWL. In Kenya, the ICERs were $482 per discounted life year gained (assuming protection lasts 3 years) for ITWL and $139 for IRS. As these ICERs were below Kenya's GDP per capita ($795), both technologies are highly cost-effective based on World Health Organization criteria. Although the ICER of ITWL was initially less favorable than that for IRS in Kenya, falling prices of LLINs suggest ITWL should also likely become less costly and more cost-effective.

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LAND COVER DETERMINANTS OF *PLASMODIUM FALCIPARUM* PREVALENCE IN URBAN AND PERI-URBAN AREAS OF NORTHERN BIOKO ISLAND

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Beginning in 2015, the Bioko Island Malaria Control Project (BIMCP) adopted a strategic and targeted approach of indoor residual spraying (IRS) for malaria prevention. Evidence suggests that hotspots of malaria transmission to be targeted are best evaluated by finding increased exposure to infectious mosquito bites. Despite routine vector monitoring throughout the island, mosquito collection in urban and peri-urban areas of northern Bioko has been logistically challenging. Remote sensing and geographic information systems (GIS) are frequently used to explore associations between land use/land cover (LULC) and mosquito-borne diseases by functioning as a proxy for mosquito abundance, while spatial scan statistics are used to detect spatial clustering of malaria prevalence. Data on prevalence of *P. falciparum* parasitaemia were collected from a representative sample of 5,286 households throughout the island during the 2015 Malaria Indicator Survey. Seven LULC types were classified through supervised classification of remotely sensed high resolution satellite imagery. Areas at higher risk of transmission were evaluated using a Bernoulli purely spatial scan statistic. Spatial associations of LULC and *P. falciparum* parasitaemia were performed using geographically weighted logistic regression analyses to determine case environmental risk factors. Complete data were available for 19,666 individuals. Although several statistically significant clusters were detected, two clusters of excess risk appeared to have been driven by one to two households with several cases. The most likely spatial clustering of malaria prevalence was detected in peri-urban areas, particularly in the northwestern region of the island, which appears to be temporally heterogenous. Results of geographic weighted regression will be analyzed further.*Plasmodium falciparum* malaria prevalence is heterogeneous in space in this urban and peri-urban study area. Geographical and housing risk factors associated with prevalence will be explored further. This analysis improves the planning of IRS interventions targeting high-risk areas.

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ENTERIC PATHOGEN SURVEILLANCE IN CHILDREN AND ADULTS IN *A* CASE-CONTROL STUDY OF ACUTE DIARRHEA IN BATTAMBANG, CAMBODIA

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Diarrhea has continued to be a major public health problem in developing countries and surveillance for for a wide range of enteric pathogens is necessary to understand frequency of pathogens. Stool samples, demographic and clinical data were collected from cases and asymptomatic controls in children (< 5 years old) and adults seen at Battambang Referral Hospital, SvayPor Health Center and Military Hospital 5 located in Battambang, Cambodia from July 2014 - April 2016. Standard microbiology for stool culture, ELISA and PCR were applied to detect enteric bacteria, virus and parasites. Antimicrobial susceptibility testing (AST) was tested by using disk diffusion method. In children < 5 years old, 226 cases and 226 controls were enrolled in the study in which rotavirus (20%), norovirus (16%) and *Shigella* (9%) were detected significantly more in the cases than controls. Enteroaggregative *E.coli* (24%), *Salmonella* (17%), *Campylobacter* (11%) were found in relatively similar proportions of cases and controls. Of 57 adult cases and 54 controls, bacterial pathogens including *Vibrio* (7%) and *Campylobacter* (4%) were identified significantly more in cases than controls. Enteric viruses were infrequently detected among the adult population. AST demonstrated multidrug resistant *Shigella* as well as co-resistance to extended spectrum cephalosporins and fluoroquinolones. Additionally, fluoroquinolone resistant *Campylobacter* was found in 80% of the isolates. Continued surveillance will provide data on etiologic agents and antimicrobial resistance patterns that are critical for treatment guidelines, prevention and control of diarrheal disease in Battambang, Cambodia.

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VIRULENCE PROFILE OF ENTEROTOXIGENIC *ESCHERICHIA COLI* (ETEC) STRAINS ISOLATED FROM PERUVIAN CHILDREN

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Enterotoxigenic *Escherichia coli* (ETEC) is a major cause of diarrhea in children and travelers. The aim of this study was to determine the presence of virulence factors (VF) of ETEC strains isolated from Peruvian children. We analyzed a total of 205 ETEC strains previously isolated from two cohort studies in children <24 months of age in Lima, Peru. ETEC was identified by a multiplex real-time PCR for lt and/or st genes. The presence of sixteen colonization factor (CF) types, ST toxin subtypes (STh and STp), adhesins (Tia, TibA, EtpA), a GTPase (LeoA), an autotransporter (EatA), an enteroaggregative *E. coli* heat-stable enterotoxin (EAST1) and an *E. coli* common pilus (ECP) genes were evaluated by PCR. LT-positive (ETEC-lt) strains (99/205, 48%) were the most frequent, followed by strains with only ST (ETEC-st) (63/205, 31%) and strains positive for both LT and ST (ETEC-lt-st) (43/205, 21%). Among ST-positive strains (with or without LT) STh (estA+) was the ST-toxin type most frequently identified (64/106, 60%). The most common CFs were CS21 (34%), CS6 (20%), CS3 (15%), and CS1 (12%). Presence of at least one CF were more frequently detected in isolates from diarrheal than control samples (81% vs. 52%, p<0.001). Whereas CS6 (26% vs. 13%, p<0.001), CS5 (13% vs. 2%, p<0.01) and CS1 (16% vs. 6%, p<0.05) were more frequently detected from diarrheal than control samples, respectively. STh+ CS21

genotype (with or without other CFs) was the most prevalent among all strains (19%, 38/205). On the other hand, the most common nonclassical VF (other than CFs and LT/ST toxins) were ECP (89%), EAST1 (44%), EatA (42%) and EtpA (32%). EatA was significantly detected in ETEC isolates from diarrheal than control samples (53 vs. 27%, p<0.01). Strains positive to at least one nonclassical VF were more frequently detected in isolates from diarrheal than control samples (100% vs. 95%, p<0.05). Using a prototype vaccine (with LT toxoid, CFA/I, and CS1 to CS6) as a model, the estimated vaccine coverage rate in children in Lima will be 92% (189/205). Further studies are needed to determine the utility of these antigens as well as other autotransporters in ETEC vaccines.

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VACCINATION FOR THE CONTROL OF TYPHOID FEVER: ESTIMATING THE POPULATION-LEVEL EFFECTS OF HISTORICAL TY21A FIELD TRIALS IN SANTIAGO, CHILE

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In the absence of water and sanitation interventions, vaccination remains a primary control measure for typhoid fever. Evaluating the herd protection of these vaccines is challenging, and is often compounded by changes in the environment. From 1982 to 1986, over 300,000 school children were vaccinated with at least one dose of Ty21a in the metropolitan region of Santiago, Chile. The incidence of typhoid fever declined in this area from over 100 per 100,000 at the beginning of this trial, to less than 50 per 100,000 at the end of 1990. Without a control population cluster, herd effects of the vaccine could not be directly estimated, or contrasted with the water and sanitation changes that occurred during this period. We use a mathematical modeling approach to estimate the vaccine's contribution to the decline in typhoid fever, both through the direct protection of the vaccine, as well as indirect protection through herd effects. Results from this study highlight methods for estimating the impact of typhoid vaccination in populations undergoing environmental change, as well as outline features for prospective typhoid vaccination trials important for the evaluation of both direct and indirect effects. These results can help inform strategies for global typhoid fever control, including the planning for new conjugate vaccine initiatives.

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ESCHERICHIA COLI PATHOTYPES FROM ECUADOR: ASSOCIATION WITH DIARRHEA AND ANTIBIOTIC RESISTANCE

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Diarrheagenic *Escherichia coli* (DEC) is an important cause of diarrhea in the developing world and the detection of these bacteria and their antibiotic resistance profiles are necessary for effective therapy. In this study, we conducted a microbiological survey of DEC in 233 stool samples, collected during a case control study in a hospital and health center in a low income neighborhood of Quito, Ecuador from April to September 2014. We used 8 sets of PCR primers to detect distinct DEC pathotypes. The overall prevalence of DEC was 30.5% in cases and 20.2% in controls (OR 1.76 CI 95% 0.96-3.20, p=0.06), Diffusely adherent E.coli (DAEC) was the most frequently detected pathotype in cases and controls (15.3% vs. 6.1% respectively) and was the only pathotype with a statistically significant association with diarrhea (OR 2.78, CI 95% 1.11-6.96, p=0.03). To our knowledge this is the first study investigating this pathotype in Ecuador. Additionally, pathotypes isolated from cases exhibited significantly higher levels of antimicrobial resistance to specific antibiotics, as well as higher levels of multidrug resistance, than isolates obtained controls.

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QUALITATIVE MOLECULAR DIAGNOSTICS MAY IMPROVE MEDICAL MANAGEMENT OF HOSPITALIZED SEVERELY MALNOURISHED CHILDREN WITH DIARRHEA: PRELIMINARY ANALYSIS FROM HÔPITAL DE L'AMITIÉ IN N'DJAMENA, CHAD

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Accurate diagnosis of infectious disease and rational use of antibiotics is challenging in resource-limited contexts where microbiological laboratory services are virtually absent. Diarrhea, for example, is typically present in at least 50% of children with severe acute malnutrition (SAM) admitted to hospital and is associated with increased risk of death. Current diarrheal treatment protocols for hospitalized SAM children rely on a syndromic approach, prioritizing fluid replacement and correction of malabsorption over specific treatment for pathogens. Antibiotic use is reserved for protracted or bloody diarrhea and is guided by a UNICEF protocol template applied without accounting for local or regional epidemiology. Studies suggest that multiplex molecular diagnostics can provide reliable clinically relevant information in field laboratories where infectious diseases like diarrhea represent a major portion of disease burden. Automated qualitative polymerase chain reaction (PCR) systems do not require a high level of expertise to operate and may provide actionable microbiologic information even though it gives no information regarding pathogen burden. ALIMA, with the support of the Institut Mérieux, is using qualitative PCR to test stools from hospitalized SAM children with diarrhoea in N'Djaména, Chad. Each sample is tested simultaneously for 22 distinct bacterial, protozoal and viral molecular targets. The trial expects 600 inclusions total over 12 months. Within the first 4 months, stool from 146 children have been analyzed. A total of 537 pathogens have been detected, an average of 3.8 per child. 70% are bacteria, 18% viruses and 12% parasites. Within the bacterial group 52% are E coli pathogens with entero-invasive E coli (EIEC)/Shigella detected in 57 of 146 samples (39%). Clinicians have adapted antibiotic prescription for 54/146 children (37%); 42/54 prescription changes result from the identification of EIEC/Shigella. Preliminary analysis of this trial demonstrates that the technology is accessible and reliable in a resource-limited context and that clinicians use the information to modify treatment.

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DRIED BLOOD SPOTS: AN ALTERNATE TOOL FOR THE ASSESSMENT OF IMMUNE RESPONSE TO CHOLERA INFECTION AND VACCINE

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Analyzing Dried Blood Spots (DBS) is an attractive tool for measuring antibody responses as it overcomes the challenges associated with supplies and expertise needed for venipuncture and sample processing, especially in resource-limited and challenging settings such as during cholera vaccine programs. The goal of this study was to evaluate DBS as a tool for the measurement of *Vibrio cholerae* O-specific polysaccharide (OSP)-antibody responses and to determine vibriocidal titers in volunteers immunized with oral cholera vaccine (OCV) Shanchol. Specifically, sera and blood spots were obtained from persons receiving OCV on day 0 (pre-vaccination), day 21 (21 days post 1st vaccine dose) and day 35 (14 days post 2nd vaccine

dose) in South Sudan. The results of assays involving DBS were correlated with traditional approaches utilizing sera. Blood spots were generated on Whatman 903 DBS cards, and stored at ambient temperature for up to 100 days. 4 dried blood soaked spots were punched out per volunteer specimen, and eluted overnight in a 24 well plate containing buffer. The eluates were subsequently used for determination of OSP-specific responses by ELISA. In a preliminary analysis involving 15 individuals, we noted statistically significant positive correlations between DBS and simultaneously sampled serum OSP-specific IgG (r=0.64, p<0.001), IgM (r=0.68, p<0.001) and IgA (r=0.64, p<0.001) antibody responses. Fold rises after the 1st OCV dose correlated between DBS and serum ELISA's for OSP IgG (r=0.61, p=0.01) and OSP IgA (r=0.76, p<0.001). Seroconversion rates assessed by the two methods were also similar. In addition, preliminary experiments suggest DBS can be used for the determination of vibriocidal titers using drop plate culture methods as determined by 50% growth reduction in samples compared to DBS free controls. Further assay optimization and validation is pending and more complete results will be available at time of presentation. Our data suggest a potential use of DBS as an inexpensive and convenient tool for the assessment of OCV immunogenicity and seroprevalence surveys, including in resource-limited settings.

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THE STUNTING SYNDROME DEVELOPS IN CHILDREN WITH INCREASED MICROBIAL TRANSLOCATION AND ATTENUATED EVOLUTION OF THE GUT MICROBIOME

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Stunting due to malnutrition is estimated to affect 165 million children under 5 years old. However, its pathophysiology remains elusive. We hypothesized that in low resource settings stunting is mediated by systemic inflammation due to increased microbial translocation ensuing from chronic intestinal damage and microbiome perturbation. We enrolled 81 healthy infants living in rural villages of Peru and followed them for 6 months with monthly growth assessments. Blood samples for markers of intestinal damage (intestinal fatty acid binding protein [I-FABP]) and systemic inflammation and stool samples for microbiome analysis were collected at months 0 (enrollment) and 6. Biomarkers were measured by Luminex or ELISA. Microbiome analysis was performed via 16SrDNA sequencing. Non parametric statistics were used to compare distribution of continuous variables and to measure correlations. Multivariate odds ratios of stunting were estimated by logistic regression. By 6 months, 18 (22%) children became stunted. I-FABP was high in cases and controls at month 0 and 6 but was not significantly different between the 2 groups at any time point. Tumor necrosis factor α, interleukin-1 and -6 levels were comparable between the 2 groups at month 0 and 6. Greater increases in soluble CD14 (monocyte activation) and lipopolysaccharide binding protein were associated with increased odds of stunting after adjusting for month 0 age and HAZ (ORs 7.24 ([95% CI 1.05- 49.84] P= 0.04) and ORs 6.32 ([95% CI 1.40-28.36] P=0.02 respectively). Children who became stunted had arrest of the physiologic increase in microbiome diversity over time and a different distribution of bacterial taxa compared to controls. Among Peruvian children younger than 2 years of age 1) markers of enterocyte damage are high 2) stunting is associated with increased microbial translocation/innate immune system activation and slower evolution of the gut microbiome. Interventions to prevent or repair intestinal damage may prevent the stunting syndrome.

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ANTIMICROBIAL RESISTANCE PROFILE IN ENTEROBACTERIAE ISOLATED FROM CHILDREN UNDER-2-YEARS-OLD IN PERI-URBAN COMMUNITIES IN LIMA, PERU

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Antimicrobial resistance is a major and growing problem worldwide, specially affecting low and middle-income countries. There is no recent data regarding antimicrobial resistance in enteric pathogens in children in Lima. The aim of this study was to determine the antimicrobial resistance patterns of important enteric bacteria isolated from stool samples of children under 2 years old. Stool cultures were obtained during a community trial in the District of Independencia, Lima, between 2008 and 2011. A total of 711 diarrheal samples and 348 controls (from asymptomatic children) were collected. *Shigella* and *Campylobacter* were identified by routine microbiology; diarrheagenic *E. coli* strains were indentified by real time-PCR. Antibiotic susceptibility was tested by disk diffusion. Diarrheagenic *E. coli* strains included 346 enteropathogenic (EPEC), 253 enteroaggregative (EAEC) and 177 enterotoxigenic *E. coli* (ETEC). Diarrheagenic *E. coli* pathotypes (n=776) were resistant to ampicillin (68%), trimethroprim-sulfamethoxazole (61%) and tetracycline (49%); with low resistance rates to ciprofloxacin and ceftriaxone (<4%). *Shigella* isolates (n=96) were resistant to ampicillin (65%), trimethroprim-sulfamethoxazole (83%), tetracycline (70%) and chloramphenicol (49%); resistance to ciprofloxacin and ceftriaxone was not found. *Campylobacter* isolates (n=187) were resistant to tetracycline (90%), ciprofloxacin (88%) and azithromycin (17%). There was no difference in the resistance rates between diarrheal and control samples. Antimicrobial resistance of enteric pathogens is high in this setting. There is an urgent need to implement intervention strategies to control the emergence and spread of resistant strains and large scale, prospective, multicenter surveillance studies to document the current trends.

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ENTEROAGGREGATIVE *ESCHERICHIA COLI* IS SYNERGISTIC WITH OTHER ENTERIC PATHOGENS TO IMPAIR GUT ABSORPTION, CAUSE INFLAMMATION AND IMPAIR GROWTH

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Enteroaggregative *Escherichia coli* (EAEC) is common in children in developing countries. We evaluated EAEC infections in monthly stools tested in 1226 asymptomatic children with over 90% of twice weekly follow up for their first 2 years of life across 8 MAL-ED sites in Asia, Africa and Latin America. When children with EAEC alone were compared with those with no pathogens, other pathogens, or EAEC with 1, 2 or 3 other pathogens, those with EAEC or any other pathogen had inadequate sanitation compared with those with no pathogen at any of the three periods. Poor sanitation, percent of mothers with <6 years of education, lower socioeconomic assessment or percent with income <$150/month were associated with EAEC coinfections compared to the other groups including those with pathogens other than EAEC. Antibiotic use and low percent of breastfeeding were also associated with EAEC coinfections. Myeloperoxidase was increased with EAEC coinfections compared also to all groups including the group with pathogens other than EAEC. Alpha-glycoprotein and neopterin were reduced with EAEC coinfection compared to all other groups. EAEC coinfections also showed gut dysfunction as measured by lactulose:mannitol absorption, driven mainly by decreased mannitol absorption, reflecting reduced mucosal absorptive area. EAEC

coinfections also associated with lower changes in weight-for-age, weight-for-length and length-for-age z scores compared with all other groups. The most frequent cumulative EAEC coinfections were *Campylobacter*, Giardia and atypical enteropathogenic *E. coli* (EPEC). We conclude that EAEC coinfections associate with lower socioeconomic status, sanitation, antibiotic use and breastfeeding. The most frequent EAEC coinfections were Campy, Giardia and aEPEC. EAEC interactions with pathogens also associate with intestinal inflammation decreased local and systemic immune responses and gut dysfunction especially reduced intestinal absorptive function. Thus subclinical EAEC infections appear to synergize with other pathogens more than they synergize without EAEC, to impaired growth in children across this multisite study.

1676

DOUBLE JEOPARDY: CHOLERA OUTBREAKS IN PRISONS IN THE 21ST CENTURY

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Cholera outbreaks in prisons have been described since the 1800s. In most countries, prisoners are among the most susceptible and neglected risk groups, and among the least likely to receive immediate care. To identify cholera outbreaks in prisons globally and the control measures used, we conducted a systematic search of medical journals and news sources for reports of cholera in prisons. Reports of outbreaks identified through personal communication with public health officials were also included. We identified 27 cholera outbreaks in 13 countries between 2000 and 2015 (18 in Africa, 8 in the Americas, and 1 in Asia). Five outbreaks were reported in medical journals, 16 by media sources, 4 in non-governmental organization blogs or reports, and 7 by other sources. The number of cases ranged from 5 to 450, with case fatality rates of 0%- 50%. Control measures included: isolation of ill prisoners; water, sanitation and hygiene interventions; prophylactic use of antibiotics; suspending visits and halting food deliveries; and setting up an emergency treatment center. Antibiotics reportedly controlled the spread of cholera in 3 of 4 outbreaks. Oral cholera vaccines were used in two outbreaks, in one as a direct control measure, and in another as a preemptive measure to prevent the spread of the outbreak to unaffected prisons. Vaccine impact was not assessed. Our search identified at least 27 cholera outbreaks that had been reported in prisons since 2000, sometimes resulting in high case counts and case fatality rates. Because of limited surveillance in prisons, reported outbreaks, cases and deaths are likely to considerably underestimate the scope of the problem. Though prisons can be a challenging setting, chemoprophylaxis or vaccination can be delivered quickly, effectively and at low cost, and can supplement other measures which often take longer to implement. Enhanced surveillance and a systematic approach to cholera prevention, preparedness, and response in prisons, as well as rigorous post-response evaluation, could demonstrate more precisely the impact of various combinations of interventions and inform future prevention strategies.

1677

DISTRIBUTION OF *E. COLI* PATHOTYPES ALONG AN URBAN RURAL GRADIENT IN ECUADOR

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Pathogenic *E. coli* is one of the primary causes of diarrhea in developing countries. We report on the results of the EcoZUR study (*E. coli* en Zonas Urbanas y Rurales - *E. coli* in Urban & Rural Areas), a case-control study of diarrhea at four sample collection sites in Ecuador, along a rural-urban gradient. We sampled ~100 subjects with diarrhea and ~100 age-matched controls without diarrhea at each of the four Ecuadorian Ministry of Health clinics, including a large clinic in Quito, Ecuador's capital (Pop. 1.62 million), a hospital in the capital of Esmeraldas Province (Pop. 162,000), the town of Borbón (Pop. ~5,000), and outlying rural communities in the Borbón region, along the Onzole, Cayapas, and Santiago Rivers (Pops. ranging from ~10-500). The urban-rural gradient also represents a gradient of access to clean and safe water. We cultured *E. coli* from the fecal samples and used a set of 8 PCR primers to test them for virulence factors associated with diarrheagenic *E. coli* pathotypes. We found very high rates of diffuse adherent *E. coli* (DAEC) in study subjects in Quito and Esmeraldas, with ~10% and ~20% of study subjects, respectively, positive for this pathogen. Atypical enteropathogenic *E. coli* (EPEC) was the second most common pathotype detected. DAEC was the only pathotype significantly associated with diarrheal disease, although this may have been a function of sample size constraints, as the numbers for the other pathotypes were limited. We also report on association between a) diarrhea and b) presence of pathogenic *E. coli* and risk factors such as water treatment, sanitation type, and recent travel. Our study allows us to understand how factors that differ along a rural-urban gradient—such as diet, access to clean water and sanitation facilities, and travel patterns—affect the set of enteric pathogens circulating within a population.

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EPIDEMIOLOGY AND RISK FACTORS FOR CRYPTOSPORIDIOSIS IN CHILDREN IN THE MAL-ED STUDY

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Cryptosporidium spp are enteric protozoa that cause significant morbidity and mortality in young children worldwide. The Etiology, Risk Factors, and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health and Development Project (MAL-ED), a cohort study across eight sites, allowed an ideal opportunity for defining the epidemiology of *Cryptosporidium spp* in children living in resource-limited settings. Children were enrolled within 17 days of birth. Data on illness, socioeconomic variables, and nutritional intake were collected by survey. Stool samples were tested for *Cryptosporidium parvum* by ELISA. Anthropometric measurements were taken monthly. The final analysis included 1,659 children with 24 months of follow up. Across the eight sites, 51.1% (848/1659) of children had at least one infection with *Cryptosporidium spp*, and 48.9% (811/1659) remained infection-free. *Cryptosporidium* diarrheal episodes were more likely to be associated with dehydration (16.5% vs 8.3%, $p < 0.01$), and to meet the GEMS definition of moderate-to-severe diarrhea (20.3% vs 11.9%, $p < 0.01$). Rates of *Cryptosporidium* diarrhea were highest in the Peru (10.9%) and Pakistan (9.2%) sites. Detection rates in surveillance stools ranged from 2.7% in Brazil to 6.6% in Tanzania and 7.3% in Peru. Children in rural sites had a significantly quicker progression to first *Cryptosporidium* infection than children from non-rural sites. Stunting at baseline was not associated with higher risk of infection (mean HAZ -0.92 vs -0.99, $p = 0.16$). Lower family income (146.94 (132.97) USD vs 199.22 (188.96) USD, $p <0.001$), overcrowding (29.6% vs 21.1%, $p<0.001$), fewer years of maternal education (6.25 (3.99) vs 7.35 (3.96), $p < 0.001$), and unimproved sanitation (chi squared 29.64, $p < 0.0001$) were all associated with *Cryptosporidium* infection. In this cross-site analysis, rural sites had greatest burden of disease, earliest onset of disease, and highest prevalence of unimproved sanitation, suggesting that interventions targeting spread of cryptosporidiosis should focus on improved sanitation infrastructure.

1679

THE SPECTRUM OF *CHROMOBACTERIUM VIOLACEUM* INFECTIONS FROM A SINGLE GEOGRAPHIC LOCATION

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Chromobacterium violaceum is a bacterium associated with soil and water exposure in tropical regions and causes rare and serious clinical infections that are often fatal. We reviewed the demographic and clinical details of twenty-eight patients with *C. violaceum* detected over fifteen years from 2000 to 2015, from the Top End of the Northern Territory. Eighteen patients had infections attributable to *C. violaceum*. Patients with infections were more commonly male (55.6%), and in the 16-to-60 year (61.1%) age group. Skin and soft tissue infections (50%), predominantly involving the limbs, were the major clinical manifestation. Water, mud exposure and trauma were all noted as precipitating circumstances and co-morbidities were present in 61.1% of the patients with infections. Ten of the twenty-eight patients (35.8%) had *C. violaceum* isolated as an incidental finding or as asymptomatic colonisation; these ten patients did not require, or receive therapy for the presence of *C. violaceum* bacteria. There were no relapsing infections in this group. *C. violaceum* remains a serious infection, with seven patients (25%) in our series requiring intensive care management. However, the mortality rate (7.1%) in our series was far lower than previously described. This case series of *C. violaceum* infections from a single geographic area provides additional information of the characteristics of infection with this pathogen.

1680

TARGET PRODUCT PROFILE FOR A DIAGNOSTIC ASSAY TO DIFFERENTIATE BETWEEN BACTERIAL AND NON-BACTERIAL INFECTIONS TO GUIDE ANTIMICROBIALS USE IN RESOURCE-LIMITED SETTINGS: AN EXPERT CONSENSUS

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Acute fever is one of the most common presenting symptoms globally. In order to reduce the empiric use of antimicrobial drugs and improve outcomes, it is essential to improve diagnostic capabilities. In the absence of microbiology facilities in low-income settings, an assay to distinguish bacterial from non-bacterial causes of fever would be a critical first step. To ensure that patient and market needs are met, the requirements of such a test should be specified in a target product profile (TPP). To identify minimal/optimal product characteristics for a bacterial vs. non-bacterial fever test, experts from academia and international organizations with expertise in infectious diseases, diagnostic test development, laboratory medicine, global health, and health economics were convened. TPP characteristics were proposed and reviewed by a working group, and consensus characteristics were defined. This working group defined non-severely ill, non-malaria infected children as the target population for the desired assay. To provide access to the most patients, the test should be deployable to community health centers and informal health settings, and staff should require <2 days of training to perform the assay. Further, given that the aim is to reduce inappropriate antimicrobial use as well as to deliver appropriate treatment for patients with bacterial infections, the working group agreed on minimal diagnostic performance requirements of >90% and >80% for sensitivity and specificity, respectively. Other key characteristics, to account for the challenging environment at which the test is targeted, included: i) time-to-result <10 min (but maximally not >2 hrs); ii) storage conditions at 0-40°C, ≤90% non-condensing humidity with a minimal shelf life of 12 months; iii) operational conditions of 5-40°C, ≤90% non-condensing humidity; and iv) minimal sample collection needs (50-100µL, capillary blood). This expert consensus approach to define assay requirements for a bacterial vs. non-bacterial diagnostic assay should guide product development, and enable targeted and timely efforts by industry partners and academic institutions.

1681

INVASIVE NON-TYPHOIDAL *SALMONELLA* INFECTIONS IN ASIA: CLINICAL OBSERVATIONS, DISEASE OUTCOME AND DOMINANT SEROVARS FROM A TERTIARY REFERRAL HOSPITAL IN HO CHI MINH CITY, VIETNAM

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Invasive non-typhoidal Salmonella (iNTS) infections are now a well-described cause of morbidity and mortality in children and HIV-infected adults in sub-Saharan Africa. In contrast, the epidemiology andclinical manifestations of iNTS disease in Asia are not well documented. We retrospectively identified >100 cases of iNTS infections in an infectious disease hospital in SouthernVietnam between 2008 and 2013. Clinical records were accessed to evaluate demographic and clinical factors associated with iNTS infection and identify risk factors associated with death. Multi-locus sequence typing and antimicrobial susceptibility testing was performed on all organisms. Of 102 iNTS patients, 71% were HIV-infected, >90% were adults, 71% were male and 33% reported intravenous drug use. Twenty-six/92 (28%) patients with a known outcome died; HIV infection was significantly associated with death (p=0.004). S. Enteritidis (ST11) (48%, 43/89) and S. Typhimurium(sequence types (STs)19, 34 and 1544) (26%, 23/89) were the most commonly identified serovars; S. Typhimurium was significantly more common in HIV-infected individuals (p=0.003). Isolates from HIVinfected patients were more likely to exhibit reduced susceptibility against trimethoprim-sulfamethoxazole than HIV-infected patients (p=0.037). We conclude that iNTS disease is a severe infection in Vietnam with a mortality rate similar to sub-Saharan Africa. As in sub-Saharan Africa, HIV infection is the major risk for death, with the majority ofthe burden in this population in HIV-infected men. Although the STs of iNTS organisms identified in this study were common globally, we suggest continued surveillance across Asia to monitor for the presence of multi-drug resistant STs.

1682

COXIELLA BURNETII ANTIBODIES ARE PREDOMINANT AMONG PATIENTS WITH UNDIFFERENTIATED FEVER IN AFGHANISTAN

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Diagnosis of infectious diseases in Afghanistan remains a challenge with limited ability for pathogen isolation and identification. Baseline data on the prevalence of etiologies causing undifferentiated fever is lacking in Afghanistan. Herein we screened serum of Afghan patients suffering from undifferentiated fever (UF) for antibodies against number of pathogens, including *Coxiella burnetii, Leptospira* spp.and typhoid fever. Patients > 5 years old with UF who meet the WHO case definition and presented at

two provincial hospitals: Kandahar (KDH, n=178), Helmand (LG, n=82) and a third quaternary level hospital in Kabul (KID, n=303) were enrolled and consented into a surveillance study between 2007 and 2012. A single serum sample was collected and tested by ELISA for the detection of IgM and IgG against Q fever (*C. burnetii), Leptospia* spp. IgM and total immunoglobulins of *Salmonella enterica* serovar typhi. A total of 563 patients were screened and 50.3 % were seropositive against at least one pathogen. Cases from KDH showed the highest frequency of *C. burnetii* antibodies (n=80, 37 % IgG and 8.4 % IgM), followed by those from LG (n= 21, 20.7% IgG and 4.9% IgM) and KID (n= 55, 16.5% IgG and 1.7% IgM). Leptospia IgM was evident in 11.4% of patients, 13.2% in KID, 10.7 in KDH and 6.1 in LG. Typhoid fever titers >320 were found in11.2% of all patients, being higher in LG (15.9%) and KDH (12.9%) than KID (8.9%). Almost half of the *C. burnetii* IgM-positive cases (12/22) did not mount immune responses to other pathogens. The data suggest that both acute and past Q fever infections were evident within patients tested. The increased seropositivity rates in cases from KDH and LG provincial hospitals compared to those of KID in Kabul city may be attributed to limited sanitary measures for typhoid fever. While typhoid fever is transmitted via ingestion of polluted food and water, both Q fever and Leptospira are spread by contact with animals, their contaminated products or excreta. The current results provide initial disease burden data for Afghanistan and will be useful to health authorities in guiding hygiene improvement plans and disease prevention strategies.

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DETECTION OF *SALMONELLA BACTEREMIA* IN RURAL KENYA USING FIELDABLE DIAGNOSTICS

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Invasive Non-Typhi Salmonella (NTS) are a major cause of infections in sub-Saharan Africa, with a case fatality rate of 4-27% in children and 22-47% in adults. The extensive presence of other co-morbidities in the patients complicates diagnostics. There is a need for rapid, reliable, and fieldable diagnostics that can be used at the point-of-care. We are developing such diagnostic assays for Salmonella detection using two approaches - pathogen biomarker detection using a waveguide biosensor, and real-time PCR. The former requires minimal handling of samples, and allows for the rapid and specific detection of antigen using fluorescent probes and uses a novel assay strategy called lipoprotein capture. Salmonella lipidic biomarkers are taken up by lipoproteins in serum, which are immobilized on sensor surface, and the associated biomarker is identified using Salmonella-specific antibodies. The assay was optimized using lipid lysates prepared from either control strains of Salmonella or clinical strains from patients in Kenya. The optimized assay was tested on four pediatric samples from Kenya. We saw excellent (100%) corroboration with culture results for the samples. Future work includes identification and characterization of the antigen, and testing more patient samples from Kenya. Real-time PCR requires more handling of samples and expertise to run the assays but are useful for the characterization of isolates and identification of resistance. For PCR assays, primers for Salmonella detection were designed and tested with DNA from control and clinical isolates. Testing is underway with a Gram detection assay to differentiate between Gram-positive and -negative bacteremia. We are also working on identifying antimicrobial resistances in the isolates from Kenya to design assays for their detection, and have identified resistance to first, second and third-line antibiotics in patients. Further validation for the PCR assays will be done on clinical samples. Our goal is to deploy these technologies to our clinical site in rural Kenya, and train local personnel to run them, thereby improving health care infrastructure in country.

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LEPTOSPIRAL DNA IN FEBRILE PATIENTS FROM SEMI-RURAL COMMUNITIES IN MANABÍ-ECUADOR

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The aim of this study was determine whether fever is associated with the presence of leptospiral DNA in human sera detected with Polymerase Chain Reaction (PCR). DNA was extracted from 576 samples of human serum (513 febrile and 63 non-febrile) obtained between February 2014 to July 2015 from semi-urban parishes Calderón and Santa Ana in Portoviejo city. DNA was analyzed first with real time PCR (PCR-RT), followed by conventional PCR and finally amplicon sequencing. The 16s ribosomal RNA sequences were detected in 2 out of 513 (0.5%) febrile patients and 0 of 63 (0.0%) from non-febrile patients.

1685

PROGRESS AND CHALLENGES OF TRACHOMA ELIMINATION IN THE FAR NORTH REGION OF CAMEROON

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In Cameroon, trachoma mapping conducted in 2010-2011 identified 13 health districts (HDs) in the Far North region with a prevalence of trachomatous inflammation-follicular (TF) of over 10% in children aged 1-9 years. These HDs qualified for district mass drug administration (MDA) as well as implementation of other components of the SAFE (Surgery, Antibiotic treatment, Facial cleanliness and Environmental improvement) strategy recommended by World Health Organization. These HDs benefitted from support from HKI with funding from USAID's ENVISION Project, managed by RTI International. Of these 13 HDs, 5 successfully passed impact assessment in 2014 and stopped MDA. In 2015, 5 additional HDs completed 3 rounds of MDA with coverage of >80% and qualified for impact assessment. However, due to continued insecurity caused by Boko Haram attacks, only 2 HDs (Mokolo, Guidiguis) were evaluated. A cross-sectional, cluster randomized survey was conducted to estimate the TF prevalence to determine if the stopping MDA criteria had been met. A sample of 1,961 children aged 1-9 years was surveyed. The WHO simplified trachoma grading system was used. The results showed that the TF prevalence decreased from 16.9% (95% CI: 15.4-18.5%) and 13.1% (95% CI: 11.7-14.5%) in 2010 to 1.0% (95% CI: 0.5-1.8%) and 0.8% (95% CI: 0.3-1.7%) in 2015 in Guidiguis and Mokolo respectively. These 2 HDs reached a TF prevalence of <5% and hence met the criteria of stopping MDA. While these results represent further positive steps towards trachoma elimination in the region, the threat of terrorism in these regions has formed a barrier to timely completion of surveys and hence measuring the progress of the program. Uncertainty remains for the 3 HDs not yet assessed, and according to the plan, 2 more HDs are scheduled for evaluation in 2016 and 1 additional HD in 2017. It is not clear whether these surveys can go ahead as planned. In addition, insecurity makes it difficult to evaluate the progress of the other SAFE components such as TT surgery and environmental improvements. These challenges are a serious threat to achieving the year 2020 trachoma elimination goals in Cameroon.

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THE BURDEN OF TRACHOMA IN EASTERN EQUATORIA STATE, REPUBLIC OF SOUTH SUDAN

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Eleven counties in the Republic of South Sudan required trachoma impact surveys in order to evaluate the success of program interventions and to determine if additional rounds of mass drug administration (MDA) with azithromycin were needed. Despite security issues and population displacement The Carter Center supported the South Sudan Ministry of Health (MoH) to conduct surveys in five counties in Eastern Equatoria state. These were the first known trachoma impact surveys since the country's independence and the first population-based clinical trachoma data to be collected in this region since 2006. Owing to limited in-country personnel capable of serving as trachoma grader trainers, the MoH reached out to neighboring countries Ethiopia, Sudan and Uganda who provided experienced grader trainers for the training. For each survey, a multi-stage cluster-random sampling method was used. Households within a cluster were selected with equal probability and all present household members were examined using the WHO simplified trachoma grading scheme for all 5 clinical signs of trachoma. Despite remoteness of villages, difficult terrain and weather conditions, a total of 14,462 individuals in 3,446 households were surveyed across the five counties in Eastern Equatoria state. The burden of trachoma was high in these 5 counties. The prevalence of trachomatous inflammation follicular (TF) in children age 1-9 years ranged from 21.1%, (95% Confidence Interval (CI):12.1, 34.1) to 47.6%, (95%CI: 42.2, 53.1). Trachomatous trichiasis was also highly prevalent, ranging between 2.9% to 4.5% in those 15 years and older. The prevalence of water and sanitation indicators were low in all five counties, including two counties which had a complete absence of latrines in all surveyed villages. The results of the survey showed that all 5 counties will require at least 3 to 5 more years of MDA and surgical interventions. This experience also shows that surveys can still be carried out in extremely resource poor and difficult areas, and that neighboring countries are willing to provide valuable technical assistance.

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IMPACT OF MASS TREATMENT WITH AZITHROMYCIN FOR TRACHOMA ON SEXUALLY TRANSMITTED INFECTIONS AND ANTIMICROBIAL RESISTANCE AMONGST WOMEN IN THE SOLOMON ISLANDS

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Chlamydia trachomatis is the most common bacterial sexually transmitted infection and is frequently asymptomatic. Population based interventions aiming to increase the coverage of screening for C. trachomatis and behavioural interventions to reduce high-risk sexual behaviour have shown limited efficacy in reducing its prevalence. Resistance to first line antimicrobials is an increasingly significant problem in the management of sexually transmitted infections and threatens the effectiveness of current treatment regimes. Ocular infection with C. trachomatis is the cause of trachoma, which is also endemic in the Pacific. The WHO strategy for the elimination of trachoma as a public health problem includes community mass treatment with azithromycin. Mass drug administration (MDA) of azithromycin for trachoma might reduce the prevalence of genital C. trachomatis but might also drive the emergence of antimicrobial resistance. We conducted a study in the Solomon Islands alongside a Ministry of Health trachoma elimination programme to establish the impact of MDA with azithromycin on sexually transmitted infections and look for evidence of emerging drug resistance. Women attending outpatient clinics before or after MDA were enrolled. Self-taken high vaginal swabs were tested by PCR for C. trachomatis and Neisseria gonorrhoeae. Whole genome sequencing was attempted on all samples that were positive by diagnostic PCR for either pathogen. Following MDA, we noted a significant decrease in the prevalence of genital C. trachomatis infection, but not of N. gonorrhoeae, by diagnostic PCR. We will present data from whole genome sequencing of genomic evidence of the presence/absence of antimicrobial resistance obtained from both the pre and post-MDA samples.

1688

QUALITY ASSESSMENT OF THE IMPLEMENTATION OF THE TRACHOMATOUS TRICHIASIS SURGERY IN POLI HEALTH DISTRICT, CAMEROON USING SWPO METHOD (SUCCESS - WEAKNESSES - POTENTIALS - OBSTACLES)

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Poli is a trachoma endemic district in the North Region of Cameroon, with a total population of 88,513. The TT prevalence was 1.08% at baseline mapping. As standardized by sex and age, the estimated backlog is 625 cases, corresponding to an Ultimate Intervention Goal (UIG) of 536. A TT surgery campaign was organized in February 2016, supported by the United States Agency for International Development's MMDP Project, managed by Helen Keller International. This was preceded by a series of training of trainers, surgeons and nurses using the HEAD START mannequin, supply of drugs and consumables, community meetings, and dissemination of awareness messages. During the campaign, 3203 patients were examined and 92 TT cases were managed, including 84 cases operated and 8 cases that refused the surgery, but received tweezers and advices for adequate epilation. With the aim to appreciate the quality of the implementation of the campaign, a self-assessment using SWPO method (Success - Weaknesses - Potentials - Obstacles) was carried out by stakeholders at different levels of the health pyramid. This consisted of reviews of all steps of implementation of each activity conducted, to identify strengths, weaknesses, obstacles and potentials that could be explored and taken into consideration for the planning of upcoming campaigns. The community meetings held in all villages were identified as a success that allowed the dissemination of sensitization messages. Challenges with timing of the surgeon training, the availability of cases for the surgeon training, and the procurement of surgical supplies constituted the main weaknesses. The spatial distribution of TT cases in the district does not reflect the forecasts resulting from baseline data, and this constituted a significant obstacle. There is a potential to use community meetings for preliminary screening of suspected TT cases before deploying surgical teams to the field. Experiences and opinions of stakeholders involved in the TT surgery campaign in Poli allowed us to collect information that will be taken into account in planning next TT surgery campaign in the North region.

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FACTORS PREDICTING TRACHOMA IMPACT SURVEY OUTCOMES

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Trachoma-endemic countries are committed to achieving the elimination of trachoma as a public health problem by 2020. To do this, multiple years of the WHO-endorsed SAFE strategy are recommended, depending on baseline prevalence of trachomatous inflammation-follicular (TF) and trachomatous trichiasis (TT). Trachoma impact surveys (TIS) have been implemented in 9 countries supported by USAID between 2012-2015 to determine whether TF and TT prevalence have decreased to a point where further intervention is no longer required. We measured the effects of baseline TF prevalence, number of rounds of MDA, and median MDA coverage on the likelihood of passing a TIS. Results from one hundred forty-three TIS conducted between 2012 and 2015 across 9 countries were analyzed using logistical regression analysis. Out of 102 surveys implemented in 8 countries, 83% showed that the TIS passed (range 60-100%). Preliminary logistic regression analysis of baseline TF prevalence, number of MDA rounds and median coverage variables showed that baseline TF prevalence was statistically associated with passing TIS (alpha=0.05): odds ratio =0.934; 95% confidence interval 0.898-0.970. However, when the results from 70 TIS implemented in one highly endemic country—all but 2 of which failed—are included in the analysis, the model no longer holds. Median baseline TF prevalence in these 70 districts was 39%, compared to 11.8% in the other 102 districts, and the median number of MDA rounds was 6 compared to 3. Median coverage in this country was high, at 94.5%, compared to 82.9% in the other 8 countries. Districts with low baseline prevalence were significantly more likely to pass trachoma impact surveys in 8 countries; for every percentage point increase in baseline prevalence, the odds of passing TIS decrease by 6.6%. Despite high MDA coverage over multiple rounds in one country, the TIS did not pass. This analysis confirms that especially in areas of very high baseline prevalence, high coverage of antibiotics alone is not sufficient for decreasing TF prevalence; F&E interventions should also be prioritized for sustainable elimination of blinding trachoma to be achieved.

1690

CAN WE ELIMINATE TRACHOMA AS A PUBLIC HEALTH PROBLEM BY 2020?

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Trachoma remains the world's leading infectious cause of blindness. The World Health Organization (WHO) is aiming to eliminate trachoma as a public health problem by 2020. In order to achieve this they have outlined the ultimate intervention goals, the first of which aims to achieve a reduction in Trachomatous Inflammation - Follicular (TF) to less than 5% in children 1-9 years old within all endemic communities by 2020. We use data collected by the International Trachoma Initiative on the prevalence of TF in 44 trachoma endemic districts to assess the feasibility of achieving this goal by 2020, using a statistically validated dynamic mathematical transmission model. For each endemic district that had not reached the current target by the most recent time point of surveillance, we assess whether the ultimate intervention goal will be met by 2020 using the current WHO guidelines alone and, if not, what additional interventions may be required. We find that for regions with greater than 30% TF prevalence, current mass drug administration (MDA) regimes are not sufficient to achieve this goal. Our work suggests that an increased frequency of MDA treatment, in addition to enhanced facial cleanliness and environmental improvements (resulting in long-term transmission reduction) will be essential in these regions. Moreover, in regions where TF is less than 30% at least some degree of long-term transmission reduction, through enhanced facial cleanliness and environmental improvements, is required in order to prevent re-emergence in the community in the absence of sustained and on-going MDA. Our findings suggest that in most districts where TF is less than 30% the first ultimate intervention goal is achievable with the current recommended WHO guidelines. However, considerably more resources will be required in high prevalence settings in order to ensure full elimination as a public health problem and elimination timelines are likely to exceed past 2020 in these settings.

1691

TRAINING OF MASS DRUG ADMINISTRATION (MDA) DISTRIBUTORS, COMMUNITY MOBILIZATION AND COMMUNITY KNOWLEDGE OF MDA: A QUALITATIVE POST-MDA ASSESSMENT AMHARA, ETHIOPIA

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In communities where the prevalence of trachoma exceeds a predetermined threshold annual community-wide mass drug administration (MDA) of antibiotics is warranted. Training of MDA distributors, community mobilization and knowledge of MDA are instrumental to effectively implement MDA. Despite this, most coverage surveys only focus on self-reported medication use by beneficiaries while ignoring the methodologies and structures through which MDA is implemented. To evaluate the training of MDA distributors, assess the role of health workers in mobilizing the community, and to assess the level of community awareness about the MDA campaign, a qualitative post-MDA assessment was conducted in Amhara, Ethiopia, an area hyperendemic for trachoma. Following an antibiotic MDA in 2015, four districts were selected for the administration of key stakeholder interviews with health workers at various levels of the health care system and focus group discussions (FGDs) with community members within randomly selected villages. A total of 12 interviews and 4 FGDs were conducted which included a total of 35 participants and results were recorded, transcribed, and analyzed using MaxQDA qualitative software to identify the primary themes described by participants. The themes included key stakeholder engagement, training, and community mobilization. More specifically the results showed multi-sector involvement of stakeholders during the MDA was thought to be very important and although training of drug distributors was consistently implemented in accordance with standardized procedures, respondents believed more time was needed for the training. With respect to community mobilization, health education was described as "essential" to ensure participation, and a greater involvement of community-level health volunteers would help governmental health workers increase mobilization and MDA coverage at the village level. The results from this study can be used to improve MDA distribution throughout the Amhara region, and will be instructive in other regions of Ethiopia currently scaling up trachoma MDA.

1692

MORTALITY FOLLOWING DISCHARGE IN CHILDREN ADMITTED TO A RURAL MOZAMBICAN HOSPITAL: DEVELOPMENT OF A PREDICTION MODEL TO IDENTIFY CHILDREN AT RISK OF DEATH

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An important but neglected contributor to child mortality is the vulnerable period following hospital discharge. The objective of this study is to determine the burden of post-discharge mortality and what are the predictors of mortality following discharge in a rural Mozambican hospital. A systematic review of the paediatric deaths taking place at community level over the last 11.5 years was done through a demographic surveillance ongoing in a southern district of rural Mozambique. We used a morbidity surveillance system ongoing in Manhiça District Hospital to exclude hospital deaths. We determined post-discharge mortality over three different time-periods: 1st: 0-30 days, 2nd: 31-60 days and 3rd:61-90 days following hospital discharge. We identified predictors of post-discharge mortality and derived a simple prediction tool that uses some of the collected variables to identify children at high risk of death after discharge. Data from 21227 children were reviewed and analysed (initial sample were 45700 children, of which 23725 lived out of study area, 555 were hospital deaths and 55 had missing outcome and were excluded). Mortality at 1st period was 2.3% (484/21227), at 2nd period was 1.4% (289/21227) and 3rd period was 1.0% (212/21227). Overall mortality was 4.6% (985/21227). The final adjusted model for the prediction of post-discharge mortality included the variables non breastfeeding among children <2 years (OR 2.4, 95%CI 1.7-3.1), orphan of both parents (OR 9.2, 95%CI 5.4-15.9), severe dehydration (OR 3.3, 95% CI 2.2-5.2), oral candidiasis (OR 8.1, 95% CI 6.1-10.7), Blantyre Coma Scale score <2 (OR 3.2, 95% CI 1.0-10.5), HIV-positive status (OR 12.1, 95% CI 5.1-28.4) and being <2years old (p<0.001). Mortality following discharge is a poorly recognised contributor to child mortality. A simple prediction tool that uses several easily collected variables can be used to identify children at high risk of death after discharge.

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LYMPHATIC FILARIASIS TRANSMISSION INTENSITY ASSESSMENT IN THE URBAN AREA OF BAMAKO, MALI

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Anopheles gambiae and *An. funestus* are the main vectors of LF in Mali. *Culex* spp is the most common mosquito species in urban areas of this region. The 2004 mapping survey using ICT found that all regions in Mali were endemic for lymphatic filariasis (LF). We aimed to determine if *Culex* species are incriminated in *Wuchereria bancrofti* (Wb) transmission in the capital city, Bamako, and to assess the endemicity level in Bamako after 3 mass drug administration (MDA). Vectors were trapped using CDC light traps and tested for Wb infection by PCR. Night thick smears were collected from volunteers >14 years in the 6 national LF elimination program sentinel sites in Bamako and in two additional urban sites selected because of a family with 3 cases of elephantiasis (Faladie) and the high frequency of *Culex* breeding sites (Bozola). A total of 6,174 *Culex* spp (85.2%), 16 *An. gambiae* (0.2%), 26 *Aedes* spp (0.4%), 858 *Ceratopogonidea* (11.8%) were collected. No positive *Culex* pools were detected among the 252 tested by PCR. None of the 1,002 volunteers had detectable Wb microfilariae. Mp microfilariae were detected in 5 individuals in two of the localities (0.5%). These data provide no evidence of active LF transmission requiring intervention in Bamako. The presence of Mansonella perstans microfilaremia in residents of two of the eight localities is consistent with migration from rural to urban areas in this population.

1694

SCRUB TYPHUS AS A MAJOR CAUSE OF ILLNESS FOR PATIENTS WITH UNKNOWN FEVER ORIGIN IN GALLE, SRI LANKA

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Scrub typhus (ST) is an acute febrile illness that is caused by the gram negative intracellular bacteria, *Orientia tsutsugamushi.* Traditionally, the diagnosis of ST mainly relies on serologic tests. IFA is considered the gold standard for cases of seroconversion or a >4-fold rise in antibody titers between acute- and convalescent-phase serum specimens. ST is under-reported due to the nonspecific symptoms and the lack of simple diagnostic tests. Recent studies of acute febrile illness (AFI) in Southern Sri Lanka suggest a broad spectrum of infectious agents including *Orientia*. Here we used the 3-patented-recombinant-protein ELISA for the detection of *Orientia*-specific antibodies in AFI patients from Sri Lanka. Among all 460 pairs of serum tested, 80 of them were positive for IgG in the convalescent sera (17.4%). The acute sera of these 80 patients were further analyzed. Nine of them appeared to have IgG serocoversion, 40 of them had positive IgM for acute sera and 3 of them were both IgG seroconverted and acute IgM positive. Taken together, 58 (12.6%) patients were considered ST positive. Additional assays for the detection of other disease-specific antibodies of the remaining 85% patients without a definitive diagnosis are needed.

1695

LEPTOSPIROSIS IS ONE OF THE MAJOR DISEASES FOR PATIENTS WITH UNKNOWN FEVER ORIGIN IN GALLE, SRI LANKA

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Leptospirosis is caused by spirochaetes of the genus *Leptospira*. It is considered to be the most widespread bacterial zoonotic disease in the world. Timely diagnosis is essential for antibiotic therapy provides the greatest benefit when initiated early in the course of illness. Currently,

MAT is the standard serological method for the diagnosis of leptospirosis. However, it is technically complex and time-consuming. We have developed a 4-recombinant protein-based ELISA to detect *Leptospira*-specific antibodies. A total of 460 pairs of serum collected from febrile patients was anlayized. Among all 460 paired serum, 148 of them were positive IgG in the convalescent sera (32.1%). The acute sera of these 148 patients were further analyzed. Among these patients, 73 of them appeared to have IgG serocoversion between acute and convalescent sera, 16 of them had positive IgM for acute sera and 11 of them were both IgG seroconverted and acute IgM positive. Taken together, 82 (21.7%) patients were considered leptospirosis positive. While these results suggest that leptospirosis is a significant cause of illness with unknown fever origin in this cohort, there are still high percentage of patients without a definitive diagnosis.

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ANTIVENOM INDUCED ANAPHYLAXIS FOR TREATING NEUROTOXIC SNAKE ENVENOMATION IN NEPAL

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Intravenously (IV) administered snake antivenom is a life-saving treatment for snakebite envenomation but adverse reactions are common, including life-threatening, antivenom related anaphylaxis (ARA). Herein, we report our experience of ARA when treating neurotoxic envenomation caused mostly by Naja naja (Indian cobra) and Bungarus caeruleus (Indian krait) in Nepal. We conducted a double blind, randomised, trial of lower vs. higher dose of antivenom (IV push followed by an infusion). Patient monitoring involved symptoms, vital and neurotoxic signs and oximeter measured oxygen saturation. Prestudy training emphasised immediate ARA treatment when first recognised: stopping antivenom, administering intramuscular (IM) adrenaline, IV hydrocortisone and IV chlorphenamine, and salbutamol nebulisers, supplemental oxygen, intubation and hospital transfer, as clinically indicated. Results From April 2011 to November 2012, 154 envenomed patients were recruited of whom 13 [8.4 (95% CI: 4.6-14.0)]% had clinical features consistent with ARA: 3 children (5, 6, 11y) and 10 adults (18-52y). Nine had clear cut ARA whilst in four differentiation from envenomation was difficult. Median (range) time to first ARA was 5 (19-115) minutes. Typical ARA features included urticaria/erythema (n=10), hypotension/shock (5), dyspnea with (3) or without (4) wheezing. Unusual features were laryngeal oedema (2) and bradycardia (4). Sudden deterioration with cardio-and/or respiratory arrest occurred in 5 patients. Eight patients died, five within 4h and three at 13h (late laryngeal oedema, 24h (cardiac arrest while ventilated) and 11 days (ventilator associated pneumonia). Three deaths occurred in transit to hospital. In conclusion, ARA was relatively common and associated with a poor prognosis in resource restrained Nepal. More training, better anaphylaxis management and quality antivenom may save more lives.

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LUNG NODULES IN CHRONIC SCHISTOSOMIASIS: A RARE CONDITION?

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Schistosomiasis is a neglected tropical diseases caused by the fluke worms of the genus Schistosoma. The infection can cause damage to the liver and to the genitourinary tract, depending on the species involved. The lungs can be affected both in the acute presentation of the infection (Katayama fever), and in the chronic phase. In the latter case, the damage is usually described as pulmonary hypertension; nodular patterns have been described rarely.Between May 2014 and October 2015 six immigrants of African origin were diagnosed at the Centre for Tropical Diseases (CTD) of Negrar (Verona), Italy, with lung nodules due to chronic schistosomiasis. A further case was found at the Infectious Diseases Clinic of Udine, Italy. The patients were screened for parasitic infections because of eosinophilia. An ELISA assay for *Schistosoma mansoni* resulted positive for all of them, and all but one had eggs (of *S. mansoni* and/or *S. haematobium*) in stool and/or urine. Four patients complained of respiratory symptoms (cough, chest pain), while the others underwent a routine, screening chest x ray. The radiological investigations (x ray and then CT scan) demonstrated, in all patients, multiple pulmonary nodules. The first five patients underwent a biopsy of the nodules (TB was the main suspected cause), and the histological examination revealed schistosome eggs. The last two patients had a presumptive diagnosis. All patients were treated with praziquantel 40 mg/Kg/day for three days, obtaining complete resolution of the radiological picture. In the same period, at the CTD, 120 cases of schistosomiasis were diagnosed; hence, the pulmonary nodular presentation represented 5% of all cases of Schistosoma infection in the study period. In conclusion, schistosomiasis should be in the differential diagnosis of pulmonary nodules in patients coming from endemic areas. A specific screening is recommended and, if the index of suspicion for other severe conditions is reasonably low, invasive procedures can be avoided or postponed. Treatment with praziquantel seems to be efficacy, and the response can be monitored with CT scan.

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WEIGHT-FOR-AGE GROWTH-RATE FAILURE IN INFANTS IS ASSOCIATED WITH AN ALTERED BLOOD GENE EXPRESSION PROFILE INDICATING REDUCED IMMUNE RESPONSIVENESS

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Undernutrition is associated with an increased risk of morbidity and mortality due to infectious disease. In addition to mucosal barrier dysfunction, a systemic immunodeficiency is likely at play; however, the molecular basis of immune dysfunction in undernutrition is largely unknown. Here using weight-for age Z-score (WAZ) growth trajectories in the Bangladeshi PROVIDE birth cohort, we analyzed the cumulative

effect of growth rate failure (GRF) over the first year of life on the baseline peripheral blood gene expression profile at one year of age. GRF was defined as a WAZ curve that deflected downward across at least two Z-lines without recovery during the 53-week observation period. RNA from whole blood was extracted and cDNA target preparation was optimized to avoid globin or ribosomal RNA transcripts. Using microarray transcriptomic analysis, we found 146 genes that were differentially regulated ($P <$ 0.05 and at least 2-fold absolute change) between the age-and gender-matched control (n = 10) and GRF (n = 10) groups. Bioinformatics analysis indicated involvement of these genes in diverse cellular processes including metabolism, regulation of growth and apoptosis, and response to viral infection. These results suggest that a GRF trajectory may be associated with an altered baseline blood gene expression profile skewed towards reduced immune responsiveness. Further pathway analysis with additional specimens will inform novel *in vitro* experimental conditions for mechanistic studies.

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MATERNAL IRON DEFICIENCY ANEMIA, MALARIA AND SOIL TRANSMITTED HELMINTHS ARE A MAJOR RISK FACTOR FOR ANEMIA IN EARLY CHILDHOOD

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Iron deficiency is common in pregnant women and young children worldwide and can lead to clinically significant anemia and impaired neurological development in children. We measured hemoglobin (Hb) levels in parallel birth cohorts (measured monthly then every three months from birth to age 3 years) of Kenyan children living in rural (N=246) and nearby urban areas (N=353). Mean Hb levels were identical in the two cohorts at birth, then diverged by an average of 2.6 gm/dL lower Hb levels in rural cohort from 6 to 18 months of age (P<0.0001), but converged by 21-24 months reflecting similar diets in the two cohorts. Malaria and soil transmitted helminth infections (STH) infections were low in both cohorts and did not differ. Since erythropoiesis in infancy depends heavily on iron stores acquired prenatally we hypothesized differences in Hb levels between the two cohorts arose from impaired maternal transplacental transfer of iron to the fetus. We examined the impact of maternal risk factors on Hb levels in their offspring between ages 6 through 18 months using a generalized linear model. Maternal risk factors included infections (malaria, HIV, schistosomiasis, filariasis, STH), Hb, parity, age, and markers of anemia (serum ferritin, soluble transferrin receptor [sTfR], C-reactive proteins). Three maternal risk factors independently accounted for the majority of reduced Hb levels in their offspring; i) sTfR >8ug/ml (-0.73 gm/dL reduction in Hb levels, p=0.04), ii) malaria (-0.80 gm/dL Hb reduction, p=0.02), and iii) STH (-0.79 gm/dL Hb reduction, p=0.03). The other maternal risk factors were not significantly associated with low in Hb levels in their children. Thus iron deficiency anemia arising from malaria and/or STH in mothers' results in reduced iron stores in their offspring and significant anemia. Improved iron supplementation and prevention of malaria and STH infection in pregnant women could have a profound impact on their child's health.

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MAPPING AND MANAGEMENT OF A LARGE SCALE DROUGHT-ASSOCIATED SCABIES OUTBREAK IN ETHIOPIA

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The impact of the severe drought in Ethiopia, attributed to El Niño weather conditions, has led to increase in the potential for outbreaks of communicable diseases. In September 2015, reports from drought affected regions indicated that drought affected areas were experiencing scabies on a very large scale. Given the likelihood that living conditions to be compromised by the drought, and reports of major social and personal impacts of scabies, we undertook a comprehensive assessment of scabies prevalence to plan interventions. Training was given to the health extension workers (HEW), who provide front-line community health services at the subdistrict level. A resource containing diagnosis and management guidelines was developed and distributed. The HEW were asked to conduct a house to house survey, and collect data using a simplified tool on the prevalence of scabies. Specificity of scabies diagnoses made by the health extension workers has been assessed. Ivermectin based treatment management has been done. A total of 450 HEW were trained in the 3 zones, and undertook scabies screening in a population of 1,125,770 across 68 districts. The prevalence of scabies across districts ranged from 0.2 to 60.7%., with 379,000 overall confirmed cases for a total prevalence of 33.7%. In the specificity assessment, the diagnosis of scabies made by the HEW in 251 cases was reviewed and 248 confirmed to have scabies (98.8%). The mean reported duration of illness was 5 months. Severe scabies was found in 42% of those with scabies, and 75.1% of cases had another family member scabies. Of all scabies cases, 39%were school aged children and 30 % of affected children had bacterial super infection. 11% of the students with scabies had dropped out from school because of scabies or/and drought, and 85% of those who dropped out had bacterial super infection. Treatment has been given to 800,000 patient and contacts. In conclusion, the scabies burden in the region is enormous, and complicated by the nutritional shortage emergency and water scarcity. A coordinated response is urgently needed to control the epidemic.

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PROGNOSTIC CLINICAL INDICATORS FOR FATAL DENGUE IN TWO ENDEMIC AREAS OF COLOMBIA: A HOSPITAL-BASED CASE CONTROL STUDY

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The WHO estimates that about 98% of dengue fatal cases could be prevented; however, countries such as Colombia have recorded higher rates during recent epidemics. Our aim was to identify predictors of mortality that allow risk stratification and timely intervention of dengue patients. We conducted a hospital-based, case-control (1:2) study in 2 endemic areas of Colombia (2009-2015). Fatal cases were defined as having one the following: 1) positive serological test (IgM); 2) positive

virological test (NS1 or RT-PCR or viral isolation); 3) autopsy findings (macro and microscopic) compatible with death from dengue. Controls were inpatients with a positive serological or virological dengue test (frequency matching by state and year). Exposure data at admission and during hospitalization were extracted from medical records by trained physicians. We used multiple regression methods (adjusting for age, sex, and disease's duration) to estimate the association between exposures and the case-control status. We evaluated 110 cases and 217 controls (mean age: 35.0 vs. 18.9 [p<0.001]; disease's duration pre-admission: 5.9 vs. 6.7 days [p=0.562]). History of previous hospitalization (27.9% vs. 11.2%) and hypertension (17.8% vs. 1.4%); respiratory distress (38.5% vs. 5.2%) and impaired consciousness (32.1% vs. 20.6%), were more frequent in cases than controls (p<0.05). In a model based on medical history, hypertension but not diabetes increased the odds of mortality (OR: 12.3; 95%CI: 1.41, 108.3). Further, a model that included respiratory distress (OR: 9.54; 95%CI: 2.56, 35.5), impaired consciousness (OR: 3.72, 95%CI: 1.18, 11.7), and heart rate (OR: 1.54, 95%CI: 1.37, 1.74 [per 5 bpm]) at admission had excellent predictive accuracy (AUC: 0.94, 95%CI: 0.90, 0.98). During hospitalization, controls but not cases showed increments of systolic (1.7 vs. 0.7 mmHg/day, p=0.045) and diastolic blood pressure (1.5 vs. -0.1 mmHg/day, p<0.001), as well as platelet count (11,605 vs. -333 per mL/day). Our results highlight the importance of medical history and easily measured clinical indicators in triaging dengue patients for mortality in endemic areas.

1702

AN EVALUATION OF MOLECULAR DIAGNOSTIC TOOLS FOR TRAVELERS' DIARRHEA: THE HOSPITAL FOR TROPICAL DISEASES EXPERIENCE

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Travellers Diarrhoea (TD) is the most common illness reported in returned travellers to the UK. A wide range of pathogens including bacteria, viruses and protozoa are responsible for TD. There are limited UK-based data on the aetiology of TD in our returned traveller population. The highly sensitive and specific detection and quantification of pathogen-specific nucleic acids in faeces, alongside cost-effective high throughput screening, makes PCR desirable as a diagnostic tool in ascertaining the aetiologic agents and informing the clinical diagnosis of TD. We present the first UK-based data investigating the aetiology of TD in travellers presenting to the Hospital for Tropical Diseases walk-in travel clinic. The objectives of the study were to evaluate the utility of an enteric pathogen PCR panel developed by Public Health England to investigate the aetiology of TD compared to standard microscopy and culture-based methods. A total of 124 unique stool specimens collected from immunocompetent patients presenting with symptomatic TD were processed using single and multiplex PCRs. Gastrointestinal pathogens were detected in 52% (64/124) of stool samples. The primary pathogens causing TD in this cohort were Giardia lamblia (12%) and norovirus (12%), followed by enteropathogenic *Escherichia coli* (EPEC) (4%), enteroaggregative *E. coli* (EAEC) (4%), Shigella spp. (2%), rotavirus (2%) and Entamoeba histolytica (1%). PCR diagnostics identified nine extra cases of giardiasis (increasing case detection from 5% to 12%) and a single case of amoebic dysentery. Use of PCR allowed identification of the viral causes of TD and showed greater sensitivity in the identification of parasites, which has public health and clinical implications. In conclusion, PCR diagnostics improved the detection of enteropathogens, allowing better assessment of the aetiology of TD. These important pilot data show that there is clearly a role for the routine use of molecular diagnostics in the clinical assessment of TD in the UK.

1703

RAPID, AUTOMATED EXTRACTION AND PURIFICATION OF NUCLEIC ACIDS FROM PATHOGENS DIRECTLY FROM WHOLE BLOOD SAMPLES

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Syndromic, PCR-based diagnostics that identify multiple pathogens in a single sample are quickly replacing the time-consuming gold-standard methods of culture, plating, and microscopy. While whole blood is an ideal sample type to use with this technology due to its richness in diagnostic targets, it is also rich in PCR inhibitors. Thus, whole blood samples typically require extensive sample processing and nucleic acid extraction before analysis. We developed the FilmArray® System (BioFire Diagnostics) to automate and integrate sample processing and nucleic acid extraction with nested multiplexed PCR to identify multiple pathogens in a single sample. Presently, there are four FDA-cleared FilmArray® panels that directly test nasopharyngeal secretions, blood culture media, stool, and cerebral spinal fluid. We are currently working to expand our first whole blood-based product, the Biothreat-E test for Ebola virus, to include other pathogens that cause febrile illness. We report the efficacy of FilmArray® sample preparation for a variety of intracellular and extracellular blood pathogens including Gram-positive and Gram-negative bacteria, parasites and viruses simultaneously from a single 200 µL whole blood sample. In addition, the Injection Vial allows dried blood spots to be used directly in the system without any added extraction steps. In whole blood, the estimated LOD was 1 x10^3 CFU/mL for *E. coli* and S. agalacatiae, and between 1 and 15 TCID50/mL for enterovirus, human parechovirus and herpes simplex virus 2. Extraction efficiency was between 25-90% depending on organism and was comparable to stand alone extraction systems. PCR inhibition was undetectable or at a low level that did not affect sensitivity. Additional evaluation with more diverse pathogens is ongoing and includes the causative agents of malaria, chikungunya, Zika, dengue fever, visceral leishmaniasis, leptospirosis, and Salmonella. This abstract contains data that have not been reviewed by regulatory agencies.

1704

PREVENTING *MYCOBACTERIUM LEPRAE* - ASSOCIATED DISABILITY: IDENTIFYING SOCIAL AND CLINICAL FACTORS ASSOCIATED WITH NERVE DAMAGE IN AN ENDEMIC AREA OF BRAZIL

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Hansen's disease (leprosy) remains a significant cause of morbidity and disability, with India and Brazil carrying the highest number of cases worldwide. *Mycobacterium leprae* infection affects skins and nerves and can cause permanent disabilities, which can have lasting effects on individuals' health and productivity. Addressing these preventable outcomes from various angles is crucial. We hypothesize that socioeconomic variables, such as income, occupation, and education level are associated with disability in patients with Hansen's disease (HD). Between July and December 2015, we enrolled patients at an HD reference clinic in Belo Horizonte, Minas Gerais, Brazil to identify variables associated with morbidity of HD. Patients with multibacillary disease were recruited, a questionnaire on several demographic & socioeconomic variables administered, and data abstracted from the medical chart. A

cross-sectional analysis was performed to determine associations with Grade 1 or 2 nerve disability according to World Health Organization (WHO) criteria. Seventy- three patients were enrolled (73% male). The majority of patients had nerve damage with Grade 1 disability found in 19 (26%) patients and Grade 2 in 29 (40%). On univariate analysis, older age (p=0.048) and lower education levels (OR = 5.4; 95% CI 1.4, 22.9) were associated with disability. Occurrence of reactions, clinical type of HD and other clinical and demographic variables were not found to be associated on preliminary analysis. Overall, our patients had a high burden of nerve damage consistent with prior studies in endemic areas. Additionally, older age and lower education were associated with disability grades of 1 or 2. While these findings are also consistent with other studies, overall data, to date, are limited and most of the literature has focused on clinical risk factors. These findings, along with planned multivariable analyses that may uncover other associations, will add to the body of knowledge on social factors associated with disability. This can then lead to strategies to target at-risk groups to reduce the burden of disease from this debilitating infection.

1705

NODDING SYNDROME/EPILEPSY IN THE SANAGA RIVER BASIN (CAMEROON): AN UNNOTICED EPIDEMIC?

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Nodding Syndrome (NS) is a severely debilitating form of epilepsy affecting children between the ages of 5 and 15 years in Northern Uganda, South Sudan and Tanzania. Unconfirmed cases of head nodding have also been reported in Cameroon and the Democratic Republic of Congo. The cause of the disease is unknown and there is no cure. Evidence suggests that there is an association between epilepsy/NS and onchocerciasis. However, relevant factors for the development of the condition are urgently needed. In order to explore these further, ethnographic research was carried out in 5 villages in the onchocerciasis-endemic Mbam Valley of the Sanaga river basin, Cameroon. Participant observations, in-depth interviews, informal conversations and focus group discussions suggested that there was a sharp increase of epilepsy about 40 years ago. Reports from older residents (50 years +) showed that epilepsy was uncommon during the 1970's, and that its prevalence increased dramatically during the 1980's and has decreased in recent years. These findings suggest the existence of environmental or social triggers for the occurrence of epilepsy and potentially NS. Relevant factors may be: (i) the construction of dams upstream of the study area (affecting the seasonal population dynamics of blackflies, and increase the transmission of onchocerciasis); (ii) changes in the patterns of human-water contact; (iii) changes in climate (rainfall and temperature); (iv) changes in nutritional habits and the variety of foods available; (v) the annual mass ivermectin treatment through the African Programme for Oncocerciasis Control (APOC) launched in the late 1990's. These factors may be key in determining the reported sudden occurrence of epilepsy/NS and need to be assessed further to contribute to the identification of the causes and conditions under which NS develops and becomes epidemic in certain locations and at specific times.

1706

HELMINTHS AND UNDERNUTRITION: FACILITATORS OF *MYCOBACTERIUM LEPRAE* MORBIDITY OR INNOCENT BYSTANDERS?

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While 30-50% of patients with Hansen's disease (HD) suffer from immunological Type 1 and Type 2 reactions that can lead to irreversible nerve damage, large gaps in knowledge exist about susceptibility to these complications. We hypothesize that helminthic co-infections and micronutrient deficiencies may be risk factors for reactions. Between July and December 2015, we performed a pilot case-control study at an HD clinic in Belo Horizonte, Minas Gerais, Brazil. Adult patients with multibacillary disease were recruited and were considered cases if they had an active Type 1 (T1R) or Type 2 reaction (T2R) or controls if free of reactions. Data were abstracted from the medical chart, and a demographic questionnaire was administered. Stool was collected for ova and parasite testing and venipuncture was performed for *Schistosoma mansoni* serology, complete blood count, C-reactive protein, Vitamin D level, and biomarkers for iron and vitamin A status. Statistical analyses were performed with adjusted odds ratios calculated for T1R and T2R as separate outcomes, controlling for age, sex, race socioeconomic status, rural residence, type of clinical HD, bacillary index, presence of anemia, other co-infections and smoking status. Seventy-three patients were recruited with 73% male and an average age of 51.2 years. Helminth infections were found in 4 patients with reactions and 1 patient without reaction, with total prevalence of 6.9%. Helminth co-infections were not found to be associated with T1R (aOR =3.5; 95% CI 0.17, 73.15) nor T2R (aOR = 0.07; 95% CI <0.001, 80.49). Micronutrient results are pending. While this pilot study did not show a statistically significant association with helminth infections and reactions, the total numbers of co-infections were small. Given the overall prevalence of low socioeconomic status, micronutrient deficiencies may play a role in the risk of reactions in our study. The nutrition results, future epidemiologic studies on co-infections in areas with higher helminth endemicity and immune studies hold promise in identifying strategies to reduce the significant morbidity of reactions in susceptible populations.

1707

CHITOSAN MICROPARTICLES TO DNA DETECTION IN URINE SAMPLES

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Chitosan is the second most abundant natural polymer in nature, derived by partial deacetylation of chitin. Chitin is part of the support structure of many living organisms, such as arthropods (crustaceans and insects), mollusks and fungi. Chitosan is being widely studied because of their advantages of biocompatibility, high charge density and non-toxicity. During the last years, has been reported the use of chitosan particles has the ability of association to peptides, proteins, oligonucleotides, due to the abundance of amino groups in its structure, thus allowing adsorption. Urine is a valuable non-invasive sample, studies report the presence of DNA fragments in urine, however the low concentration is not detectable by conventional methods, an alternative is the use of chitosan biopolymer to concentrate the small amount of nucleic acids and their future application in the diagnosis of infectious diseases. We infected

urine samples with DNA and applied the particles chitosan, previously we analized the pH interaction with DNA, we evaluated by PCR in real time. Chitosan particles has efficient to capture DNA .We hope to find the use of the particles could be used as a biomarker through DNA in urine samples.

1708

ASSESSING POSSIBLE EXPOSURE TO ZIKA VIRUS IN A HOSPITAL POPULATION THROUGH A TRAVEL SCREENING QUESTION

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Responding to possible exposures to infections with potential importation, Mount Auburn Hospital incorporated a travel screening question "Have you traveled outside the US within the past 30 days" into patient registration for each visit. We describe characteristics of patients registered from November 1, 2015 to January 31, 2016 who responded "Yes" to estimate the number of patients potentially exposed to Zika virus. De-identified data collected prospectively in MIDAS database is analyzed. Services are in 5 categories: 1) Inpatients, 2) ED/ Walk-In Center, 3) Travel clinic 4) Other outpatients, and 5) Laboratory/ radiology. Destination countries are categorized by Zika transmission: 1) Confirmed autochthonous transmission in past 9 months [ECDC 041516], 2) Documented prior presence (virus or serologic evidence), and 3) No documented Zika. All analyses are performed using SPSS 17.0. Of 1267 total visits, 1065 with complete destination and diagnosis data were included.The mean age was 47y, 62% were female, 83% reported English as primary language, and 75% were white. The most common contact point was diagnostics (radiology/lab; n=584). Seventeen percent registered to the ED/ Walk-in clinic. Thirty-eight patients were admitted (3.6%). Top destinations were Canada (n=132), Mexico (n=79) and France (n=78).310 patient visits reported travel to countries with Zika transmission (29%). The Caribbean was the most visited region with active Zika transmission (n=168). Among 660 female patient visits, 51% (n=341) were of child-bearing age (15-49y; WHO criteria). Ninety-seven of 341 (28%) patient visits reported travel to an active Zika transmission area. Fifty-three visits were by pregnant women. Eighteen pregnant women had visited Zika transmission area (1st trimester=12; 2nd trimester=4; trimester unknown=2).A travel screening question at patient registration allowed for analysis of potential Zika exposure. We found over a quarter of patients had visited Zika transmission areas. A significant number of women were of child-bearing age and most pregnant women who visited Zika areas were in their first trimester.

1709

PODOCONIOSIS: GENETIC PREDISPOSITION NORTHERN PROVINCE, RWANDA, AFRICA

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Imididio Project, Musanze, Rwanda

Non-filarial elephantiasis (also known as podoconiosis) is a noninfectious neglected tropical disease caused by prolonged exposure of bare feet to irritant volcanic soils. It is a disabling and debilitating condition that left untreated can lead to the severest stage of lymphedema (elephantiasis). The Imidido Project based in Musanze Town, Rwanda, Africa has been providing care for those suffering with Podoconiosis since 2009. Through data gathering during the clinic registration process, the research indicates that there is a genetic predisposition to susceptibility of acquiring this condition through chronic exposure to irritant volcanic soils. To date, we have registered 342 patients with Podoconiosis in various stages of advancement within the Northern Province of Rwanda Africa. Of these 342 patients, 183 (53.5%) indicated there were other family members that were suffering from this condition, including grandparents, parents, and siblings. The remaining 159 (46.5%) patients reported no familial link to the condition. The genetic linked group of high risk individuals has been a focus of our prevention education program in an effort to reduce the number of new cases in Rwanda, Africa.

1710

SYMPTOMS AND CLINICAL CORRELATES AS A PREDICTIVE MARKER FOR THE OUTCOMES IN TROPICAL FEVER: A TWO YEAR RETROSPECTIVE STUDY FROM CENTRAL INDIA

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There has been a resurgence tropical fever in India. Management protocols at our specialist centre have been aligned to the tropical fevers management guidelines issued by Indian Society of Critical Care Medicine. We aim to study the trends in symptomology and relate with predictive outcomes. We retrospectively evaluated the cohort of tropical fever patients including dengue, malaria, rickettsial infections, leptospirosis, typhoid, bacterial sepsis and viral infections. The syndromic approach with presenting feature as acute undifferentiated febrile illness was utilised to identify patients from the SCOUT integrated database of our tertiary care hospital. ANOVA was used for stratified analysis. Symptoms and outcomes were retrospectively corroborated as a predictive tool across 281 patients admitted during 2014-15. Year 2014 n= 180, 2015 n=101, Males n = 182, Females n=99. Overall, mortality rate was 4.62% (n=13; 2014 n=7, 2015 n=6). 38.7% (n=109) were within 20 km vicinity and included treatment naïve patients. Majority (n=172) were referred from remote, rural geography and had already received primary care for the severe grade symptoms. Stratified analysis was performed across the duration of hospital stay, age, symptoms, GCS on admission, diagnosis, complications ($p<0.0001$). Hospital stay was < 10 days in 89.6% of patients, 49.8% were < 5 days in hospital. Mortality rate was 6.42% with length of hospital stay of <5 days Vs 17% in 16-20 days. Patients with breathlessness at admission had highest mortality 20% (n= 6/30), followed by cough and cold 6.4% (n=2/31), headache 5.6%, vomiting 5.38%, abdominal pain 5.17%. 95% (n=267) had GCS > 8 at time of admission with mortality rate of 4.12%. ($p<0.0001$). Highest mortality (29%) was noted in dengue with IgM/IgG (2/7) followed by *Plasmodium vivax* 20% (1/5). Patterns and the outcomes of tropical fever could be the benchmark for the rest of the country. The early triage of tropical fever patients could optimise the utilisation of the hospital resources. Symptoms at hospital admission with the clinical correlates could be an important predictor for outcomes.

1711

COMPREHENSIVE CARE OF CHAGAS DISEASE IN A NON-ENDEMIC COUNTRY: THE EXAMPLE OF SPAIN

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Spain is the most affected country of Chagas disease (CD) in Europe, and the second non-endemic country globally (after US). Europe still faces an underdiagnosis of 90%. Among population from endemic areas, lack of knowledge, stigma and fear are still linked to CD. Community health activities are needed in order to reach population at-risk (par) and to overcome barriers for diagnosis and treatment. Activities have been performed synergistically by several institutions/organizations, in different Spanish regions. Highlighted: - CD patients' associations since 2008: Barcelona (ASAPECHA), Valencia (ASAPECHAVAE) and Murcia (ASAPECHAMUR). - Catalonian Expert Patient Program® on CD: initiative started in 2011 within the Chronic Disease Program. It aims to boost responsibility of patients for their own health and to promote self-care. Results: 15 participants completed the program. Knowledge about disease improved after sessions. - Mothers committed to Chagas' disease: taking action here and there®: community health workers (CHW) specialized in CD's training program, performed in Madrid in 2013. A second edition is currently in progress. Results (2014-2015): 1,401 par informed (185 in Bolivia); 60 par phoned the free-phoneline (900 103 209), asking mainly where to go for testing; 50 accompanied to the consultation by CHW; more than 7,000 received informative material. - Community screening campaigns performed in non-clinical settings on Sundays, on the occasion of CD International Day or Bolivia National Day's celebrations. Prior to the event, intense communicational campaigns are led by CHW and patients' associations. Results (2012-Feb. 2016): 3,474 par were screened in Barcelona, Madrid, Valencia, Murcia and Alicante; 775 were positive (CD prevalence 22.3%). - Access to treatment. 2013-2015: more than 4,000 treatments were administered among 155 healthcare centers all over the country. CD requires interdisciplinary approach including prevention, control, strategies and programs, being CHW and patient's associations key factors. Spain has reduced underdiagnosis and offers comprehensive care for CD patients.

1712

IMPACT OF MICRONUTRIENT SUPPLEMENTATION COMBINED WITH MALARIA CHEMOPREVENTION ON MALARIA, ANAEMIA AND COGNITIVE DEVELOPMENT IN EARLY CHILDHOOD: FINDINGS FROM A CLUSTER RANDOMIZED STUDY IN SOUTHERN MALI

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Early childhood is a time of rapid growth and development and public health interventions during this period could yield substantial benefits across several developmental areas: physical, cognitive and linguistic. Iron is important in brain function, and interventions that reduce iron-deficiency and anemia may improve cognitive function and learning. A randomized intervention study was undertaken to examine the combined impact of two newly-recommended interventions in early childhood: seasonal malaria chemoprevention and home fortification with micronutrient powders. Although each intervention has previously been shown to improve malaria morbidity, anemia and/or physical growth in children, the impact of combining these two complementary interventions is not known. No previous studies have examined effects on cognitive and linguistic development. A cluster-randomized controlled study of this combined strategy has been carried out in 60 rural communities in southern Mali since 2013. Children aged less than 5 years living in the 30 intervention communities receive seasonal malaria chemoprevention during the months of peak malaria risk, followed by daily supplementation of micronutrients for four months each year. Children living in control communities receive seasonal malaria chemoprevention only. The impact of the combined intervention after three consecutive years of implementation will be evaluated in May-June 2016 through cross-sectional surveys to compare malaria infection, nutritional indices and cognitive performance in children aged 3 and 5 years living in intervention and control communities. The results of this evaluation will be presented and discussed.

1713

A COHORT STUDY TO ESTIMATE THE RISK MERS-COV POSES TO TRAVELERS TO THE MIDDLE EAST

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Since its appearance in 2012, the Middle East respiratory syndrome coronavirus (MERS-CoV) has emerged as a serious public health threat of global concern. As of September 2015, the World Health Organization has been notified of 1,626 laboratory confirmed cases and the case fatality rate is estimated at approximately 36%. Beyond its high fatality rate, significant concern lies in the potential for MERS-CoV to spread beyond the Middle East, as was recently witnessed in the Republic of Korea which saw an outbreak of 185 confirmed cases and reported 36 deaths. The

spread of MERS-CoV may be facilitated by high population mobility and mass gatherings such as the Hajj pilgrimage that an estimated two million Muslims make each year to the region most impacted by the virus. Indeed, two of the most frequently visited cities during the Hajj (Mecca and Medina), have contributed nearly 10% of the known cases to the present epidemic. Despite the global concern over the virus and its potential for spread, many questions about MERS-CoV remain unanswered such as the number of asymptomatic cases that go undetected by current surveillance activities. Here, we describe an established multi-year cohort of pilgrims departing for Hajj from Malaysia, a country that sees an annual average of 20-25,000 Muslims make the pilgrimage each year. Within this cohort, pre- and post-pilgrimage serological analysis is paired with questionnaire data to estimate the risk of exposure to MERS-CoV during Hajj and assess its potential to spread beyond the Middle East.

1714

ROTAVIRUS AMONG MEDICALLY-ATTENDED CHILDREN YOUNGER THAN FIVE YEARS OF AGE WITH AND WITHOUT DIARRHEA IN LIMA, PERU FOLLOWING UNIVERSAL ROTAVIRUS VACCINE IMPLEMENTATION

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Before the monovalent vaccines Rotarix™ was added to the national immunization program in 2009, rotavirus A (RVA) was the leading cause of acute gastroenteritis among children in Peru. Although vaccination confers a high level of protection against several genotypes, continued monitoring of the prevalence of circulating strains that could affect vaccine efficacy is recommended. A case-control study was conducted in children younger than five years of age with and without diarrhea seeking medical care at Instituto Nacional de Salud del Niño in Lima, Peru between October 2013 and May 2015. Clinical data was gathered to determine the severity of gastroenteritis episodes, and stool samples were collected. Presence of stool RVA was determined using RT-qPCR, and positive samples were genotyped by a multiplex hemi nested PCR assay. During the study period, 1032 children (757 diarrhea cases 275 and controls) were analyzed. A total of 87.9% participants received the complete vaccine series. Prevalence of RVA was higher among cases (8.7%) than controls (3.6%) (p=0.0086). The emerging heterotypic G12P[8] was the most prevalent (54.8%) genotype. Among cases, no difference in the clinical severity, using either the Vesikari or Clark scales, was observed between RVA positive and RVA negative children. RVA infection remains an important cause of acute gastroenteritis among Peruvian pediatric populations. The identification of new circulating genotypes and their association with more clinically severe symptoms should continue to be evaluated.

1715

PREVALENCE AND ASSOCIATED RISK FACTORS OF DIABETES, CHRONIC KIDNEY DISEASE, HYPERTENSION AND OBESITY IN THE PERUVIAN AMAZON: THE AMARAKAERI RESERVE COHORT STUDY

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The Peruvian Amazon is in the midst of an epidemiological transition. Large urban population centers are growing rapidly, with non-communicable diseases (NCDs) beginning to outpace incidence of infectious diseases. However, in rural and peri-urban environments, population-level disease burdens are undefined with both infectious and NCDs being reported in increasing numbers. In this study, we determine the prevalence of NCDs in rural areas of the Peruvian Amazon to observe differences in prevalence between males and females and between native and non-native communities, and to identify risk factors associated with hypertension, obesity, diabetes, and chronic kidney disease (CKD). We conducted a cross-sectional study of 2,268 randomly selected adults (18-96 years old) in 1,122 households in communities surrounding the Amarakaeri Communal Reserve in the southern Amazon region of Madre de Dios. Disease prevalence was estimated using a finite population correction. WHO/ISH risk prediction charts were used to indicate the 10-year risk of a fatal or non-fatal major cardiovascular event. Comparing males vs. females, prevalence rates were: 7.0% vs. 1.8% for hypertension; 19.7% vs. 35.7% for obesity; 1.7% vs. 2.5% for diabetes; and 10.2% vs. 3.3% for CKD. Significant differences between males and females were detected for obesity, hypertension, and CKD prevalence. Among adults positive for diabetes or hypertension, 30.7% self-reported having diabetes, and 18.6% having hypertension. Multivariate analyses indicate that education, sex, and community location were important risk factors for each of these NCD outcomes. Physical activity and waist circumference were additional risk factors for hypertension and obesity. Only 3% of the population was at moderate or high risk for a major cardiovascular event. NCD burden is high and differential among males and females. Risk factors identified in this region indicate that disease burden may increase and have severe cardiovascular consequences. As many of these risk factors are modifiable, interventions should be implemented immediately to lower the NCD burden in this region.

1716

DEVELOPMENT AND PRELIMINARY CLINICAL EVALUATION OF A MOBILE TECHNOLOGY FOR DIARRHEAL DISEASE OUTBREAK MANAGEMENT

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The emergence of mobile technology offers new opportunities to improve access to clinical guidelines, especially in resource-limited settings during acute health crises. We conducted a multi-year design initiative to determine how best to adapt diarrheal disease outbreak management guidelines to smartphones. End-user design sessions with medical staff in rural Bangladesh resulted in the development of a rehydration calculator for use during initial resuscitation and a surveillance platform for real-time syndromic reporting and data visualization. The calculator was evaluated in a pre/post pilot study at a generalizable government district and sub-district hospital in Northern Bangladesh in an area prone

to cholera outbreaks (Netrokona). Inclusion criteria were patients with uncomplicated diarrheal disease (>= 3 loose stools per 24 hours) and an age >= to 2 months. The baseline and interventional arms were six weeks each. The primary outcome was adherence to guidelines for antibiotics (azithromycin for moderate and severe dehydration for suspected cholera), zinc (<5 years) and intravenous (IV) fluids. A total of 327 and 521 patients were enrolled during the baseline and interventional arms. For the district and sub-district sites, guideline adherence increased for antibiotics (13% to 82%, p<0.01; 63% to 99%, p<0.01, respectively), zinc (82 to 89%, p>0.01; 91 to 98%, p>0.01; respectively) and the use and administration of IV fluids. No adverse events related to the intervention were detected during admission and at 10-days post discharge. The surveillance platform (aka Outbreak Responder) was durable and reported clinical and laboratory endpoints in real-time. A randomized control trial is in development to accommodate for study limitations that included the lack of an independent control and implementation challenges. In this study, we report the successful technical first steps towards a smartphone-enabled platform for diarrheal disease outbreak management.

1717

DIFFERENTIAL CLINICAL AND LABORATORY CHARACTERISTICS AMONG ADULT DENGUE PATIENTS WITH DIABETES

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Dengue results in significant public health burden globally. It is usually a self-limiting disease, but about 1-5% of those symptomatic dengue infections results in dengue hemorrhagic fever, dengue shock syndrome (WHO 1997) or severe dengue (WHO 2009). Diabetes has been significantly associated with severe dengue progression. However, there is a lack of understanding of the differential clinical, laboratory, and immunological characteristics of these high risk group of dengue adult patients at presentation and during hospitalization, that potentially may provide insights in the disease pathogenesis of the virus and provide guidance on improved clinical management. Dengue patients with diabetes were significantly associated with hypertension, hyperlipidemia and severe dengue outcome. Several warning signs such as abdominal pain, clinical fluid accumulation and hematocrit rise and rapid platelet count drop were significantly associated with dengue patients with diabetes. Levels of white blood cells and neutrophils were significantly associated with dengue patients with diabetes. Immunologically, several chemokines and cytokines were significantly associated with dengue patients with diabetes. In conclusion, dengue patients with diabetes may have different immune responses against dengue virus, resulting differential clinical manifestations and disease severity. Much more immuno-pathogenesis studies are still required to provide understanding of how diabetes pre-dispose a patient with severe dengue outcome.

1718

ANTHELMINTHIC SCREENING FOR PARASITIC NEMATODES

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For many parasitic diseases, high-throughput phenotypic screening is an important tool in finding new drugs. Some of the most important parasitic diseases are caused by nematodes. However, these parasitic nematodes are not typically amenable to high throughput screening. Due to the ease of its maintenance and suitability for high throughput assay, the nematode *Caenorhabditis elegans* is instead used. To address whether *C. elegans* is a good model for nematode drug discovery, we compared the drug susceptibility of *C. elegans* relative to the human hookworm nematode parasite *Ancylostoma ceylanicum* at several developmental stages using a library of FDA approved drugs. I will present results of these studies that point to how well *C. elegans* efficacy correlates with hookworm efficacy and how early larval stages (easier to get) correlated with adult stages (more representative of what stage is targeted in human therapy). In addition, we are working on moderate-high throughput system for screening adult parasites. Using Union Biometrica, Copas, worm sorter we were able to sort adult parasites into 384 well format. Here I will discuss the capabilities of this system as well as how we are building de novo, in collaboration with the Albrecht laboratory at WPI, an imaging and image analysis platform for screening adult parasitic nematodes against large drug libraries.

1719

WHAT'S IN A NATIONAL PLAN OF ACTION? EVALUATING PROGRESS TOWARD GLOBAL CONTROL OF SOIL-TRANSMITTED HELMINTHIASES?

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Soil-transmitted helminthiases (STH) are estimated to affect more than 2 billion people worldwide. In effort to minimize this disease burden, the World Health Organization (WHO) has outlined milestones to monitor the progress of global STH control. Specifically, WHO has advocated for the development of national plans of action (PoA) for integrated control of neglected tropical diseases (NTDs) by all countries requiring preventive chemotherapy (PC) for STH by 2015. To measure progress on this indicator, we collected national PoA from WHO, national programs, and implementing partners. WHO indicates that 84 national PoA exist; of these, we were able to confirm and analyze 41, representing 40% of the 102 countries requiring PC for STH in 2014. All available PoA included STH control. Yet, a substantial proportion failed to address key roles for intersectoral collaboration with education, water and sanitation, and nutrition sectors. Although WHO recommends deworming to reduce morbidity from STH in both preschool-age children (PSAC) and school-age children (SAC), the majority of available PoA did not address PSAC as a target population. Our findings suggest that developing a national PoA is an effective step in STH control. Of countries with reported STH treatments for SAC in years prior to and during an active PoA (N=33), the average national coverage increased by 15.4% (95% CI: 6.8 - 24. 0%) under PoA implementation. However, even with this increase, only 16 of the 41 countries with available PoA reported coverage greater than 75% in the year 2013 or 2014. Our analysis is limited by the difficulty in collecting PoA. However, to date, this is the first collective review of available PoA for integrated control of NTDs. Most notably, our review suggests that if WHO milestones on STH control are to be met, improved efforts in developing and updating national PoA may be required.

1720

PREVALENCE OF MALARIA, GEOHELMINTHS AND ANAEMIA AMONG SCHOOL CHILDREN IN MUHEZA DISTRICT

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Malaria and intestinal helminths are an important public health issue with malaria-geohelminths co-infections commonly occurring in school aged children. A consequence of these co-infections in humans is anaemia. This cross sectional study aimed to determine the prevalence of malaria, geohelminths, co-infections and anaemia and associated factors among school children. The prevalence of malaria was 21.5% (82/381), (95% CI: 20.5 to 24.1) geohelminths (6.7%) 26/387 (95%CI: 8.4 to 12.9), co-infections (malaria-geohelminths) (1.8%) 7/381 (95%CI: 1.7 to 2.1) and anaemia was (39.1%) 149/381 (95%CI: 37.2 to 51.7). Non-use of insecticides treated nets (aOR 4, 95% CI: 2.24 to 8.51 P= 0.0012) was associated with malaria infection. Eating unwashed raw food (aOR 2.9,

95%CI: 1.9 to 9.2 P-value = 0.032) and not washing hands before eating (aOR 5.81, 95% CI: 1.92 to17.54 P-value =0.0002) were associated with geohelminth infections. Malaria and anaemia are prevalent in the study area while geohelminths and co-infections among school children are low. Further studies are required to explore the reasons why primary school children do not use insecticide treated nets and hygienic practice.

1721

CRYSTAL PROTEIN CRY5B AS A NOVEL AND POWERFUL ANTHELMINTIC

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Soil-transmitted helminths (STHs), most notably, hookworms, whipworms, and *Ascaris*, are nematodes that infect more than 1.5 billion of the poorest people and are amongst the leading causes of morbidity worldwide. Only two classes of de-worming drugs (anthelmintics) are available for treatment, and only one is commonly used in mass drug administrations. New anthelmintics are urgently needed to overcome emerging resistance and to produce higher cure rates. Crystal (Cry) proteins, in particular Cry5B, made by *Bacillus thuringiensis (Bt)* are promising new candidates. Cry5B has excellent anthelmintic properties against many free-living and parasitic nematodes, including *in vivo* efficacy against multiple STH infections in rodents (*Heligomasmidoes polygyrus* and *Ancylostoma ceylanicum*) and in pigs (*Ascaris suum*). An enormous challenge for STHs, very different from most diseases worked on in the developing world, is the requirement that therapies be very cheap (the people infected are very poor and current drugs costs pennies a dose), massively scalable (over 4 billion people are at risk from infection), and have a long shelf life in harsh environments, that have high temperature and humidity and no cold chain. We will update our progress in several key areas. We will present new data on the *in vivo* activity of Cry5B against a major human parasite of humans. We will also present data on the whether or not the immune system is required for Cry5B action *in vivo*. We will also present on our development efforts to produce a deployable version of Cry5B that is cheap, safe, scalable, and stable. These efforts are focused on bacterial engineering, expression, and formulation, and we believe we hit upon a novel bacterial expression system that meets these key requirements.

1722

PLANT DERIVED COMPOUNDS AS 'RESISTANCE-BUSTING" ANTHELMINTIC DRUG

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There is an urgent need for new therapies for parasitic helminthic diseases affecting 1.5-2 billion people worldwide due to the threat of wide-spread resistance development to existing treatments and due to problems of incomplete efficacies. Plants and plant secondary metabolites have been used historically to treat STH infections. Although they can be effective, we hypothesize that the active ingredients in plants may be absorbed prematurely, which limits their efficacy. Our hypothesis is that modern formulation techniques could be used to overcome limitations. We screened a number of plant extracts and metabolites for anthelmintic activity *in vitro* against adult stages of the hookworm and whipworm parasitic nematodes *Ancylostoma ceylanicum* and *Trichuris muris*. Here we will present results of this work, which shows the promising potential for some of these as pan-nematode anthelmintics. This work has allowed us to classify plant materials into at least two groups based on their *in vitro* killing kinetics. We have also shown that some are effective against an albendazole-resistant *Caenorhabditis elegans* strain suggesting that they may play an important role in overcoming helminthic drug resistance. We will also present our work on optimizing lead formulations *in vitro* and *in vivo* in animal models of parasitic nematode infection in order to overcome the challenges and realize the potential of "resistance-busting" plant-based anthelmintic therapies.

1723

INVESTIGATING THE DIFFERENTIAL IMPACT OF SCHOOL AND COMMUNITY-BASED INTEGRATED CONTROL PROGRAMS FOR SOIL-TRANSMITTED HELMINTHS IN TIMOR-LESTE: THE (S)WASH FOR WORMS PILOT STUDY

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Soil-transmitted helminth (STH) infections remain a significant global health issue, with an estimated 1.45 billion people infected worldwide. Water, sanitation and hygiene (WASH) interventions are thought to be important in sustainable STH control, alongside regular distribution of anthelminthic drugs. Currently, large-scale STH control programs are most often targeted to children, through school-based delivery systems. However, a recent meta-analysis shows greater reductions in STH prevalence in children following community-wide deworming, compared to child-targeted deworming. The (S)WASH for WORMS pilot study aims to compare the impact of school- and community-based integrated WASH and deworming programs on STH in school-aged children. This pilot study includes six remote communities in Timor-Leste. STH prevalence and intensity were measured in school-aged children at baseline in June 2015, using both a flotation-based microscopic technique and a quantitative PCR technique. All communities then received a WASH and deworming program at the primary school, and three communities additionally received a community-wide WASH and deworming program. STH prevalence and intensity will be re-evaluated in May 2016, six months after anthelminthic delivery. Cumulative incidence and intensity of STH infections at six months will be compared between the two study arms. At study baseline, 522/563 (91%) children present were recruited for the study. Stool samples were obtained for 483/522 (93%). STH prevalence was 37.7% (95%CI 33.2-42.3%) using microscopy, and 50.3% (95%CI 45.7-54.9%) using quantitative PCR. In summary, baseline results show that this pilot study achieved high participation rates and that STH are prevalent among school-aged children in Timor-Leste. Preliminary analyses suggest that quantitative PCR is more sensitive than microscopy for diagnosing STH infections. This presentation will discuss the pilot study in more detail, and will include final results comparing the two study arms.

1724

IS THERE EVIDENCE THAT THE SEASONAL TIMING OF MASS DE-WORMING FOR *ASCARIS* IS IMPORTANT?

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Regular mass treatment programs are commonly used in areas of high *Ascaris* prevalence. It is well known that that seasonal variables can affect maturation and mortality of *Ascaris* eggs, yet the potential implications for incidence, transmission and control are poorly understood. A recent field study of 477 individuals in Sri Lanka has shown significant correlation between *Ascaris* infection rates and a number of seasonal variables, including temperature. To demonstrate the implications that seasonal variation in climate could have for mass treatment, and the potential for intervention optimization, we have used statistical modeling techniques to simulate the results of seasonal timing of mass chemotherapy in different settings. Using historical experimental data on *A. suum* eggs to fit relationships between temperature and egg developmental parameters,

we find that the optimal temperature for Ascaris eggs lies in the range of 25°C-30°C. Higher temperatures facilitate egg development, but temperatures above 30°C show a steep drop in the proportion of eggs that survive. Using these relationships, a mathematical model is developed to represent seasonal mean worm burden in a population under various mass treatment conditions. We demonstrate the ability of this model to predict seasonal *Ascaris* prevalence using historical data from a study of approximately 600 individuals in Korea, across six neighbouring villages. Applying this model to prevalence data from the Sri Lanka field study allowed us to predict the outcome of mass treatment programs with different annual timing. Results suggest that tuning treatment timing could have significant consequences for program impact, with the optimal annual treatment date providing up to a 55% comparative decrease in prevalence after four treatment rounds. Whilst different seasonal patterns would give different results, this implies that we may have previously under-estimated the importance of seasonality in driving Ascaris infections. Further investigation into seasonal timing of treatment could result in long-term global implications for helminth control and elimination programs.

1725

SPONTANEOUS SEDIMENTATION IN TUBE TECHNIQUE IS AS SENSITIVE AS KATO-KATZ FOR THE DIAGNOSIS OF SOIL-TRANSMITTED HELMINTHS AND SUPERIOR FOR THE DETECTION OF *STRONGYLOIDES STERCORALIS*: A COMMUNITY-BASED STUDY IN THE AMAZON BASIN OF PERU

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Soil-transmitted helminthiasis (STH) constitute a major health problem especially in developing countries, where the lack of parasitologists and limited laboratory resources may be contributing factors to underestimate the burden of disease by these parasites. According to the WHO, Kato-Katz (K-K) method is the *gold standard* for the diagnosis of STH; however its sensitivity has been reported as low when compare to other methods. This study aimed to compare the sensitivity of K-K against Spontaneous sedimentation in tube technique (SSTT); a low-cost and rapid diagnostic technique for the diagnosis of STH. Fresh stool samples from residents of a rural community in the Amazon (Peru) were collected and analyzed by both techniques within 6 hours from emission. In addition, Agar plate culture was used as the *gold standard* for the diagnosis of *Strongyloides stercoralis*. One hundred seventy stools samples were collected in this study, mostly children. Overall, the prevalence of STH was 24.7%. In an individual analysis, the prevalence of these parasites by means of K-K or SSTT was as follows: *Ascaris lumbricoides* (12.4% vs. 13.5%), *Hymenolepis nana* (8.2% vs. 7.7%), *Trichuris trichiura* (1.2% vs. 1.2%) and hookworm (1.2% vs. 2.4%). Furthermore, the prevalence of *S. stercoralis* through K-K, SSTT and Culture was 0% vs. 4.1% vs. 10.6% ($p<0.001$). In conclusion, SSTT was as sensible as K-K for the diagnosis of STH and superior for the detection of *S. stercoralis* larvae in this specific population. Our results suggest that SSTT may be an alternative for the diagnosis of STH in tropical areas, and this finding warrants especial consideration, given the high prevalence of *S. stercoralis* in such regions. In addition, the SSTT may need further modifications in order to quantify the intensity of infection. Major advantages of this technique are the simplicity (no need for centrifuge) and low cost.

1726

POTENTIAL IMMUNOLOGICAL MARKERS FOR DIAGNOSIS OF HUMAN STRONGYLOIDIASIS USING HETEROLOGOUS ANTIGENS

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Strongyloides venezuelensis is a parasitic nematode of rodents that is frequently used to obtain heterologous antigens for immunological diagnosis of human strongyloidiasis. The aim of this study was to identify antigens from filariform larvae of *S. venezuelensis* for immunodiagnosis of human strongyloidiasis. Soluble and membrane fractions from filariform larvae of *S. venezuelensis* were obtained in phosphate saline (SS and SM) and Tris-HCl (TS and TM), and were analysed by western blotting (WB). Different antigenic components were recognized by IgG antibodies from the sera of strongyloidiasis patients. Highest recognition was observed for a 30-40 kDa band in all antigenic fractions. This band was then excised and subjected to mass spectrometry for protein identification. Immunoreactive proteins identified in the soluble fractions corresponded to metabolic enzymes, whereas cytoskeletal proteins and galectins were more abundant in the membrane fraction. Thus, these results represent the first step towards identification of *S. venezuelensis* antigens for use in immunodiagnostic assays for human strongyloidiasis.

1727

THE EFFECT OF MATERNAL POSTPARTUM DEWORMING ON INFECTION STATUS, ANEMIA AND FATIGUE

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Anemia and fatigue are common consequences of infection with intestinal parasites in women of reproductive age living in parasite-endemic areas. To date, no previous study has evaluated symptoms of fatigue in infected individuals using standardized scales. A randomized controlled trial conducted in 2013-2014 in Iquitos, Peru recruited 1010 mother-infant pairs. One objective of the study was to determine the effectiveness of maternal postpartum deworming on the prevalence and intensity of intestinal parasites, anemia, and fatigue in lactating women up to 6 months postpartum. Following delivery, women were randomly allocated to receive single-dose deworming (albendazole) or matching placebo. At 6 months postpartum, mothers provided stool specimens for detection of intestinal parasite infection, and finger-prick blood samples for assessment of blood hemoglobin concentration. The Fatigue Assessment Scale (FAS) was used to ascertain the self-reported presence of physical and cognitive symptoms of fatigue. A total of 970 (96.1%) participants attended their 6-month follow-up visit. The risk of parasite infection at 6 months postpartum was significantly lower in the group who received albendazole compared to placebo (RR: 0.5; 95% CI: 0.4, 0.6). At 6 months postpartum, no statistically significant benefit of deworming on maternal anemia (48.1% *vs.* 48.6%) or elevated fatigue (61.3% *vs*. 64.1%) was observed. Results were similar when analyses were restricted to mothers who tested positive for helminth infection at baseline. In the present study population, where baseline soil-transmitted helminth infection prevalence and intensity were low, deworming was highly effective at reducing the

burden of infection at 6 months postpartum; however, benefits in terms of maternal anemia or fatigue could not be detected at this time point. Further research is needed to determine which interventions, either during pregnancy or during the postpartum period, provide the most benefit to mother and infant at this critical time.

1728

SYSTEMATIC REVIEW AND META-ANALYSIS OF SOIL-TRANSMITTED HELMINTH TREATMENT EFFICACY STUDIES AND THE CASE FOR SHARING INDIVIDUAL PATIENT DATA

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In 2014, over 271 million schoolchildren were treated with benzimidazoles as part of the World Health Organization's plan to scale up mass drug administration (MDA) programmes targeting the soil-transmitted helminthiases (STHs) caused by roundworm, whipworm, and hookworms. There is consensus that drug efficacies should be monitored for signs of decline that could jeopardise the long-term effectiveness of MDA strategies. Efficacies are mostly calculated and reported as averages in groups of patients. However, heterogeneities in trial design and reporting hinder straightforward meta-analysis of these data, which could otherwise be used to explore varying efficacy among populations with different MDA histories or particular sub-populations of interest. Some heterogeneity issues could be avoided if individual participant data could be accessed, as this would facilitate the execution of standardized, state-of-the-art statistical analyses. Such data would also allow examination of the distributions of individual responses to anthelmintic drugs, offering a more sensitive means to identify reduced efficacies potentially caused by emerging drug resistance. To assess the trial landscape, we systematically search the STH literature for published anthelmintic trials. We collate locations, study sizes, methodologies, reported drug efficacies and other aspects of the reported data. We quantify these characteristics and create an overview of the variety therein, exploring the limits to analysing aggregated data. The results indicate the volume and characteristics of individual patient data that may exist and could be used to create a database on the efficacy of the anthelmintics that are the cornerstone of MDA targeting STH infections.

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USING TRANSMISSION MODELS IN STUDY DESIGN: DETECTING ELIMINATION AND THE IMPACT OF PRE-EXISTING TREATMENT PROGRAMS

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The DeWorm3 project aims to investigate the feasibility of eliminating soil transmitted helminth (STH) infection using mass drug administration, in particular by leveraging existing treatment programs such as for lymphatic filariasis (LF). The project uses mathematical models of transmission to aid study design. An important component of this work is to identify a statistic to identify when sufficient treatment has been delivered to achieve long-term elimination. We discuss a number of possible candidates, their sensitivity, specificity and their requirements in terms of sampling strategy. We also examine the potential impact of existing LF treatment platforms on the possibility of STH elimination and its detection. We investigate how STH elimination efforts can best be coordinated with such programs to maximise the possibility of success, particularly in cases in which LF programs have achieved their targets and are being discontinued.

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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, 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, and this leaves STH infections under-notified. Diagnostic testing for the STH relies mainly on the WHO recommended Kato-Katz method, which has been shown to lack sensitivity. Other economical, feasible, and more accurate 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 from four different areas of the city, one rural site, two peri-urban sites and one urban site. We compared the traditional Kato-Katz thick smear to two newer diagnostic methods, the mini-FLOTAC kit and a multi-parallel quantitative polymerase chain reaction (qPCR) technique. The mini-FLOTAC kit allows for quick analysis of fresh or preserved feces with minimal equipment needed. The multi-parallel qPCR can accurately detect and quantitate parasites within the stool with high specificity and sensitivity, and is optimized to allow for inexpensive analysis of each sample. All three diagnostic methods were analyzed for both parasite detection and quantification. Both the mini-FLOTAC and multi-parallel qPCR offer feasible, higher-accuracy diagnostics, which will enable a shift away from morbidity control and towards elimination, especially in areas of low STH endemicity.

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ANTIPARASITIC METABOLITES OF *DALEA* SPP (PLANTAE, FABACEAE)

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About a billion people are infected worldwide with hookworms. These intestinal parasites are the major cause of iron-deficiency anemia, weight loss, stunted growth and malnutrition in endemic areas. Despite control strategies using mass drug treatment combined with water, sanitation and hygiene, hookworm infection remains a major public health threat to the overall wellbeing of populations in endemic countries. Moreover, there is increasing concern with reports of lower efficacy of current drugs used in the treatment of hookworms and other soil-transmitted nematodes. Consequently, there is an urgent need of new tools to control the transmission of these helminths. We have explored the anthelminthic potentials of natural products from plants including those of the genus *Dalea* spp. The activity of *Dalea* metabolites against the adult *Ancylostoma ceylanicum* hookworm was assessed using an *ex vivo* assay. Whole extracts, chromatographically-enriched fractions and pure compounds from eight plant species (Dalea spp) were evaluated. Worm mortality due

to plant extracts varied from 0 to 100% by day 5 post-incubation. Some extracts recorded 0% worm survival i.e 100% mortality by 24 hours after incubating worms and plant products. Toxicity of pure compounds to mammalian cells was evaluated by flow cytometry and their effects on cell proliferation by BrdU. In vivo evaluation of pure compounds using our hamster laboratory model of hookworm infection is also underway. Detailed results will be presented.

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A COMPARATIVE ANALYSIS OF STOOL PRESERVATION TECHNIQUES FOR THE MOLECULAR DETECTION OF SOIL TRANSMITTED HELMINTHS

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Accompanying the growing successes of the world's soil transmitted helminth (STH) treatment and elimination programs is an increasing need for sensitive and species-specific molecular diagnostic techniques. While the continued development of new tools is helping to meet this need, sample preservation remains a largely understudied, yet crucial aspect of the stool-based diagnostic process. Diagnostic test sensitivity is not only critical at the level of the individual, but is equally important for programmatic decision making. However, poor sample preservation renders all testing insensitive, making this aspect of diagnostics one of utmost importance. Accordingly, we have performed a comparative analysis of eight different literature-cited stool preservation techniques. Utilizing human stool samples spiked with hookworm eggs at concentrations of 60 eggs per gram (epg) and 200 epg, samples subjected to each preservation methodology were analyzed for the presence of detectable levels of hookworm DNA following storage for one, two, four, and eight weeks at 32 °C. Results have indicated variable preservation efficacy across methodologies as measured by the real-time PCR-based detection of parasite DNA. These results will help program managers to more appropriately structure their future survey efforts, allowing for the more informed balancing of performance needs and budgetary constraints.

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COST ASSESSMENT OF FIVE PARASITOLOGICAL TECHNIQUES FOR THE DIAGNOSIS OF *STRONGYLOIDES STERCORALIS*: EVALUATION IN A HIGHLY ENDEMIC REGION

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Inexpensive, easy to carry out and highly sensitive diagnostic techniques are needed to estimate the real global burden of *Strongyloides stercoralis* infection. This is especially important in tropical areas, where high prevalence rates of this parasite have been reported. We conducted a cost assessment for the detection of *S. stercoralis* by five parasitological techniques: direct microscopic examination (DME), Kato- Katz (K-K), Spontaneous Sedimentation in Tube (SSTT), Modified Baermann technique (MBT) and Agar plate culture (APC), by using fresh stool samples collected from an Amazonian rural community in Peru. The cost of a single sample was estimated considering the costs of laboratory materials and the time consumed in each technique. Out of 234 samples, 207 met the criteria for analysis (sufficient stool amount for examination). The prevalence of *S. stercoralis* was 0.48% (n=1) by DME, 0% (n= 0) by K-K, 3.86% (n= 8) by SSTT, 9.66% (n=20) by MBT and 10.14% (n=21) by APC. The total cost per a single exam was 0.48$ for DMS, 0.59$ for K-K, 0.70$ for SSTT, 0.79$ for MBT and 1.18$ for APC. The cost per case of *S. stercoralis* detected was 99.36$ (DME), 18.11$ (SSTT), 8.18$ (MBT) and 11.63$ (APC), respectively. Analysis of cost per positive case was not performed on K-K, as no larva of *S. stercoralis* was detected by this method. In conclusion, MBT and APC represent low-cost techniques when taking into account the rate of cases detected. However, in poor-resource settings where technicians and laboratory resources are scant, MBT and SSTT may represent cost effective parasitological techniques for the detection of *S. stercoralis*.

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PREDICTING INFECTION DISTRIBUTION AND BURDEN OF DISEASE USING SPATIOTEMPORAL MODELS FOLLOWING A SEVEN YEAR MASS DRUG ADMINISTRATION PROGRAM AND LONGITUDINAL STUDY IN BURUNDI: 2008 - 2014

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Spatiotemporal models (STM) of soil transmitted helminth infections (STH-I) prevalence provide important information for developing effective control strategies. We aimed to quantify the impact of a 7-year mass-drug administration programme (MDA) on the geographical distribution of STH-I and burden of disease (BOD). Longitudinal data on STH-I were collected from 2008-2014 in Burundi. Remotely sensed environmental variables (EV) were used. Associations between EV and STH-I were assessed using univariate and multivariate generalised linear regression models. Bayesian binomial geostatistical models were built to quantify the propensity, cluster size and produce predictive STM for each STH species. Our study found that after accounting for EV, trends in spatial-dependency in STH-I still existed. Model validation found that STM for all parasites were accurate, with 95CI-ROC values between 0.3-1. Predicted infection rates/1000 children fluctuated in accordance with the prevalence. A. lumbricoides showed a maximum of 319/1000 in 2008, 239 in 2011 and 324 in 2014; T. trichiura presented 173 in 2008, 103 in 2011 and 225 in 2014; Hookworm presented 571 in 2008, 262 in 2011 and 219 in 2014. Our findings indicate that using BOD predictions, STM based on longitudinal data may be helpful in maximising the effectiveness of MDAs through optimized resource management.

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PSYCHOSOCIAL ADJUSTMENT IN PERINATALLY HUMAN IMMUNODEFICIENCY VIRUS INFECTED OR EXPOSED CHILDREN

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This study was undertaken to determine whether perinatal HIV infection/exposure adversely affected psychosocial adjustment (PA) between 6 and 18 years of life (i.e. during school-age and adolescence). We enrolled 58 perinatally HIV infected, 56 HIV-exposed uninfected and 54 unexposed controls from Kampala, Uganda. Perinatal HIV status was determined by 18 months old using DNA-polymerase chain-reaction test and was confirmed via HIV rapid diagnostic test at psychosocial testing when children were 6–18 years old. Five indicators of PA (depressive symptoms, distress, hopelessness, positive future orientation and esteem) were measured using validated, culturally adapted and translated instruments. Multivariable linear regression analyses estimated HIV-status related percent differences (β) in PA indicators and corresponding 95% confidence intervals (CI). During school-age and adolescence, positive outlook (β=-3.8, 95% CI:-7.2,-0.1) and self-esteem (β=-4.3, 95% CI:-6.7,-1.8) scores were significantly lower whereas depressive (β=11.4, 95% CI:3.3,19.5) and distress (β=12.3, 95% CI:5.9,18.7) symptoms were elevated for perinatally HIV-infected compared to unexposed controls and HIV–exposed uninfected children. Similarly, positive outlook (β=-4.3, 95% CI: -7.3,-1.2) and self-esteem was lower for exposed controls vs. HIV-unexposed children. Hopelessness was similar by perinatal HIV status. Likewise, the distress and depressive symptom levels were comparable for HIV-exposed uninfected and HIV-unexposed children. In conclusion, perinatal HIV infection predicted higher distress and depressive symptoms, while HIV-affected status (infection/exposure) predicted low self-esteem and diminished positive outlook in the long-term. However, HIV-affected status had no impact on hopelessness suggesting that psychosocial interventions as an integral component of HIV-care for infected children or primary care exposed uninfected children may improve PA and quality of life in these vulnerable groups.

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PERCEPTION OF HUMAN IMMUNODEFICIENCY VIRUS SCREENING AMONG PASSERSBY ALONG A STREET CONNECTING THE EAST AND WEST GATES OF THE ADVENTIST UNIVERSITY AT CARREFOUR, HAITI, AUGUST 2ND 5TH, 2015

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Screening for HIV can be a health indicator for the population. Boulevard Perin is the lane artery borrowed by students university. This alley is the junction between East and West exit out of the university campus. However, this artery is also used by local residents who consider this passage as a shortcut, Diquini and Bizoton. This survey was conducted among thoseestimate the importance ofscreening, and describe some associate risk factors. A descriptive survey conducted among 258 passers who borrowed this path on August 2nd5thin 2 hours of time. These passers-by were divided into three categories, those who enter into the eastgate, west gate, and those who were scattered elsewhere on campus. Questions were asked about their last date of testing andthought of importance of HIV screening. The data were collected on Google forms, processed and analysed on EPI info 7. 258 passerby, 129 (50%) were women. The average age of passerbyyears for a standard deviation of + / - 9 years. For marital status,(68.5%) were single, and 108(41.86%) ofto havethan one sexual partner. For the perception of HIV testing,80.71% (205) think it is important to get tested, though 63(29.72%) say they have never been screened.(10.85%) do not to recall the date of their last screening. In conclusion, this study allowed passerby to understand the importance of the testing. However, prevention campaigns should be conducted by Carrefour health officials in order to increase their screening frequency for HIV.

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PREVALENCE OF CERVICAL CANCER (CC) SCREENING AND THE ROLE OF KNOWLEDGE OF CC RISK AND SCREENING GUIDELINES FOR WOMEN LIVING WITH HIV IN LIMA, PERU

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Cervical cancer (CC) incidence of women in Peru is twice the worldwide average. Awareness by women living with HIV (WLHIV) of their increased risk and Papanicoloau (Pap) smear frequency is understudied, particularly in Peru. We assessed the prevalence of adherence to the recommended CC screening guidelines among WLHIV and if knowledge of CC risk and screening was a factor. 80 HIV-infected women receiving care at Via Libre, a HIV clinic, were surveyed from 2014 - 2015 by a self-administered questionnaire. Knowledge of CC for WLHIV was assessed through 4 questions regarding CC risk and Pap smear frequency for WLHIV. A correct response was worth 1 point leading to a possible score out of 4 per participant. "Adequate knowledge" was judged as ≥ 3/4 correct answers. The Wilcoxon rank sum test was used for bivariate analysis. Nearly all (91.3%; 73/80) WLHIV were enrolled in a HAART program. 21.3% (17/80) of WLHIV were adherent to the guidelines by obtaining a Pap smear within 12 months of study enrollment. 78.8% (63/80) were not adherent by having their most recent Pap smear outside of 12 months, including 11.3% (9/80) who had never been screened. The median composite score for knowledge of CC risk and screening was 3 (IQR 2 -4) for adherent WLHIV and 2 (IQR 1-3) for non-adherent WLHIV. 58.8% (10/17) of adherent WLHIV met "adequate knowledge" criteria as did 41.3% (26/63) of non-adherent WLHIV. When asked how often WLHIV should get a Pap smear, 76.5% (13/17) of adherent WLHIV answered "Every year" correctly while 55.6% (35/63) for non-adherent WLHIV were able to. Overall, bivariate associations found between knowledge score and adherence were not significant. Prevalence of WLHIV who had on-time Pap smears was three times less than WLHIV who did not. Knowledge of CC risk and screening guidelines influenced adherence as more adherent WLHIV met the criteria for adequate knowledge. Larger studies of this population are needed to assess the educational, social, and structural barriers to screening and potential benefits of HIV and gynecological care integration services.

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HIV/NCD INTEGRATED CARE: A LITERATURE REVIEW

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With advances in antiretroviral therapy, people living with HIV (PLHIV) are living longer lives and HIV itself is becoming a chronic disease. Since PLHIV are living longer lives, they are increasingly contracting other diseases such as diabetes and hypertension. Despite the prevalent view that NCDs are ailments faced only by the wealthy, NCDs burden low-and middle-income countries (LMICs) at ever increasing rates. In fact, 80% of global deaths from NCDs occur in LMICs due to weak health systems, limited access to information, medication, and services. As individuals, communities, and clinics move towards treating HIV as a chronic illness, opportunities arise to deliver both NCD and HIV services in an integrated way and to build

stronger health systems capable of delivering more patient-centered care. Several health-focused organizations have pursued the opportunity to enhance patient care by integrating HIV and NCD services. The goal of this paper is to present findings from a literature review of these programs, identify best practices, and provide recommendations for policymakers. We studied 23 programs from 11 different organizations operating across Sub-Saharan Africa and examined 4 aspects of each program: which NCDs were addressed, what services were offered, the level of health system involved, and whether the programs engaged in policy work. Diabetes and cardiovascular disease were the most commonly targeted diseases, followed by cancer and mental health illness. Services commonly included screening, diagnosis, and treatment for NCDs in conjunction with existing HIV care. Most integration occurred at the lowest level of care including at the home, community and HIV clinic. Services were occasionally offered at the tertiary level as well. Few of the programs we studied engaged in policy interventions. Some programs, however, did engage Ministries of Health at the national level on NCD protocols. Linking HIV/AIDS and NCD care regimens is a new concept and more research is needed. This review is intended to aid policymakers and program implementers design further integrated programs and stronger health systems.

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HIV CO-INFECTION WITH *PLASMODIUM VIVAX* MALARIA AND OTHER TROPICAL INFECTIOUS DISEASES IN THE PERUVIAN AMAZON

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Interactions between HIV and other infectious diseases have the potential to alter course of disease, response to therapy, and epidemiology of some or all agents involved. In the tropics, geographic overlap of the "big three"–HIV/AIDS, tuberculosis, and malaria - result in millions of deaths and disability-adjusted life years annually. Loreto, a region in the Northern Peruvian Amazon, is responsible for 90% of the country's malaria burden and has the second-highest prevalence of HIV. In an attempt to understand the overlap of infectious diseases, we began a cohort study of outpatient HIV patients from Loreto Regional Hospital assessing infectious disease history, including malaria. Out of 337 enrolled patients, 33% were female and 75% defined themselves as heterosexual. Most (59%) attended high school and were employed (65%). The median years since HIV diagnosis was 3.83 years (IQT=1–7.83 years) and 278 (86%) had disclosed their status. Patients reported on average 2 (IQT=0-2) respiratory infections and 2.9 bouts of diarrhea (IQR=0-2) in the last year. Further, 162 (49.39%) reported having had tuberculosis, 120 (36.59%) hepatitis, and 51 (15.55%) dengue. Five individuals had cerebral toxoplasmosis and live with lasting neurological sequelae. Microscopy detected one case each of *Plasmodium vivax, P. falciparum*, and filaria. ELISA for *P. vivax* using PvMSP1-19 confirmed microscopy result and detected 14 low (OD 0.25-0.50) and 11 high positives (OD >0.50), a total of 25 positives by ELISA (7.65%). PCR confirmed both cases of malaria identified by microscopy and identified one unconfirmed case of *P. malariae*. Other ELISA *P. vivax* positives were PCR negative. Of the 27 malaria ELISA or PCR positives, 22 self-reported cases of malaria, 7 of which occurred less than 6 months of enrollment. Patient reports suggest multiple infectious diseases affect this population, especially individuals that live and work in peri-urban and rural environments. *P. vivax* malaria co-infection is frequent but its consequences are little known. Research should try to more accurately identify the interactions and burden of co-infections in this neglected population.

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PRESENTATION, ETIOLOGY, AND OUTCOME OF FEBRILE INDIAN PATIENTS DIFFERS BY HIV STATUS

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Despite a <1% HIV prevalence, India has the third largest burden of HIV worldwide. Acute febrile illness is one of the most common reasons for hospital admission in India, but the clinical differences between HIV infected and uninfected patients are unknown. Patients >= 12 years of age admitted to general medicine wards at BJ Medical College - Sassoon General Hospital, in Pune, India with >1 day of fever were enrolled into a prospective cohort between July 2013 and December 2015. We compared clinical characteristics, laboratory data, treatment course, and mortality between HIV positive and negative patients using fisher exact test and a multivariable logistic regression model adjusted for modified Apache II score, age, and sex. Of 970 participants enrolled, 127 (13%) had HIV; 37 were newly diagnosed. Median CD4 count was 161 cells/cumm; 61 (49%) were on ART. Vector-borne infections among patients living with HIV included dengue (n=5), malaria (n=2), chikungunya (n=2), and leptospirosis (n=1). Additional infectious disease diagnoses included bacteremia (n=6), pneumonia (n=7), meningitis (n=16), and microbiologically confirmed tuberculosis (n=10). Two patients with mosquito-borne illnesses also had microbiologically confirmed tuberculosis. Compared to patients without HIV, patients with HIV were more likely to have meningitis (13% vs 4%, $p < 0.01$), diarrhea (42% vs 16%, $p < 0.01$), tuberculosis (8% vs 1%, $p < 0.01$), and alcoholism (21% vs 10%, $p < 0.01$), and were less likely to have dengue (4% vs 24%, $p < 0.01$) and malaria (2% vs 8%, $p < 0.01$). Patients with HIV more frequently received fluoroquinolones (24% vs 10%, $p < 0.01$) and antituberculosis drugs (24% vs 4%, $p < 0.01$). Mortality was more than two times higher in HIV infected patients (adjusted odds ratio 2.1, confidence interval 1.1-3.8). People in India living with HIV who develop acute febrile illness more commonly have diarrhea, tuberculosis, and meningitis, and less commonly have mosquito-borne illnesses. Clinicians must recognize that patients living with HIV present differently with acute febrile illness, and are more likely to die.

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CONDOMLESS INSERTIVE ANAL SEX AND GENDER IDENTITY AMONG MEN WHO HAVE SEX WITH MEN IN TOGO

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Consistent condom use continues to be an effective preventive tool to control HIV transmission among men who have sex with men (MSM). Few studies have explored the association of condomless insertive anal sex (CIAS) and gender identity among African MSM. Our aim was to identify factors associated with CIAS among MSM living in Lomé and Kara, Togo. A total of 683 MSM ≥18 years of age were recruited using respondent driven sampling (RDS) for a cross-sectional survey (354/683 in Lomé and 329/683 in Kara). Participants completed a structured questionnaire and were tested for HIV and syphilis. Statistical analysis included t-test, RDS-weighted (RDS-w) proportions, bootstrapped confidence intervals and logistic regression models. Overall median age was 23.9 years; 62.8% were between 18 and 24 years (RDS-w Lomé=79.1%, RDS-w

Kara=55.5%, p<0.01). Most participants identified themselves as being males (RDS-w male=67.9% vs. 91.2%, p<0.01, female=18.1% vs.1.3%, p<0.01 and intersex=13.9% vs. 7.4%, p<0.01 in Lomé and Kara, respectively), were single/never married (RDS-w Lomé= 91.7% vs. Kara=95.4%, p=0.11), and reported their sexual identity as gay/homosexual (RDS-w Lomé=61.2% vs. Kara: 62.6%, p=0.59). Consistent condom use in the past 12 months was reported by 30/270 MSM who had insertive anal sex (RDS-w Lomé=30.5% and Kara=6.2%, p<0.01), 17/198 who had receptive anal sex (RDS-w Lomé=13.4% and Kara=not available (NA)), and by 100/223 who had vaginal/anal sex with a woman (RDS-w Lomé=58.6% and Kara=not available (NA)). HIV prevalence was 62/683 (RDS-w Lomé=10.4%, RDS-w Kara=0.2%, p<0.01). Multiple logistic regression analysis showed a positive association between CIAS and intersex gender (adjusted OR=3.0, 95% CI=1.2-7.4). In Togo, local cultural and social norms could increase the number of condomless insertive anal sex acts among MSM who self identify as being of intersex gender. Strategies to address gender inequity should be included in HIV prevention programs aiming to address the needs of MSM.

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COMPARISON OF ASYMPTOMATIC AND CLINICAL MALARIA FREQUENCIES BETWEEN HIV POSITIVE AND HIV NEGATIVE INDIVIDUALS LIVING IN GABON

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This study was undertaken to compare the frequency of clinical and asymptomatic *Plasmodium falciparum* parasitaemia between HIV-positive and HIV negative individuals living in Gabon. Prospective screening for malaria parasitaemia of HIV-infected Gabonese adults on antiretroviral therapy (ART) and HIV negative individuals were performed at two health centers and during a survey on malaria knowledge performed in asymtomatic volunteers in 2015. Clinical malaria was defined as fever with a positive blood smear, and asymptomatic malaria as a positive blood smear in the absence of fever and history of fever the preceeding 7 days. Data from three hundred thirty seven asymptomatic and 76 febrile HIV-1-positive patients were compared to those from 439 asymptomatic and 130 symptomatic HIV negative adults. Clinical malaria frequency was 31.6% among HIV positive and 29.3% among HIV negative patients. Asymptomatic malaria prevalence rate was comparable between both groups (9.6% in HIV positive and 8.4% in uninfected population). Cotrimoxazole prophylaxis was non significantly associated with a lower malaria prevalence : 4.3% versus 1.2% among asymptomatic HIV patients and 42.9% versus 27.8% in symptomatic ones. HIV1 infection is not associated with a higher frequency of asymptomatic or symptomatic malaria prevalence in patient on ART. The present results also suggest a protective effect of cotrimoxazole prophylaxis on malaria occurence in HIV-positive individuals.

1743

LEVERING HIV DIAGNOSTIC AND CARE INFRASTRUCTURE IN RWANDA TO ACCELERATE THE ROLL-OUT OF NEW PEDIATRIC TB TREATMENT FORMULATIONS

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Since the end of the genocide in Rwanda, the country has made impressive gains in health system strengthening. In particular, the rapid scale up of antiretroviral therapy has been a great success story for Rwanda. Tuberculosis remains an important health concern in sub-Saharan Africa, and infants and children in particular, are at the highest risk for severe forms of the disease because of immature immune systems. New pediatric formulations of anti-tuberculosis drugs have renewed calls for aggressive roll-out of an aggressive diagnostic and treatment campaign in affected regions to reduce the burden of TB among infants and children. The success of Rwanda's scale-up of ART can greatly inform the process of accelerated roll-out of new pediatric anti-TB formulations. Through a review of the history of HIV treatment scale-up in Rwanda, as well as a field assessment of current interventions and available evaluation data, we developed a framework to identify the successes of ART expansion in Rwanda and how lessons learned can be applied to the accelerated roll-out of new pediatric anti-TB formulations. Key themes of this framework identify a decentralized form of care administration and a centralized system of data collection that supports real-time monitoring and evaluation. Innovative health system financing strategies, including a universal community health insurance scheme, have improved access to primary care services for prevention and treatment of HIV and TB. Furthermore, cost-effective and responsive supply chains for drug delivery have permitted scale-up with limited stock outs. This framework of successes in the delivery of ART in Rwanda can be leveraged to improve pediatric care for TB immediately in Rwanda, and lessons learned can be applied to other countries with a high burden of infant and child TB across the sub-Saharan African region.

1744

SEXUALLY TRANSMISSIBLE INFECTIONS (STI'S) AMONG HIV CLIENTS ATTENDING AN URBAN UGANDAN HIV CLINIC

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Naguru Hospital was established as a Regional Referral Hospital in February 2012.In July2012, an HIV clinic was started to take care of the many patients who live with the infection in Kampala City and its surrounding areas. To date the cumulative number of clients stands at 6480 while the still active clients stand at 3750.We were concerned about the possible complications caused by sexually transmissible co-infections so we set out to find out their prevalence among our clients. Of the 6480 records looked at,139 had a clinical or laboratory diagnosis of an STI. We found the commonest diagnoses to be: Herpes Simplex 2 (47=33.8%) candidiasis (38=27.3%),syphilis(20=19.4%), other genital ulcer diseases-other than Herpes 2 (20=19.4%), genital warts(7=5%), gonorrhea (5=3.5%) , Hepatitis B(5=3,5%), Bacterial Vaginosis (1=.7%). Genital chlamydial disease was not identified by any of our clinicians implying that it was probably missed. A prospective study to ascertain the true burden of STI's among our HIV patients is required to avert complications and possible mortality from these treatable co-ailments.

1745

TRYPANOSOMA CRUZI INHIBITION OF SIRT1/PGC1 ACTIVITY CONTRIBUTES TO ANTIOXIDANT/OXIDANT IMBALANCE BUT NOT TO MITOCHONDRIAL BIOGENIC DEFECTS: BENEFITS OF SIRT1-TARGETED THERAPY IN CHAGAS DISEASE

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Increased oxidative/inflammatory stress and decreased mitochondrial function are the hallmarks of chronic chagasic cardiomyopathy. SIRT1/PGC1 signaling of NRF1 and Nrf2 regulates mitochondrial biogenesis and antioxidant response. C57BL/6 mice were infected with *Trypanosoma cruzi* (Tc) and monitored during chronic phase (~150 days post-infection). SIRT1 and PGC1 protein levels were normal; however, SIRT1 activity and PGC1α deacetylation (active-form) were decreased significantly in chagasic myocardium. Tc-infected mice were treated with SIRT1 agonist SRT1720 for 3-weeks after control of acute parasitemia (i.e. 45 days post-infection). SRT1720 therapy provided the maximal benefits in restoring the SIRT1/PGC1 activity, and subsequently left ventricular (LV) function (stroke volume, cardiac output, ejection fraction etc.) was significantly improved

in chronically-infected/SRT1720-treated mice. SIRT1-targeted therapy did not improve the PGC1/NRF1-dependent mitochondrial biogenesis (i.e., mitochondrial DNA content, expression of subunits of the respiratory complexes and mtDNA replication machinery) and the disproportionate synthesis of collagens and LV mass in chagasic mice. Instead, SRT1720 therapy restored the Nrf2 level and antioxidant capacity; and subsequently resulted in 2-10-fold inhibition of Tc-induced oxidative (H2O2 and advanced oxidation protein products), nitrosative (inducible nitric oxide synthase, 4-hydroxynonenal, 3-nitrotyrosine), and inflammatory (IFNγ, IL1β and TNFα) stress and inflammatory infiltrate in chagasic myocardium. These results suggested that Tc-inhibition of SIRT1/PGC1 activity inhibition was not the key mechanism in mitochondrial biogenic defects during Chagas disease. SIRT1/PGC1 activation enhanced the antioxidant capacity, and subsequently controlled the oxidative/nitrosative and inflammatory pathology and LV dysfunction in chronic chagasic cardiomyopathy. These findings indicate that activators of the sirtuin family of proteins will provide promising new therapeutic strategies for treating cardiac dysfunction in chronic chagasic disease.

1746

THE *LEISHMANIA* METAPHYLOME: A COMPREHENSIVE SURVEY OF *LEISHMANIA* PROTEIN PHYLOGENETIC RELATIONSHIPS

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Leishmaniasis is a neglected parasitic disease with diverse clinical manifestations and a complex epidemiology. It is known that the infecting *Leishmania* species is responsible for the distinct clinical presentations and treatment needs due to virulence factors that mediate host parasite interaction, infectivity and pathogenicity. However, our understanding of these species-specific adaptations and their evolutionary background is still limited. To improve our knowledge regarding the biology and adaptive mechanisms of different *Leishmania* species, we conducted a proteome-wide phylogenomic analysis to gain insights into *Leishmania* evolution. The analysis of the reconstructed phylomes (totaling 45,918 phylogenies) allowed us to detect genes shared in pathogenic *Leishmania* species, such as calpain-like cysteine peptidases and 3'a2rel-related proteins, or genes that could be associated with visceral or cutaneous development. Our findings demonstrated that gene duplication constitutes an important evolutionary force in *Leishmania*, acting on protein families that mediate host-parasite interactions, such as amastins, GP63 metallopeptidases, cathepsin L-like proteases, and our methods permitted a deeper analysis of their phylogenetic relationships. Our results highlight the importance of proteome wide phylogenetic analyses to detect adaptation and evolutionary processes in different organisms and underscore the need to characterize the role of expanded and species-specific proteins in the context of *Leishmania* evolution by providing a framework for the phylogenetic relationships of *Leishmania* proteins.

1747

POLYMORPHISMS IN CASPASE-1 ARE ASSOCIATED WITH CHAGAS CARDIOMYOPATHY IN SANTA CRUZ, BOLIVIA

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Trypanosoma cruzi infection is usually acquired in childhood in endemic areas. Chagas cardiomyopathy (CC) develops in 20-30% of infected individuals over decades. CC pathogenesis involves the host inflammatory response to *T. cruzi*, in which upstream caspase-1 activation prompts the cascade of inflammatory chemokines/cytokines, cardiac remodeling and myocardial dysfunction. To date, no reliable early biomarkers of CC risk have been identified. However, polymorphisms of caspase -1 have shown to be associated with susceptibility to myocardial infarction and cardiovascular death risk. We recruited infected (Tc+) patients (n=151) and uninfected (Tc-) patients (n=85) in a hospital in Santa Cruz, Bolivia, to examine the association of two caspase-1 single nucleotide polymorphisms (SNPs) with cardiomyopathy. Cardiac status was categorized as A: normal EKG without systolic dysfunction and/or segmental wall motion abnormalities; B: ECG with abnormalities consistent with CC but normal ejection fraction (EF); CD: systolic dysfunction (EF<50%). We compared A vs BCD (all cardiomyopathy) and A vs B (early cardiomyopathy) for Tc+ and Tc-. Genotypes were determined using Taqman probes via RT-PCR in peripheral blood DNA. Genotype frequencies were analyzed by 3 inheritance patterns (dominant, recessive, additive) using logistic regression adjusted for sex and age in the SNPassoc R package. Caspase-1 SNP rs501192 showed consistent differences in AA genotype frequencies in Tc+ BCD vs A (aOR 2.25 [0.83-6.08]) for the recessive model. The association was similar but stronger for Tc+ B vs A (aOR 2.84 [0.99-8.13]). No significant associations were found in comparisons within Tc- groups or between Tc- and Tc+ patients. Our data suggests that polymorphisms within caspase-1 play a role in CC development, identifying individuals with a higher risk of developing Chagas cardiomyopathy. Further investigation into genomic biomarkers may help identify Tc+ patients for whom intensified monitoring and early medical intervention could be beneficial.

1748

ARTEMISININ DIMERS AS PROMISING NEW DRUG LEADS FOR VISCERAL LEISHMANIASIS

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Visceral leishmaniasis is a major global health problem with more than 12 million confirmed cases, about 2 million new cases being added every year and more than 350 million people at the risk of being exposed to the disease. The disease is fatal if left untreated. The current choices of therapies are highly limited and suffer from severe drug-toxicities or have become ineffective due to drug-resistance. Recent studies at our laboratories have identified a few novel artemisinin dimers with

outstanding antileishmanial activities against intracellular amastigotes of *Leishmania donovani,* the clinically relevant parasite stages for the visceral leishmaniasis. Antileishmanial activities of these dimers are several folds better as compared to the current battery of clinically used antileishmanial drugs. These dimers do not show any toxicity on differentiated THP1 cells. Selectivity index (SI) has been calculated by comparing toxicity with antileishmanial activity. Dimer piperidine (IC50 0.073 µM, SI >198), dimer morpholine (IC50 0.007 µM, SI >2052), dimer valine (IC 50 0.060 µM, >230), dimer AB acid (IC50 0.013 µM, SI >999), dimer tryptamine (IC50 0.045 µM, SI >165), dimer oxime HS (IC50 0.062 µM, SI >219), dimer benzylamine (IC50 0.099 µM, SI >141) and dimer GABA (IC50 0.013 µM, SI >1086)) have been selected as promising leads for extended evaluation. The *in vitro* antileishmanial activity of the lead analogs has been further confirmed by THP1 cells-*L. donovani* amastigotes digital image analysis counting of intracellular amastigotes. Artemisinin the parent drug from this class do not show noticeable antileishmanial activity up to 35 µM concentration. This indicates selective leishmanicidal action of the artemisinin dimers. The artemisinin dimers thus offer promising leads, which can be further optimized and developed as oral treatments for visceral leishmaniasis.

1749

IDENTIFICATION AND CHARACTERIZATION OF "YINP", A NOVEL GENE INVOLVED IN *LEISHMANIA* PATHOGENESIS. A POTENTIAL NEW TARGET FOR DRUG DISCOVERY

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Leishmaniasis is a neglected tropical disease caused by *Leishmania* spp. Current drug therapies are unsatisfactory due to their toxicity, long treatment courses and development of resistance. To improve existing treatments, the identification and characterization of novel therapeutic targets based on parasite genes involved in *Leishmania* pathogenesis is essential. One of these genes is "YinP", identified in our research group. It may play a role in the acquisition of infectivity by *Leishmania* promastigotes. This gene is highly conserved and has demonstrated to be involved in several cellular processes such as embrionary progress, ribosomal biogenesis, cellular proliferation, and genetic transcription. Our assays showed that YinP reaches its highest expression level in metacyclic promastigotes, the infective stage. Furthermore, we have performed several experiments to analyse the infectivity of parasites overexpressing YinP. Our data reveal a dramatic increase of the ratio of infection as well as a higher replication rate whithin macrophages exposed to *Leishmania* overexpressing YinP. All together, these results strongly suggest a relation between YinP gene expression and leishmaniasis pathogenesis. Moreover, to localize YinP expression in the leishmanial cell, two plasmids were constructed: pXG-mCherry12-YinP and pXG-mCherry34-YinP. In these vectors, YinP gene was inserted directly next to the gene for fluorescent protein (mCherry), to generate fluorescent fusion proteins expressed in the parasites. Fluorescent microscopy disclosed that red fluorescence of mCherry fused with YinP was localized only in a part of nucleus. Therefore, our results showed that YinP protein is expressed in the nucleus. Further experiments need to be performed to analyze if such expression is nucleolar. Finally, in order to validate YinP as a therapeutic target, we are carrying a screening of new compounds that have shown promising leishmanicidal activity. Our preliminary results have shown that YinP overexpressing parasites seem more resistant to these drugs. Therefore, this gene may be a new and good molecular target.

1750

PROTEOMIC ANALYSIS OF PLASMA-DERIVED EXTRACELLULAR VESICLES IN NATURAL INFECTIONS OF *PLASMODIUM VIVAX*, *TRYPANOSOMA CRUZI* AND *FASCIOLA HEPATICA*

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Chagas disease, fasciolosis and the malaria caused by *Plasmodium vivax*, are three neglected tropical diseases responsible for millions of infections worldwide, including disease and death. Our present research effort is focused on the molecular characterization of plasma-derived extracellular vesicles (EVs) from these neglected diseases to further study their role in intercellular communication, and the use of their selective-cargo as novel therapeutic agents and diagnostic markers, as reported previously. EVs were obtained from infected patients (malaria and Chagas) and infected cows (*Fasciola hepatica*), as well as from healthy volunteers and animals as controls of specificity. Size exclusion chromatography (SEC) using sepharose CL-2B, commercially available as qEV columns (iZONTM), was used for isolation of EVs. Molecular characterization was done by a bead-based assay with antibodies against tetraspanins CD9 and CD81, by cryo-transmission electron microscopy (cryo-TEM), and by Nanoparticle Tracking Analysis (NTA). An extensive LC-MS/MS analysis was performed using different mass spectrometers and algorithms. The proteomic composition of plasma-derived EVs from samples of these neglected tropical diseases contain parasite-specific proteins. Their identities and possible roles facilitating studies on mechanistic insights of pathology, as well as antigen discovery for vaccination and disease biomarker identification will be presented and discussed.

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IDENTIFICATION OF NOVEL INHIBITORS OF *LEISHMANIA* INITIATION FACTOR 4A AND ASSESSMENT OF THEIR BIOLOGICAL EFFECTS ON PARASITE GROWTH

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Leishmaniases constitute a group of parasitic vector borne diseases. They correspond to major public health problems that are neglected and ill controlled. The identification of novel targets and drugs for chemotherapy constitute a research priority. Different pieces of evidence point to *Leishmania* initiation factor eIF4A (LeIF), member of the DEAD-box protein family, as a potential drug target. Through a structure-based drug discovery process, we previously identified a novel inhibitor (C208) of LeIF ATPase activity (Harigua-Souiai et al., submitted). In the present work, C208 was used as a bait to search chemically related molecules. The Morgan fingerprints were used as chemical descriptors to assess the similarity between the molecules. Their use as criteria to identify moieties potentially responsible for bioactivity was validated by our group and in drug discovery in general, as reported previously. This virtual chemical

screen led to the identification of 28 analogous molecules. In a first step, fifteen of them were screened out of which a second hit was retained (CS48). It demonstrated modest levels of inhibition of LeIF activity compared to C208. Both C208 and CS48 were tested for their effects on the *Leishmania* promastigote viability. Their respective IC_{50} values were of 3 and 4 µM. Currently, as the ATPase screening is tedious, requesting high amounts of recombinant protein and compounds, we are testing the remaining 13 compounds for their effects on promastigote viability, then we will evaluate the active molecules for their effect on the ATPase enzymatic activity of LeIF. Five molecules were tested so far and a third hit (CT7) having an IC_{50}=1 µM was retained. At the IC_{50} concentration, these compounds had no to un-significant cytotoxic effect on human cells. Selectivity index (SI) was established for the C208 (SI= 6.6) in a preliminary way. Compound C208 and its analogs may constitute a novel route for drug design for anti-*Leishmania* treatments. Further work will be undertaken to understand the mode of binding and interaction between LeIF and its most promising inhibitor.

1752

GENOTYPING OF PANAMANIAN *TRYPANOSOMA CRUZI* STOCKS USING A MAXICIRCLE MULTILOCUS SEQUENCE TYPING APPROACH

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Chagas disease, caused by *Trypanosoma cruzi* is a major health problem in Central America leading to significant economic losses in this region due to reduced productivity from early-age mortality and disability. Differently from the rest of Central America, the main Chagas disease vector in Panama is Rhodnius pallescens, a sylvatic triatomine closely associated with "royal palm" trees. It has been suggested that the geographical heterogeneity in Chagas disease pathology and clinical outcome is related to parasite genotype. The present work provides information on *T. cruzi* discrete typing unit genotypes circulating in endemic areas of Chagas disease in Panama. Thirty *T. cruzi* stocks isolated from persons with different clinical profiles, as well as from vectors and different mammalian hosts were included in the study. Initial molecular analysis using mini-exon, heat shock protein 60 and glucose-6-phosphate isomerase nuclear markers confirm that DTU TcI was the predominant genotype found. To further evaluate intra-DTU diversity within Tc1, we use a multilocus sequence typing (mtMLST) approach. Six maxicircle gene fragments were amplified: ND1 (NADH dehydrogenase subunit 1), COII (cytochrome c oxidase subunit II), MURF1 (Maxicircle unidentified reading frame 1, CYT b (cytochrome b), 12S rRNA and 9S rRNA, coding regions. For each isolate, maxicircle sequences were concatenated according to their structural arrangement. Phylogenetic analysis was performed by two types of applied phylogenetic techniques: neighbor-joining method and Bayesian inference. The results showed a low level of diversity within *T. cruzi* Tc1 isolates. The epidemiological significance of these findings is discussed.

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EVALUATION OF RHOB GENE SILENCING MEDIATED BY SHRNA ON INFECTION PHENOTYPE OF U937 CELL DERIVED MACROPHAGES INFECTED WITH *LEISHMANIA (VIANNIA) BRAZILIENSIS*

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The limited knowledge of the *Leishmania* - macrophage interaction has interfered with the development of control strategies for leishmaniasis. *Leishmania* is able to modulate the immune response of the host cell by altering macrophage gene expression during infection. In a previous work, RhoB a gene that encodes for a small GTPase, was differentially expressed between non-infected and infected macrophages with L. (V.) braziliensis. The regulation of RhoB expression is related to sterols processing, pathway altered during *Leishmania* infection. The aim of this study was validate expression levels of RhoB in infected macrophages with L. (V.) braziliensis and determine the effect of RhoB silencing mediated by shRNAs on infection rate and burden load of infected macrophages. The expression levels of RhoB were measure by qRT-PCR from 0 to 120 h after *in vitro* infection. Lentiviral transduction was used to generate U937 derived cell lines bearing the constructs encoding for the expression of shRNAs against RhoB. RhoB gene silencing and active form of RhoB were assessed using western blot and pull down assay respectively. Generated cell lines with highest levels of RhoB silencing were infected and colored with fluorescent stain to determine the effect of RhoB silencing on infection rate and burden load. Results show that RhoB expression did not change between 0 and 24 h after infection while it is up regulated between 48 and 96 h. In the other hand, RhoB silencing is associated with decreased infection rate and burden load suggesting that RhoB could be related with the establishment of infection.

1754

TRANSCRIPTIONAL PROFILE OF HUMAN WHOLE BLOOD CELLS STIMULATED WITH SOLUBLE *LEISHMANIA* ANTIGENS

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Visceral leishmaniasis (VL), caused by *Leishmania infantum*, affects many people around the world. It has been estimated that only 10% of infected individuals will develop symptomatic disease and this might be primarily due to host-associated factors (*i.e.* environmental and genetic factors). This study aimed to assess the global gene expression pattern of peripheral blood cells, when challenged with Soluble *Leishmania* Antigen (SLA) stimulus. Whole blood cells from symptomatic VL patients (n=7) and individuals within 4-12 months of VL recovery (n=4) were divided into two groups, non-stimulated and SLA-stimulated, upon four hours of incubation with and without SLA, respectively. Total RNA were extracted and hybridized against ~ 48,000 probes using a microarray platform (HumanHT-12 BeadChip). The recovered group had 604 differentially expressed (DE) genes whereas symptomatic group had 128 DE genes. Overall, symptomatic individuals were able to express nearly 20% (118/604) of those genes expressed by the recovered individuals, suggesting an incomplete response under SLA stimulus. These 118 common genes were most from Cell chemotaxis (GO:0060326) and Chemokine receptor binding (GO:0042379) pathways, as shown by enrichment analysis. The list of DE genes for the recovered group was enriched with genes from Response to lipopolysaccharide (GO:0032496), TNF and Toll-like receptor signaling pathways (KEGG:04668 and 04620). Of note, 10 genes were exclusively changed in symptomatic group, which included apoptosis genes (*TNFRSF10B, CARD9*) and a galactoside-binding protein (*LGALS3)*. With a two-fold change increase under SLA stimulus (adjusted p=0.01), *LGALS3* might play important role in VL pathogenesis by regulating the response to innate immunity signals as well as proinflammatory cytokine production.

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BACTERIAL CO-INFECTION IN MURINE CUTANEOUS LEISHMANIASIS

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Leishmaniasis is a collection of human protozoan diseases caused by *Leishmania* inoculated through sand fly bites. Cutaneous leishmaniasis

(CL) causes localized skin lesions often followed by ulceration. In some human infections, response to treatment is best when initiated after ulceration. However, immune responses associated with ulceration and their effects on the course of disease are relatively unexplored. CL ulceration introduces bacteria into subdermal layers, and secondary bacterial infections are common. Thus, we hypothesized that bacterial effects are critical determinants of CL outcome. *Staphylococcus aureus*, a bacterium commonly isolated from disrupted human skin, is often found in CL lesions. We hypothesized that bacteria present during *Leishmania* inoculation, both before and after ulceration occurs, activate inflammatory responses that augment inflammation and contribute to host control of parasitic infection. We injected *L. major, S. aureus*, or both intradermally into the ears of female C57BL/6 mice. We monitored inflammation and parasitic burden by lesion volume, histology, and qPCR through 4 weeks post-infection (p.i.). During the first week of infection, there were greater lesion sizes in co-infected ears compared to ears injected with *S. aureus* alone, whereas ears injected with *L. major* alone do not yet form lesions. At 4 weeks p.i., we observed greater lesions sizes and decreased parasite burden in ears co-infected with a higher dose of *S. aureus* compared to ears infected with *L. major* alone or co-infected with a lower dose of *S. aureus*. Overall, these data suggest that co-infection with *S. aureus* increases inflammation, contributing to control of *L. major* burden but potentially greater lesion pathology. Identifying the specific inflammatory responses activated by skin microbiota in leishmaniasis, such as inflammasomes, may lead to novel therapeutic approaches to parasitic infection.

1756

PHYLOGENETIC ANALYSIS OF RNA OF CRIMEAN-CONGO HEMORRHAGIC FEVER AND WEST NILE FEVER SELECTED IN KAZAKHSTAN

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Studies performed on 14 samples of CCHF RNA extracted from ticks collected in South-Kazakhstan Oblast and 3 samples of WNF RNA extracted from mosquitos caught in Western-Kazakhstan Oblast. Prior to study,RNA samples were modified into cDNA. Sequencing was performed with the use of BigDye® Terminator v3.1 Cycle Sequencing Kit according to manufacturer's instruction with following separation of fragments on automatic genetic analyzer 3730xlDNAAnalyzer. Nucleotide sequences obtained by means of direct and reverse primers were analyzed and combined in general sequence using SeqScape 2.6.0 software. Sequences of reference strains of corresponding virus deposited in international database NCBI were used as the matrix. Construction of dendrogramm was performed with Mega 5.0 software, equalization of nucleotide sequences was performed with Muscle algorithm, construction of phylogenetic trees was performed by means of Neighbor-Joining method. Performed sequencing of S-segment nucleotide sequence (on 7 samples of ticks), M-segment (1 sample) and L-segment (9 samples) CCHF genome and E-segment of WNF virus (on 3 mosquito samples). Comparison of nucleotide sequences indicated that the samples being studied are most contiguous to strains from Afghanistan, Pakistan, Oman, Tadzhikistan, Uzbekistan and China, and are related to *"Asia 1-2"*CCHF groups. Comparison of nucleotide sequences of E-segment of WNF (with the length around 800 nucleotide pairs) indicated that WNF RNA in studied mosquito samples from Western-Kazakhstan Oblast are genetically close to the RNA of Russian isolates.

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ANTI-*LEISHMANIA DONOVANI* ANTIBODIES ENHANCE PROMASTIGOTES INTERNALIZATION INTO HOST MACROPHAGE

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This study aimed to demonstrate the role of humoral immunity in *Leishmania* parasite internalization into host macrophages. First, informed consent sera were obtained from 67 parasitologically confirmed visceral leishmaniasis patients reporting to our field treatment centre, Eastern Sudan. Then following titre determination, sera that had a titre of >102,400 were selected for parasite coating. An *in vitro* parasite internalization system was developed to enhance the *Leishmania*/ macrophage interactions. The mean parasite number per monocytes was 626 ± 91 for antibody-coated *Leishmania donovani*, compared to 412 ± 70 uncoated isolates (p= 0.01). On the other hand, the percentage of infected cells was significantly higher for all antibody-coated isolates (100%) compared to uncoated ones (40%). This evidence of high infectivity probably points to the fact that anti-*Leishmania* antibodies facilitated the parasite uptake by host macrophages and monocytes-derived macrophages (MDM). Conclusion *Leishmania* spp. promastigotes preferentially infect host macrophages, where parasite internalization is facilitated by several host and parasite surface molecules. Moreover, the rate of parasite uptake by MDM was significantly higher compared to monocytes. This could be explained by the fact that the functional capabilities of fully differentiated macrophages differ from monocytes. In conclusion, host humoral immunity probably plays a pivotal role in *Leishmania* parasites internalization into host macrophages.

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THE ROLE OF IL-10 AND IFN-γ IN VIRULENCE OF DERMOTROPIC *LEISHMANIA DONOVANI* IN SRI LANKA

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Cutaneous leishmaniasis (CL) in Sri Lanka is caused by an apparently dermotropic variant of *Leishmania donovani*. The visceralization potential of *L. donovani* in Sri Lanka and determinants of disease outcome is not yet fully understood. The current study was aimed at determining visceralization potential, cytokine response during local *L. donovani* infection and to explore the effects of route of infection on disease outcome. Parasites isolated from an ulcerated lesion of CL patient were used to inject BALB/c mice via intra-dermal (ID) and intra-venous(IV) infections (6 each with 2 controls similarly treated with normal saline). Similarly another set of mice were infected intra-dermaly with *L. donovani* wild type strain (Ld1S). Mice were euthanized after 10weeks following inoculation. Both spleen and liver were removed and cultured. The effect of infection on the animal including its weight, spleen, parasitic load and induction of IFN-γ and IL-10 in splenocytes and lymph nodes of BALB/c mice were measured. Skin lesions were observed in the ear piece of ID-infected BALB/c mice (n=4/6) and none of them showed any sign of visceral infection. However, infection of spleen was evident in 4 out of 6 IV-infected mice. All BALB/c mice infected with Ld1S showed spleen infection. High parasite burden and IL-10 levels were observed in spleen and lymph nodes of the BALB/c mice infected with Ld-1S, IFN-γ was low in these cells. Moderate levels of IFN-γ was observed only in mandibular node and splenocytes of ID-infected (with local parasites) BALB/c mice, while minute levels of IL-10 was observed of these animals. Splenocytes and popliteal lymph node of IV-infected (with local parasites) BALB/c mice

showed a moderate level of IL-10 and low level of IFN-γ. Local strain of *L. donovani* has the capacity to establish infection in BALB /c mice, inducing visceral disease. The level of IL-10, IFN-γ and the route of infection play a role in determining disease outcome. This study also may imply that, the local strain, though predominantly dermotropic in humans may acquire the ability to visceralize over time. The use of this model is being pursued for detailed investigation of this parasite.

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CUTANEOUS LEISHMANIASIS DUE TO *LEISHMANIA DONOVANI*: ROLE OF IL-4 AND IFN-γ IN LESION HEALING

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Sri Lanka is a newly identified focus of cutaneous leishmaniasis (CL) caused by the usually visceralizing *Leishmania donovani*. In situ cytokine expression plays a key role in the pathogenesis and lesion healing. This study describes the association between expression of Interleukin (IL)-4 and Interferon-gamma (IFNg) and time taken for lesions to heal. Skin biopsies from 58 patients with parasitologically or histopathologically confirmed CL and 25 healthy controls were collected in RNAlater and quantified for local tissue expression of IL-12A, IL-4, IL-10, IFNγ and TNF-α by real-time RT- PCR using SYBR green. Relative copy numbers were calculated by $2^{-\Delta\Delta Ct}$ method using β-actin as the reference gene and healthy controls as calibrators. Patients were treated with intra-lesional sodium stibogluconate. Correlation between cytokines and time taken to heal estimated with Spearman's rank correlation test. Study group consisted of 37 males (63.8%) and 21 females (36.2%) with a mean age of 35 years (SD=12.05, range 18-66) and a mean lesion duration of 6.75 months (SD=9.1, range: 1-48). Type of lesion varied from papules and nodules to non-healing ulcers. A total of 44 (75.8%) patients, consisting of 28 (63.6%) males and 16 (36.4%) females were followed up for time taken to heal. The mean treatment duration was 3.0 months (SD=1.75, range 1.5-8) and correlation coefficient between relative gene expression and time taken to heal for IL-12A, IL-4, IL-10, IFNg and TNF-α were 0.073, 0.321, -0.002, 0.257 and 0.155, respectively. A significant positive correlation was found between IL-4 and time taken to heal (p= 0.034). A tendency to have increased expression of IFNg was also observed though statistically not significant. Increased expression of both IL-4 and IFNγ are predictors of poor lesion healing in CL due to *L. donovani*.

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OXYGEN METABOLISM REGULATES MACROPHAGE SUSCEPTIBILITY TO *TRYPANOSOMA CRUZI*

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Macrophages (MΦ) are one of the early responders to control the causative agent of Chagas cardiomyopathy, *Trypansoma cruzi*. Infection and incomplete clearance of *T. cruzi* by MΦ result in parasite dissemination to peripheral tissues, and is a significant cause of chronic disease progression. Aerobic metabolism is often dependent on mitochondrial oxidative phosphorylation, and is associated with the activity of immunomodulatory macrophages (M2). Conversely, mitochondria-independent, glycolytic metabolism provides substrates to produce anti-microbial mediators by pro-inflammatory macrophages (M1). Reactive oxygen species (ROS) and nitric oxide (NO) are key M1 molecules for host defense against intracellular pathogens, which are associated with MΦ utilizing oxygen-independent metabolism. The susceptibility of macrophages to *T. cruzi* infection and incomplete clearance has been previously suggested to be due to lack of substantial pro-inflammatory activation of MΦ, however, the required mechanisms for a potent macrophage response for *T. cruzi* clearance remain unknown. In this study, we report a potent induction of the TNF-α pro-inflammatory cytokine, and deficient production of ROS and NO by macrophages infected with *T. cruzi*. Mitochondrial gene expression and cell respiration analysis suggested that *T. cruzi* infection elicit a metabolic response in MΦ which is similar to M2. These findings suggest that modulation of oxygen metabolism may improve the macrophage function for pathogen clearance to limit disease progression.

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MACROPHAGE CELLULAR IMMUNE RESPONSES IN CUTANEOUS LEISHMANIASIS AGAINST *LEISHMANIA DONOVANI*

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Leishmaniasis has a spectrum of manifestations including cutaneous, mucosal and visceral disease, caused by parasitic protozoa of the genus *Leishmania*. The clinical outcome of *Leishmania* is determined primarily by the infecting species and the immune response of the host. In Sri Lanka localised cutaneous leishmaniasis (LCL) is caused by *Leishmania donovani*; a visceralizing species. We hypothesized that the distinct alterations in the early immune response determines the outcome of infection observed in Sri Lanka. Aim of this study was to characterize the immune response in LCL by macrophages; which is central to both replication and elimination of the parasite. Peripheral blood mono nuclear cell (PBMC) derived macrophages from LCL patients (n=8) and healthy non endemic controls (n=8) were stimulated *in vitro* with *L. donovani* antigen (50µg/ml). The production of IL-10, TNFα, TGF β and Nitric Oxide (NO) were measured by ELISA and Griess test at predetermined time intervals. The two groups were compared using Student's T-test for parametric and Mann-Whitney test for non- parametric data. IL-10 production by patient macrophages was significantly higher (105.68±26.05vs 19.81±28.24pg/mL;p<0.01) at 72 hours but did not vary markedly at 24 & 48 hours. Production of TNFα by patients macrophages was significantly higher at 24 (15.63±16.44vs5.43±1.41;p<0.01),48(438.42±140.63vs30.06±24 .82;p<0.01)&72 hours (412.31±222.11vs14.41±11.68;p<0.01). TGF β production was higher at 24(1539.80±490.40vs1080.19±366.87)&48ho urs(1962.29±754.94vs1456.77±811.99)than the controls, but the values didn't vary significantly. Production of NO showed increased levels by LCL macrophages at 72 hours (5.40±1.15vs2.36±1.21; p<0.01). These data suggest IL-10; TNFα & NO play a role in determining disease outcome in LCL due to *L. donovani*. In contrast to TNFα, the contribution of IL-10, TGF - β and NO appear to be later in the infection. The findings should be interpreted in the context of changes in other imflammatory mediators, to better understand the underlying pathogenic mechanisms where a visceralizing *Leishmania* species is localized to the skin.

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IDENTIFICATION OF MICRORNA-21 AS A BIOMARKER IN LIVE ATTENUATED *LEISHMANIA* VACCINE INDUCED PROTECTIVE IMMUNITY

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No vaccine exists against visceral leishmaniasis. In an attempt to develop effective vaccines, we have reported extensively on the immunogenicity of live attenuated LdCentrin1-/- mutant in animal models. However, for the use of LdCen1-/- in humans there is a need to develop biomarkers associated with protection and safety. As a first step, we infected *ex*

vivo normal human macrophages with LdCen1-/- and compared with LdWT infection. We identified several microRNAs that regulate important cytokine genes, significantly induced upon LdWT infection compared to LdCen1-/- infection. Importantly, we found a strong induction of microRNA-21 (miR-21), which was shown to degrade mRNA encoding IL12, in LdWT infection compared to LdCen1-/- infection. IL12 produced by DCs is critical for priming a host protective Th1 cell response during *Leishmania* infection. To validate the role of miR-21 in regulating IL12 during *Leishmania* infection, we altered the miR-21 expression in murine DCs infected with LdWT or LdCen1-/-. Silencing of miR-21 using specific inhibitors resulted in an augmented induction of IL12 in LdWT infected BMDCs, illustrating the role of miR-21 in LdWT mediated suppression of IL12. In contrast, LdCen1-/- infected BMDCs, showed a strong induction of IL12, and miR-21 silencing resulted in a further increase in IL12 levels. Our data from *in vitro* human macrophages and mouse dendritic cell experiments suggests that miR-21 plays a role in early IL-12 mediated immunity and could be an important biomarker for LdCen1-/- vaccine immunity in human clinical trials.

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CTLA-4 AND ICOS COSTIMULATORS: POSSIBLE ROLE DURING ACTIVE VISCERAL LEISHMANIASIS

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Visceral leishmaniasis (VL) is an endemic disease found in many countries around the world. In Latin America, *L.* is the main etiologic agent for VL. During symptomatic VL a temporary anergy, *Leishmania* antigen-specific, is observed, which is reversed after clinical cure. Coestimulation can be involved in anergy, when T lymphocytes tend to increase expression of molecules such as ICOS and CTLA-4. We evaluated those two costimulators in T lymphocytes within whole blood samples collected from subjects with symptomatic VL (sVL) and after their clinical recovery (rVL), in addition, cytokines were also measured. We found that during sVL there was an increase in CD4 and CD8 cells expressing CTLA-4, in *ex vivo* condition (exvc), when compared to rVL group ($p< 0.05$). Moreover, CD8 T cells from sVL expressed more CTLA-4 ($p< 0.01$) after stimulation by soluble *Leishmania* antigen (SLA), but not in rVL or control groups. A 9.3 fold increase in the relative expression of CTLA-4 was observed in sVL when compared to rVL; however, there was no difference when cells from the goups were stimulated with SLA (fold change of 0.702 and 0.992, respectively). An increase in CD4 and CD8 cells expressing ICOS, in exvc, when compared to rVL ($p< 0.01$ to CD4, and $p< 0.05$ to CD8) and control group ($p< 0.01$ to CD4 and CD8) was observed. After SLA stimulation, both CD4 and CD8 cells from sVL showed an increase in ICOS, when compared to unstimulated samples ($p< 0.01$ to CD4 and CD8). The relative expression of ICOS in sVL was 3.78 fold higher when compared with rVL; whereas, opposite results were found after SLA stimulation (2.8 fold change, and 0.5 fold change, respectively). The ratio of INFγ to IL10 was higher after clinical recovery. These findings support the role of CTLA-4 and ICOS in the reversible anergy observed during sVL and might indicate pathways to be explored for immunotherapy against visceral leishmaniasis.

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BIOMARKERS OF PROTECTIVE IMMUNITY INDUCED BY LIVE ATTENUATED *LEISHMANIA DONOVANI* PARASITES IN PRESENCE OF ASYMPTOMATIC INFECTION

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Currently there is no vaccine against visceral leishmaniasis (VL). Towards developing an effective vaccine, we have reported extensively on the immunogenicity of live attenuated LdCentrin1$^{-/-}$ mutants in animal models. In VL endemic areas, asymptomatic carriers outnumber symptomatic cases of VL (9:1) and are considered to be a reservoir of infection. Vaccination of asymptomatic cases represents a viable strategy to eliminate VL. Studies in our lab using LdCen1$^{-/-}$ parasites that secrete model epitopes have shown that experimental infection of mice with LdCen1$^{-/-}$ results in robust CD4+ memory T cell induction. Protection mediated by such immune memory against virulent challenge was observed in hosts that have not previously been exposed to infection. Immunological correlates of protection thus derived might have limited applicability in conditions where the immunized host has prior exposure to virulent infection. To examine whether LdCen1$^{-/-}$ parasites can induce protective immunity in experimental hosts that have low-level parasitemia from a previous exposure mimicking an asymptomatic condition, we infected mice with wild type *Leishmania donovani* parasites expressing LLO epitope 3 weeks prior to immunization with LdCen$^{-/-}$ parasites expressing 2W epitope to characterize the immune responses in the same host. Flow cytometric analysis of the antigen experienced T cells enriched using specific tetramers showed that comparable memory T cell responses (CD4$^+$ T central memory) represented by CD62L^{hi}, CCR7$^+$, and IL-7R$^+$ T cell populations can be induced with LdCen1$^{-/-}$ in asymptomatic hosts to that of LdCen1$^{-/-}$ immunization alone. These results demonstrate that LdCen1$^{-/-}$ immunization could be efficacious for use in asymptomatic VL individuals.

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B-CELL ACTIVATING FACTOR (BAFF) IS INVOLVED IN DEVELOPMENT OF SPLENOMEGALY DURING EXPERIMENTAL VISCERAL LEISHMANIASIS

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Splenomegaly is one of the major symptoms during visceral leishmaniasis (VL). However, the mechanisms underlying splenomegaly remain unclear. We previously reported that serum levels of B-cell activating factor (BAFF) in VL patients were significantly higher than those in healthy controls, as reported previously. Since mice overexpressing BAFF are known to show splenomegaly along with increased number of B cells, we examined if BAFF is also involved in splenomegaly during VL by using an experimental model. BALB/cA mice inoculated i.v. with 1×10^7 promastigotes of *Leishmania donovani* developed splenomegaly, with higher spleen weight at 12 and 24 weeks post infection compared with naive mice. Those infected mice with enlarged spleen had significantly higher levels of serum BAFF compared with naive mice. Flow cytometric analyses of splenocytes revealed increased CD19$^+$ (B cell marker) lymphocytes as a major contributor to splenomegaly in the infected mice. When BAFF gene knockout mice were infected with *L. donovani*, the spleen weights at 12 and 24 weeks of infection were significantly lower than those of infected wild-type mice. Increase of CD19$^+$ lymphocytes in the spleen after infection was significantly suppressed in BAFF-knockout mice compared with the wild-type mice. Taken together, these results suggest that BAFF-mediated increase of B cells is the major cause of splenomegaly during experimental VL.

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DETECTION OF A FLAGELLAR ANTIGEN OF *TRYPANOSOMA CRUZI* IN URINE OF PATIENTS WITH HIV/CHAGAS CO-INFECTION USING NANOPARTICLES

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Chagas disease, caused by the protozoan parasite *Trypanosoma cruzi*, is one of the major health problems affecting Latin American population. In patients co-infected with HIV,the reactivation of Chagas disease is almost always lethal and diagnostic tests are not effective in predicting reactivation ahead of time, when a pharmacological treatment would be life-saving. Currently immunological diagnosis based on the detection of anti-*T.cruzi* antibodies have poor specificity and sensitivity because it is affected by the genetic makeup of both the parasite and the human population studied.Antigen detection tests may provide a solution to these issues because they directly detect the presence of the parasite in a body fluid. Technical and biological issues that hampered antigen test development in the past include:low abundance,masking by high abundance resident proteins and extreme labiality. In order to address these issues we developed a affinity hydrogel nanoparticles that performs in one step a)concentration of the target analyte,b)size sieving and c) complete protection from degradation.This work aims to develop a novel test for the detection of *T.cruzi* antigens in urine. For this purpose we have developed new antibodies against flagellar protein of *T. cruzi*,from rabbits and chickens. The purified antibodies were evaluated by Western blot and were used in a magnetic ELISA assay to achieve a sensitivity of 0.5ng/mL. Affinity hydrogel nanoparticles will be used to increase the analytical sensitivity 100 fold and test diagnostic specificity and sensitivity in patients co-infected with HIV and Chagas disease.In parallel,an affinity hydrogel particles enhanced quantitative protein macro array test will be developed in order to verify the concentration of the *T. cruzi* flagellar antigen in the urine of patients. Our previous results using this system for spiked urine with flagellar protein of *T. cruzi* showed a sensitivity of 0.1ng. One goal of this work is to develop a self-working,low cost,visual urine test for Chagas disease that achieves a clinical sample sensitivity ten to 100 fold higher compared to existing technology.

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IMPROVING ACCESS TO ESSENTIAL OXYGEN THERAPY AND PULSE OXIMETRY FOR CHILDREN

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An estimated 15% of children under five who are hospitalized for pneumonia—the leading infectious cause of child mortality worldwide—have hypoxemia, and yearly around 1.5 million children with severe pneumonia require oxygen treatment. Hypoxemia is a risk factor for death in pneumonia—increasing mortality by five times in some settings—and is a complication of other common childhood diseases. Accurate identification of hypoxemia and provision of oxygen are essential components of a strategy to reduce child mortality, supported by new World Health Organization (WHO) guidelines on oxygen therapy in children and technical specifications for oxygen concentrators. Improvements in oxygen supply have been shown to reduce up to 35% of child pneumonia deaths. Despite its necessity for child survival, oxygen therapy is not prioritized nor included on the WHO or most national-level essential medicines lists for children, and oxygen supplies and pulse oximetry are often not available in many pediatric wards. We conducted stakeholder consultations with over 50 key informants to assess priorities and challenges associated with ensuring availability of oxygen and pulse oximetry in settings with a high burden of childhood pneumonia. Informants represented global and national policy, procurement, manufacturing, regulatory, and programmatic decision-makers. Key findings from the consultations and literature suggest policy leadership and financial investments are needed by governments, donors, and global manufacturers to increase availability of oxygen and pulse oximetry in low-resource settings. Identifying current coverage and barriers to access for oxygen are key to developing a full understanding of how to ensure inclusion of oxygen and pulse oximetry in current normative policies, treatment guidelines, health budgeting, system infrastructure, and programmatic priorities. Utilizing this evidence to advocate for prioritization of oxygen for child health and increasing availability of appropriate oxygen concentrators and pulse oximetry could help improve access to essential therapy and reduce pneumonia mortality in children.

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DRUG RESISTANCE AND MOLECULAR CHARACTERIZATION OF *MYCOBACTERIUM TUBERCULOSIS* ISOLATED FROM PULMONARY TUBERCULOSIS SUDANESE PATIENTS

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Currently, Sudan is suffering from many factors like tribal restlessness and poverty causing its population to be in a continuous movement in and outside the country in seek for a better life. These movements mostly lead to the transmission of diseases among which is pulmonary tuberculosis. WHO estimated that one third of the world's population are infected with *Mycobacterium tuberculosis* and approximately 9.7 million new cases of tuberculosis were diagnosed globally in 2013. Sudan is considered as one of the countries having a high prevalence of tuberculosis. The current study aimed at determining the susceptibility of *M. tuberculosis* isolates to the first line anti-tuberclosis drugs. Isolated organisms were also genetically identified to be allocated and compared with species identified elsewhere in the global. Two hundred forty three sputum samples were collected in the period from May 2007-October 2013 from central, eastern and western Sudan. These were namely; Khartoum state, Portsudan and Elobeid respectively. Sputa were examined microscopically after being fixed and stained with ZN stain. Sensitivity to Rifampicin; INH, Ethambutol and Streptomycin was tested using proportion method after establishing microbial culture. Moreover, Spoligotyping and MIRU-VNTR were used to discriminate between different strains of *M. tuberculosis* and results were compared to the international database. From this work it was obvious that tuberculosis is prevalent in different parts of Sudan and all age groups can are affected. 58% of the cultured samples were positive for *M. tuberculosis* in L.J. medium. The overall resistance to anti-tuberculosis drugs was 20% and Multi drug resistant strains (MDR) were 8.7% All strains were grouped into 28 different spoligotypes. A total of 70 strains have unique patterns and were considered as orphan strains. We recommend that further studies are to be done to identify other mycobacteria species causing TB and to investigate its association with drug resistance.

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THE USEFULNESS OF OXIMETRY IN TRIAGING FEBRILE CHILDREN AT OUTPATIENT LEVEL: EXPERIENCE FROM A CLINICAL TRIAL IN DAR ES SALAAM, TANZANIA

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The objective was to determine the usefulness of oximetry in the outpatient setting in detecting febrile children with severe respiratory distress requiring referral to a higher level of care. A sub-cohort of febrile children aged 2-59 months from a larger non-inferiority trial that investigates a novel electronic algorithm for management of fever in Dar es Salaam, Tanzania, was included. Oxygen measurement was performed in using a hand-held device in all children connected to the smartphone with the built-in algorithm. Children with cough and oxygen saturation of <90% received pre-referral antibiotic and salbutamol treatment and were referred to the nearest hospital. All children were followed until clinical cure or death. 1590 children were enrolled into this sub-cohort from December 2014 to February 2016 out of which 922 (58.0%) of patients had cough, 26 (2.6%) chest indrawing, and 20 (2.0%) a respiratory rate >97th %ile for age and temperature, respectively. 4 patients presented with hypoxemia (0.4%) of which all had other signs of respiratory distress, i.e. chest indrawing and respiratory rate >97th %ile for age and temperature. Two patients were eventually diagnosed with cyanotic congenital heart disease of which one patient died at the referral hospital, likely from incorrect administration of oxygen. The other two patients had lower respiratory tract infections and fully recovered. In conclusion, in the Tanzanian outpatient setting, hypoxemia is very uncommon and clinical signs and symptoms, including chest indrawing, may have superior performance in detecting children with severe respiratory distress requiring higher level of care. In resource-poor settings, oximetry and oxygen should be implemented in hospitals but might not be useful at peripheral level.

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EARLY TREATMENT OUTCOMES FOR THE FIRST COHORT OF PATIENTS INITIATED ON PULMONARY MULTI-DRUG RESISTANT TUBERCULOSIS TREATMENT AT PUBLIC REGIONAL REFERRAL HOSPITALS IN UGANDA

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Multi-drug resistant tuberculosis (MDR-TB) is an emerging public health concern in Uganda, with over 1,000 new cases notified in 2014. Before 2013, MDR-TB treatment was provided by Mulago National Referral Hospital (NRH) while Regional Referral Hospitals (RRH) only had the capacity to do surveillance for diagnosis and would refer patients to the NRH for treatment. However, patients diagnosed at RRH had challenges accessing the NRH. Since 2013, the USAID's SUSTAIN project has supported scale-up of provision of drug resistant (DR)-TB treatment at six Ugandan RRH using a combination of ambulatory and hospitalization models of care. We analyzed data on the first cohort of patients started on MDR-TB treatment at SUSTAIN-supported RRHs. This study was a retrospective descriptive analysis of data collected on 69 patients started on MDR-TB treatment at six RRHs between 1st April 2013 and 30th June 2014. Nineteen (30.4%) patients were female, 39 (56.5%) HIV-negative, 30 (43.5%) resistant to both isoniazid and rifampicin and 57 (82.6%) category 1 or 2 failures. At the start of MDR-TB treatment, their median age was 35 years (SD ±13.5), mean time-to-treatment initiation 96 days and out of the 30 HIV-positive patients, 27 (90%) were on anti-retroviral treatment with a mean CD4 count of 258. Within six months of treatment, fifty-nine (87%) patients achieved favourable treatment outcomes (45:65.2% culture converted at two months and 14:20.3% by the sixth month) while 10 did not (1: 1.5% did not culture convert while three: 4.4% each died, were transferred out, or were lost-to-follow up). During treatment, 32 (46%) patients experienced at least one severe drug adverse event and all were managed clinically. The median weight gain was 3.0 kilograms (SD ± 4.52). Despite delays in MDR-TB treatment initiation after diagnosis, a reasonably high proportion of patients achieved early culture conversion. Reasons for the high proportion of HIV-negative patients started on MDR-TB treatment should be investigated. These encouraging interim outcomes indicate a successful scale up of DR-TB treatment from NRH to RRH.

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EVALUATION OF VITAMIN D LEVELS AND PREVALENT TB AMONG HIV INFECTED IN ZAMBIA

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Vitamin D insufficiency is highly prevalent in the general population of the United States and appears to play an important role in immune regulation and tuberculosis (TB) progression. Little is known about Vitamin D in African HIV-infected populations. We thus evaluated the association of serum vitamin D levels with prevalent TB, incident TB, HIV progression, and biomarkers of nutritional status among new enrollees at an HIV clinic in Lusaka, Zambia at baseline and up to 12 months after enrollment. All consenting adults without current or recent history of TB were screened for TB regardless of symptoms according to current World Health Organization (WHO) recommended guidelines. Two sputa, one blood and one urine specimen were cultured. For participants consented to participate in a TB specimen repository for serum and urine specimens were stored at -80 oC. Baseline serum samples were analyzed for levels of Vitamin D, pre albumin, albumin, lipid profile, CD4+ count, viral load (VL), creatinine and hemoglobin. Vitamin D insufficiency was defined as <30 ng/ml, and deficiency <20 ng/ml. A total of 285 samples were tested. Forty-three (16%) patients were diagnosed with bacteriologically confirmed TB at enrollment. Mean age was 35 years; 47% were female; and mean body mass index was 22. Median CD4+ was 205 cells/mL and mean VL 4.6 Log10. Vitamin D insufficiency was detected in 43%(127/294) and deficiency in 28% (82/294). Patients with CD4<100 had higher pre-albumin, albumin and HDL-cholesterol (p<0.01), lower triglycerides (p<0.01), and were older (p,0.01)compared to other groups. No significant associations were found between baseline vitamin D levels and prevalent TB at enrollment or incident TB at 6 month. However, a trend was observed for baseline prealbumin levels to be lower in the prevalent (median 8.0 mg/dl) and incident (10.5 mg/dl) TB groups compared to participants without TB (16.0 mg/dl). High prevalence of Vitamin D insufficiency levels were observed in this population. Although not associated with prevalent or incident TB, further analysis is required to understand the immunological effect on HIV and or recurrent TB.

1772

IGG ANTIBODY SECRETION IN LYMPHOCYTE SUPERNATANT AMONG PAKISTANI CHILDREN WITH CONFIRMED TUBERCULOSIS

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Antibody Secreting Cells (ASCs) are terminally differentiated B-cells that release pathogen-specific antibodies in response to infection. ASC activity has been harnessed as a diagnostic test for infections including tuberculosis (TB): by isolating and culturing peripheral blood mononuclear cells (PBMCs) for 24-72 hours, the secreted anti-mycobacterial IgGs can be estimated via ELISA methodology using Bacille Calmette Guerin (BCG) vaccine as the coating antigen. Here, we aim to compare the yield of the MASC assay using different incubation time points among children confirmed to have pulmonary TB. Among a cohort of children 1-14 years old being evaluated for pulmonary TB, we included children with respiratory specimens that were positive for *Mycobacterium tuberculosis* (MTB) by GeneXpert. Venous blood (3-12mLs) was obtained for the MASC test. Briefly, mononuclear cells were cultured with 10% FBS at the concentration of 5-10x10^6 cells/ml in 24-well tissue culture plate at 37°C for 24, 48 or 72 hours. Culture supernatants were collected at each time point and stored at -80°C. ELISA plates were coated with 1µg / well of BCG vaccine (Japan BCG laboratory). Culture supernatants were added in ELISA plates after blocking nonspecific sites and incubated for 2 hours at 37°C. After washing, HRP labeled secondary antibody was added for the detection of IgG. The results were expressed as relative optical densities (O.D.) of IgG. A cut-off of 0.35 was used for a positive test [1]. Eight children with a median age of 12.5 years (IQR: 3.825) had GeneXpert positive respiratory samples. MASC results from six children were consistently above the threshold for a positive test at all incubation periods. The median ODs did not significantly differ across the selected incubation periods (p=0.727, Mann-Whitney U test; see Figure). Mycobacterium-specific IgG can be detected in lymphocyte supernatant from children with microbiologically confirmed TB. The assay demonstrates consistent results across the selected incubation periods, suggesting that this assay could be optimized to provide relatively rapid results, within 2-3 days after collection of blood.

1773

CHARACTERIZATION OF AN ALGORITHM FOR LOCAL SEASONAL INFECTIOUS DISEASE OUTBREAK DETECTION USING A SIMULATION STUDY

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Control of seasonal outbreaks plays a vital role in public health, particularly in healthcare settings where the young, elderly, and immunocompromised are at the highest risk. Early detection of seasonal virus outbreaks at the level of individual hospitals and community centers are key to controlling their epidemic potential. The Above Local Elevated Respiratory Illness Threshold (ALERT; http://reichlab.github.io/alert.html) algorithm is an online tool designed for clinicians and hospital epidemiologists to use in triggering enhanced protective measures for healthcare workers and hospital visitors. In this study, we use an influenza A dataset from a pediatric hospital to compare the date-based trigger strategy recommended by the CDC to the case-count threshold trigger implemented using ALERT. We found that a threshold case count of 3 was able to reliably out-perform the date-based threshold approach by reducing the median duration by 3 weeks, increasing the median percent cases captured from 93.5% to 95.1%, and increasing the minimum cases captured from 56.2% to 68.9%. Then we fitted a model to a subset of the flu A dataset and tested its ability to make one-step ahead predictions of the reserved testing subset of the flu A data. Once we confirmed that our model was representative of real seasonal influenza time-series, we used the model coefficients to generate data for a simulation study. We then systematically characterize ALERT performance on simulated data generated across a range of endemic and epidemic values in order to develop guidelines for when ALERT may be an appropriate tool for rule-based decision-making. We conclude with a discussion of these guidelines and suggestions for how ALERT may be leveraged for other infectious diseases, such as dengue fever, to enable clinicians and epidemiologists to make evidence-based public health decisions, particularly in low-resource settings.

1774

A GLOBAL FRAMEWORK FOR STRATEGIC TUBERCULOSIS PREVENTION AND CONTROL IN THE WORKPLACE

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Tuberculosis (TB) prevention and control activities in the workplace are an important consideration for multinational corporations with workforces in mixed-risk settings. Preventing TB transmission at ExxonMobil and its affiliated companies (EM) is especially challenging due to the global nature of our workforce and varied workplace settings which can include congregate living. Furthermore, EM operates in more than 50 countries, some having a high incidence of TB. To protect the workforce and minimize operation disruptions, EM has implemented a strategic TB Control Program to mitigate the risk of TB transmission among its workforce. Components of the program include worker awareness, conducting screening, coordination of care, and contact tracing. Eligible employees and contractors live in or travel to high-risk settings for 30 cumulative days per year. High-risk settings include all EM camps or dormitories in countries with TB incidence ≥ 20 cases per 100,000 population as well as all offshore sites. Workers in the program receive a baseline screening test upon enrollment, and are subsequently screened annually. Annual screening consists of a risk-based questionnaire and, when indicated, a T-Spot or Quantiferon screening test. For those testing positive, further evaluation is conducted for potential TB diagnosis and referral to a healthcare provider. If an active TB case is identified, contact tracing is promptly initiated in conjunction with relevant health authorities. Surveillance of cases through data management is key to stewardship of the program. In 2015, 9000 TB screening tests were performed, resulting in 1220 latent TB infections cases being identified among employees and contractors. Identification of these latent infections is imperative to effective TB control in the workplace, as early detection improves outcomes and overall productivity of the workforce, preventing illness and therefore potential for operations disruption. EM's TB Control Program is a pragmatic, tiered and scalable approach to mitigate the spread of TB among workers in areas of varying TB incidence, protecting our workplaces around the world.

1775

DECIPHERING LONG TERM DYNAMICS AND ASSESSING IMMUNIZATION CAMPAIGNS OF MEASLES IN CHINA

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Industrialization and demographic transition generate non-stationary dynamics in human populations that can affect the transmission and persistence of infectious diseases. Decades of increasing vaccination and development have led to dramatic declines in the global burden of measles, but the virus remains persistent in much of the world and measles is still one of the leading causes of vaccine-preventable childhood diseases. An international collaborative study was carried out by researchers from WHO, US and China CDC, and universities to decipher the dynamics of measles in China. Based on 50-year long term various measles surveillance data from national, provincial and city level sources, here we show that a combination of demographic transition, as a result of declining birth rates, and reduced prevalence, due to improved vaccination, has shifted the age distribution of susceptibility to measles throughout China. We estimate the relative change in the force of infection in 6 focal provinces across China as well as the impact of supplemental vaccination activities on the reduction of the susceptible population. The force of infection of measles has declined dramatically in the industrialized eastern provinces during the last decade, driving a concomitant increase in adult cases who had been protected from infection as children by herd immunity, while central and western provinces exhibit dynamics consistent with endemic persistence. The shift in the age distribution of susceptibility emphasizes the importance of progressive control strategies and measures to evaluate program success that anticipate this transition in observed incidence. Further, the regional differences in the persistence of measles across China suggest the importance of targeted efforts to interrupt transmission in endemic areas. We also developed novel modeling approach for immunization intervention effectiveness assessment when surveillance data is strongly biased or not available. The theoretical understanding and analytic approach in our study could shed light on how the ongoing global measles eradication campaign reaches its goal successfully.

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RESPIRATORY OUTBREAKS DURING AN OUTBREAK INVESTIGATION COURSE

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During a one-week outbreak investigation course in South America, the faculty noted an increase of respiratory symptoms among students and then conducted a quick outbreak investigation for academic purposes. The course has both a plenary component (25 hours) and case study component (11 hours) where the class is divided in six groups of 10-12 students. In course day 5, all 70 students were interviewed using a self-administrated questionnaire assessing signs and symptoms, demographic information, and location in the plenary and workgroup. Two case definitions were used, one based on self-report of "disease" and another using the most frequent signs and symptoms. Sixteen students (23%) reported themselves as "sick", 63% with headache, 56% with sneezing, 56% with rhinorrhea, 31% with sore throat, 31% with cough and 25% with fever. Up to 3 cases started symptoms on each of course days 1-4 and seven started symptoms in day five. The attack rate differed significantly by case study groups (range: 0-75%, $p<0.001$) and also by plenary table row ($p=0.012$) but not by plenary table column, gender, age or region of origin. An alternative, parsimonious case definition of sneezing (24 cases) or headache (12 cases), led to an attack rate of 29 cases (41%), capturing 15 cases not self-identified as "sick" and all but two cases with any symptoms. Most new cases (87%) only reported sneezing. Incidence of cases found only with the second definition differed by case study group (0-60%, $p=0.001$) but not by other characteristics. The case study groups with highest incidence differed for self-defined and not self-defined cases. A similar course was taught later in Central America without noticing potential outbreaks of respiratory illness. Two clearly-differentiated outbreaks may have presented in a five-day course, suggesting person-to person-transmissions of separate respiratory viruses, both with short incubation period probably due to close proximity of students, particularly during case study groups.

1777

IMPLICATION OF SOUND RECORDING SYSTEM ON TREATMENT SUCCESS FOR TB PATIENTS IN PORT HARCOURT NIGERIA

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In 2006, the World Health Organisation (WHO) established a Global Task Force on tuberculosis (TB) Impact Measurement and one of the mandates was to strengthen national capacity in monitoring and evaluation. This requires improvement of data quality especially completeness and accuracy of records. In Nigeria as in most countries, each TB patient has a treatment card on which the most relevant processes for patient care are documented. Proper completion of all of these care processes in the patients' treatment card is essential to survey the disease dynamics, assess program progress/gaps and plan for future interventions. The aim of this study was to determine the completeness of TB patients' records

and its implication on treatment outcome. This was a facility-based cross-sectional study, using data from treatment cards of 243 patients seen from November 2012 to October 2013. Following data extraction, proportions were calculated for completeness of patient treatment cards. Chi-squared statistic was computed for dependent variables such as treatment success and independent variables such as sputum AFB tests. Logistic regression was done to determine predictors of treatment success. Of the 243 patient treatment cards reviewed, only 23.9% were complete. Assessment of the individual variables revealed the following proportions of completeness: initial AFB test - 84.8%; 2nd AFB - 74.1%; 3rd AFB - 47.6%; weight at commencement - 99.2%; 2nd weighing - 72.8%; 3rd weighing - 46.5%; intensive phase treatment - 99.2%; continuation phase treatment - 77%. The treatment success was 49%. Predictors of this outcome were: complete acid alcohol fast bacilli tests, odds ratio 5.18 and 95%CI (2.08 - 12.89); and compliance to continuation phase, odds ratio 8.47 and 95%CI (3.31 - 21.68). The WHO targets for STOP TB are dependent on the credibility of readily available data generated from the health facilities. The completeness of records in the facilities assessed for this study adversely affects the validity of the treatment outcomes recorded.

1778

ANTIMYCOBACTERIAL AND PHYTOCHEMICAL ANALYSIS OF METHYL TERT-BUTYL ETHER EXTRACTS FROM THE FRUIT SKIN AND LEAVES OF *ANNONA MURICATA* LINN

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Arising from the Millennium development goals and Stop TB strategy of the World Health organization into the Sustainable goals cum End TB strategy, there is urgent need to fast-track research along proffering solution to the agelong burden of Tuberculosis. The current study examined the antimycobacterial activity and phytochemical constituents of methyl tert-butyl ether (MTBE) extracts from the fruit skin (epicarp) and leaves of *Annona muricata* Linn. The extracts were prepared from the matured unripe fruits and leaves of *A. muricata* with MTBE for accurate lipidome profiling. Antimycobacterial activity was determined Drug susceptibility testing (DST) procedure on Lowenstein Jensen (LJ) media. Three concentrations (1, 40 and 250 µg/ml) of the extracts were prepared with the LJ media and subsequently inoculated with 10-3 and 10-5 suspensions of both control (H37Rv) strain and a clinical isolate (MTB-584) of Mycobacterium tuberculosis. LJ media prepared with Rifampicin at 40 µg/ml served as the standard drug for positive control while plain media with respective inoculum represented the negative control. Four Ziehl-Neelsen's stain slides were also prepared to confirm the presence of organisms in the two suspensions employed for the two strains tested. Plain media with drops of distilled water were employed as normal control to check for possible contaminant. The inoculated media and control slants were incubated at 37oC and observed every seven days for a period of six weeks. The antimycobacterial analysis result showed that the organism strains exhibited resistance to the extracts at tested concentrations as there was substantial growth with typical creamy non-pigmented morphology on all the LJ media prepared with extracts. There was no growth on the media with standard drug and on those with distilled water as expected. Tannins, saponins, flavonoids, anto- and betacyanins, terpenoids, phenols and steroids were present in the extract. The conclusion from the foregoing is that MTBE extracts from the fruit skin and leaf of *A. muricata* at tested concentrations have no antimycobaterial activity.

1779

A LECTIN-BASED ASSAY FOR DETECTION OF SCHISTOSOMIASIS

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Schistosomiasis is a debilitating neglected tropical disease caused by trematodes of the genus *Schistosoma*. Current serological tools based on antibody detection lack the capacity to distinguish current from former infections after successful chemotherapy. A commercially available point-of-contact test to detect adult worm circulating cathodic antigen (CCA) in urine has been developed and is being evaluated for use in control programs. However, this test only reliably detects *S. mansoni* infections and may therefore not be as useful in *S. haematobium*-endemic areas in Africa. Furthermore, it has been difficult to conclusively determine whether persons who are CCA+ but *S. mansoni* egg negative are truly infected or may have non-patent infections. Another adult antigen, circulating anodic antigen (CAA) is more sensitive than the CCA test and can detect both *S. mansoni* and S. hematobium infections. However, this optimal sensitivity of this test requires a concentration step and is not currently amenable to point-of-contact testing. Both the CCA and CAA tests use monoclonal antibodies for antigen capture and detection, which adds to the cost of the tests. Therefore, although the CCA and CAA tests show promise for use in schistosomiasis control programs, we were also interested to see if it would be possible to detect free glycans secreted from eggs of both *S. mansoni* and S. hematobium. Schistosome-specific glycans terminating with α (1-2) fucose attached to internal N-acetyl glycosamine and N-acetyl galactosamine were investigated as potential diagnostic targets for schistosomiasis. Lectins from Ulex aeropaus and wheat germ agglutinin that bind these glycans with high specificity were functionalized with gold nano particles, agarose beads or horseradish peroxidase and used to detect schistosome egg glycans in lateral flow and ELISA assays. Urine from persons with *S. mansoni* and S. hematobium infections were positive by this test but urines from persons living in non-endemic areas were not. These results show promise for development of an inexpensive point-of-contact assay that is able to detect patent *S. mansoni* and S. hematobium infections.

1780

THE PERSISTENT PARASITE: WHY DO *SCHISTOSOMA MANSONI* INFECTION LEVELS REMAIN HIGH IN THE RURAL UGANDAN VILLAGE OF WAKAWAKA EVEN AFTER OVER A DECADE OF TREATMENT?

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Despite more than a decade of mass preventive chemotherapy, age-infection profiles of *Schistosoma mansoni* have shown that prevalence and intensity of infection in Eastern Uganda are still high. With a global shift to the elimination of schistosomiasis in the 2012 World Health Assembly resolution, there is a need to identify why more than 70% prevalence and high intensities of infection are still identified in those most at-risk populations, school-aged children. The aim of this study was to identify possible non-biological contributors to this trend by interviewing 248 individuals across all age groups in Wakawaka village, a large fishing community, in the district of Bugiri. The survey explored the social, behavioural and economic background of the participants, in addition to gathering information on living environments and access to healthcare. For children aged between 3 and 15 years an additional innovative activity was carried out which involved a colouring sheet identifying transmission routes. Only half of the colouring activity questions were marked correctly. For the survey, results revealed 80% of the participants continue to

use Lake Victoria as their primary water source for activities other than drinking. Additionally 67% thought that most 'worm based infections' came from drinking dirty water and only 18% of participants correctly identified that swimming in contaminated water was a source of infection. Knowledge of STH were similar with 98% unable to identify walking barefoot and not washing hands as risk behaviours and only 30% of the survey participants forbidding their children from defecating in the open. Almost 14% of the population had lived in the area for 2 years or less. Results show that there is still a dearth of community level understanding of schistosomiasis and STH and their transmission routes despite numerous treatment rounds and high levels of infection. If programmes are to move from control to elimination, then we need to strengthen current strategies with improved treatment coverage and sensitisation, taking into account communities that are mobile, access to safe clean water and community awareness.

1781

COMMUNITY-WIDE PATTERNS OF INFECTION AFTER MORE THAN TEN YEARS OF PREVENTIVE CHEMOTHERAPY FOR SCHISTOSOMIASIS AND SOIL-TRANSMITTED HELMINTH INFECTION IN UGANDA: ARE WE READY TO MOVE BEYOND MORBIDITY CONTROL?

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In 2003, Uganda was one of the first sub-Saharan African countries to implement national-scale control programmes for schistosomiasis and soil-transmitted helminth (STH) infection, which have aimed to control infection-associated morbidity through mass drug administration (MDA) of suitable anthelmintics to school aged children (SAC) and other high risk groups. SAC harbour the highest prevalence and intensity of schistosome infections which then typically decrease with age. Control programmes often incorporate a monitoring and evaluation (M&E) component to estimate a programme's impact on population levels of infection, using data collected from SAC. More data are needed, however, to understand the impact on the wider community when only SAC are targeted; this understanding is essential to determine the feasibility of shifting the focus towards elimination. Using baseline data from 2003 and data from a two year full age-infection (AI) survey from 2014 and 2015, we aim to provide an accurate picture of the change in infection patterns in Uganda. In the full AI study, data were collected from approximately 7500 individuals each year, across a wide age-range (<1 to >50 years) from 10 different sites in Uganda, which varied by initial underlying endemicity and treatment history. Results showed that the AI profiles for *Schistosoma mansoni* and hookworm followed similar patterns as observed at baseline and in other studies. Between 2014 and 2015, no significant treatment impact was observed for *S. mansoni* and hookworm. When analysing results by underlying endemicities, the high prevalence sites and the low prevalence sites, both of which had received multiple rounds of treatment since 2003, showed no reduction between the two years. Conversely, for the low prevalence sites that had not received treatment until 2014 (i.e. previously treatment-naïve), we observed a decrease in prevalence and intensity. We will discuss the age-related changes in intensity and prevalence in each subset; possible explanations for the trends observed and their significance levels; and compare the age-specific force of infection from baseline to the recent data.

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MODELLING THE EFFECT OF A POTENTIAL VACCINE APPLICATION ON THE SCHISTOSOME PARASITE DYNAMICS

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Schistosomiasis affects approximately 258 million people, killing an estimate of 280 thousands per year. This makes the development of an effective vaccine to help, alongside mass drug administration (MDA), in the elimination of the disease within the near future a necessity. To date, the primary form of treatment is the drug praziquantel, which gives some reductions in the burden of the infection but repeated annual administration is required over many decades to eliminate the burden of disease. A potential vaccine candidate could act to reduce parasite establishment, survival and fecundity within the host population. Analysed data from experiments with a candidate prophylactic vaccine application in a nonhuman primate model, the baboon, gives evidence that the development of a partial efficacious vaccine may be a possibility. We describe the construction and use of mathematical models of candidate vaccine community based impact alongside the use of MDA. We focus on vaccination effect on both the host population and the parasite's dynamics. We run the models under different scenarios by taking into account various crucial assumptions about the vaccine candidate. These include the effectiveness of the vaccine and the rate of loss of vaccine-induced immunity. We also run the simulated models for the combination of mass drug administration (MDA) and community based cohort vaccination to test if the elimination of the disease can be achieved more quickly with a partially efficacious vaccine.

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UROGENITAL SCHISTOSOMIASIS: WHAT DO SCHOOLCHILDREN IN THE EASTERN REGION OF GHANA KNOW ABOUT THE DISEASE?

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Urogenital schistosomiasis (UGS) is endemic in the Eastern Region of Ghana, particularly amongst primary-school aged children in rural communities. Educating children about UGS through the public school system in endemic areas can be an effective primary prevention strategy that accompanies mass drug administration. However, little is known about the baseline knowledge of schoolchildren in the Eastern Region of Ghana regarding UGS, and the individual and community-wide characteristics that predict levels of knowledge. The objective of this study was to determine the baseline knowledge of students in the Eastern Region of Ghana regarding UGS, and then to determine the extent to which year in school, sex, and district of residence predict UGS knowledge among schoolchildren. Over a 17-day period, we conducted a cross-sectional study among 1813 primary and junior high school children in public schools across 37 randomly selected towns within 10 districts in the Eastern Region of Ghana. All participants were given a 22-question knowledge survey on the transmission, treatment, and symptoms of schistosomiasis and protective measures that can be taken to prevent infection. A score was assigned to each student representing the number and percentage of questions answered correctly. Overall, the average score on the knowledge survey was 57.5%. Junior high school students had a mean score of 63.0% while primary school students had a mean score of 51.5%. Responses indicate that knowledge of how the disease is transmitted and how the disease can be treated is lacking among both primary and junior high school students. Linear regression analyses indicate that sex, class year, and district of residence are predictive of student performance on the knowledge survey, with class year as the strongest predictor. Linear regression and chi-squared analyses indicate that boys systematically perform better than girls on the knowledge survey, and

junior high school students systematically perform better than primary students. These findings are valuable for officials engaged in the planning and implementation of UGS educational interventions.

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SPATIOTEMPORAL MODELING OF SCHISTOSOMIASIS IN GHANA: LINKING REMOTE SENSING DATA TO INFECTIOUS DISEASE

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More than 90% of the worldwide schistosomiasis burden falls on sub-Saharan Africa. Control efforts are often based on infrequent, small-scale health surveys, which are expensive and logistically difficult to conduct. The use of satellite imagery to predictively model infectious disease transmission has great potential for public health applications. The transmission of schistosomiasis, a disease acquired from contact with contaminated surface water, requires specific environmental conditions to sustain freshwater snails. If a connection between schistosomiasis and remotely sensed environmental variables can be established, then cost effective and current disease risk predictions can be made available. Schistosomiasis transmission has unknown seasonality, and the disease is difficult to study due to a long lag between infection and clinical symptoms. To overcome these challenges, we employed a comprehensive 15-year time-series built from remote sensing feeds, which is the longest environmental dataset to be used in the application of remote sensing to schistosomiasis. The following environmental variables will be used in the model: accumulated precipitation, land surface temperature, vegetative growth indices, and climate zones created from a novel climate regionalization technique. This technique, improves upon the conventional Köppen-Geiger method, which has been the primary climate classification system in use the past 100 years. These predictor variables will be regressed against 8 years of national health data in Ghana, the largest health dataset of its kind to be used in this context, and acquired from freely available satellite imagery data. A benefit of remote sensing processing is that it only requires training and time in terms of resources. The results of a fixed effects model can be used to develop a decision support framework to design treatment schemes and direct scarce resources to areas with the highest risk of infection. This framework can be applied to diseases sensitive to climate or to locations where remote sensing would be better suited than health surveys.

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EPIDEMIOLOGICAL MAPPING OF SCHISTOSOMIASIS AND SOIL TRANSMITTED HELMINTHIASIS IN 19 STATES AND THE FEDERAL CAPITAL TERRITORY (FCT), NIGERIA

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The coordinated mapping of Schistosomiasis and Soil Transmitted Helminthiasis (STHs) was conducted in 19 States and the FCT of Nigeria from November 2013 to May 2015.Both diseases were mapped using a novel technique; the LINKS system developed by the Task Force for Global Health (TFGH), on Android devices and cloud based data reporting and management. The application of these devices supported the transition from paper-based questionnaires to electronic data collection tools. A total of 108,472 children from 2160 schools in 433 LGAs in 19 States and the Federal Capital Territory (FCT) were examined for Schistosomiasis and STHs. The Kato-Katz, filtration techniques were used to examine stool and urine samples Also, Water, Sanitation and Hygiene (WASH) information for schools and school children were collected. The result of this survey revealed that all the States and the FCT were endemic for one or both diseases with an overall prevalence of 9.5% for Schistosomiasis and 27% for STHs. However, the data captured by LGA; the intervention unit, showed that prevalence of infections varied from low to high risk. The prevalence of infections was significantly higher in males than in females for both diseases. STHs were more prevalent among the younger age group (5-10years) while Schistosomiasis was more prevalent among the older age group (11-16 years).Heavy intensity levels were nearly equal for S.haematobium (24.31%) and *S. mansoni* (23.48%). The intensity levels of *S. haematobium* and A. lumbricoides showed statistical significant difference ($P<0.05$) with respect to sex in this survey. STHs and Schistosomiasis were seen among pupils who claimed to defeacate in the school toilets, around the school compound and outside school environment. Of the 433 LGAs surveyed the number of LGAs requiring interventions for Schistosomiasis and STHs were 359 and 237 respectively. The mapping exercise provided insight into disease distribution and intensity in 19 States and the FCT. It is recommended that Government and stakeholders should scale up mass deworming alongside WASH interventions.

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EVALUATION OF A URINE POOLING STRATEGY FOR THE RAPID AND COST-EFFICIENT PREVALENCE CLASSIFICATION OF SCHISTOSOMIASIS

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Schistosomiasis is geographically focal, making it difficult to target with mass treatment through preventive chemotherapy. The aim of the present study was to evaluate the diagnostic performance of a urine pooling strategy using a *Schistosoma mansoni* point-of-care circulating cathodic antigen (CCA) test, and then use simulation modeling to test the classification accuracy and efficiency in determining where preventive chemotherapy is needed in low burden settings. We performed a cross-sectional study in 114 children in six neighborhoods in Azaguié Ahoua, Côte d'Ivoire to characterize the sensitivity and specificity of the CCA test with urine samples that were tested individually and in pools of 4, 8, and 12. We used a latent class model to estimate test characteristics for individual CCA and quadruple Kato-Katz thick smears. We then developed a microsimulation model and used Lot Quality Assurance Sampling to test the performance of the urine pooling strategy and traditional stool microscopy in predicting the binary need for school-based preventive chemotherapy using WHO's 10% prevalence threshold recommendation. We estimated the number of tests and total cost of each strategy, and tested robustness of the simulation through sensitivity analyses. The overall sensitivity of the urine pooling strategy for pool sizes of 4, 8, and 12 was 85.9%, 79.5%, and 65.4% when CCA trace results were counted as positive, and 61.5%, 47.4%, and 30.8% when CCA trace results were counted as negative. The modeled specificity ranged from 94.0-97.7% for the urine pooling strategies (with trace CCA results

categorized as negative). The urine pooling strategies (pool size=4, 8, 12) gave comparable, and often superior, classification performance to stool microscopy for the same number of tests, and the urine pooling strategy (pool size=4) reduced number of tests and total cost compared to stool microscopy. This study introduces a rapid, cost-saving urine pooling strategy to inform where preventive chemotherapy against intestinal schistosomiasis is necessary that does not depend upon slide preparation, microscopy, or a formal laboratory.

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SIZE MATTERS: CHANGING POPULATION STRUCTURE MEANS CHANGING SAMPLING REQUIREMENTS FOR SCHISTOSOME POPULATIONS

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Eradication or local extinction of schistosomiasis is a goal for nearly all control programs today. We have demonstrated how genetic markers can be used to evaluate control programs, indicate incipient resistance and perhaps predict the extinction of a local population. Our studies have been conducted by collecting parasites from all identified infections in a population to calculate individual host differentiation indices (Di) and community effective population size (Ne). Collecting all individuals and genotyping their parasites, however, would be impractical on a large-scale, thus we sought to determine the relative error associated with different sample sizes. Using data collected in 2009 and 2012 from two villages in Bahia, Brazil, we calculated Di based on samples of different sizes. Individual infections were selected at random with replacement to produce samples ranging in size from 5-50% of the total. Each size class was repeated X 30. Di was calculated for each using SPADE. Error rates of ± 5%-10% of the true value of Di were taken as acceptable limits. The percent of groups outside this range was then calculated. We thus compared 2009 and 2012 for these communities, since the Di and Ne changed following community-wide treatment. Between 2009-2012 there was no difference in Di for one, but did increase for the other. Ne fell by 15 fold for each site. When the Di is moderate and Ne large, taking samples of 30-40% of the population was within the 10% limit 60% of the time. When the Di increased and Ne reduced, the curves were less steep, but shifted upward so that samples from composed of 30% of the infected had only a 50% chance of being within 10% of the true value and where the Di was significantly higher, there was only a 40% chance of being in this range. The chance of obtaining differentiation indices outside of the acceptable error range with smaller sample sizes increases when the population has undergone a bottleneck. In order to acquire the most representative results for population genetics of *S. mansoni* some characteristics such as population size, prevalence of the parasite, history of treatment in the community has be taken into account.

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SCHISTOSOMIASIS DIAGNOSIS AND CLINICAL MANAGEMENT: USE OF IMMUNODIGNOSIS, DNA BASED ASSY AND DETECTION OF CIRCULATING CATHODIC ANTIGEN (POC-CCA) PRE AND POST-PRAZIQUANTEL IN NON ENDEMIC AREAS

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Diagnosis of *Schistosoma mansoni* infection in travelers and immigrants living in nonendemic areas can be troublesome. Parasitological methods and tissue biopsy underdiagnosis acute and chronic infection with no or low egg counts. As an alternative to traditional methods, rapid tests (RT) like POC-CCA has been used in some institutional settings. However, no consensus toward the use of RT in clinical protocols was achieved. The study objective was to evaluate POC-CCA in both schistosomiasis diagnosis and post-therapy response in non-endemic areas. Thirteen individuals living in the non-endemic area with a history of previous exposure to S.mansoni participated in the study. Fecal samples were tested by Kato-Katz (KK) and DNA amplification by Real-Time PCR. Tissue biopsy was also performed when available. Serum IgG1 anti-adult worm (SMMA) levels were detected by ELISA (arbitrary units:a.u., positive > 1 a.u.). For CCA detection, urine samples were tested by POC-CCA (Rapid Medical Diagnostics, Pretoria, South Africa). Praziquantel (40mg/kg) was used for treatment. Responders to therapy were defined as KK and/or PCR negative (KK/PCR). Nine male and four female (mean age:34.5±15.9 years old) participated in the study. Seven individuals presented asymptomatic infection and two manifested acute schistosomiasis. Intestinal severe form and neuroschistosomiasis were diagnosed in two and one individual, respectively. Active schistosomiasis was confirmed by KK in 7/13 individuals, being 6/13 KK negative. IgG levels detected 10/13 and reactivity varied from 0.1 to 4.9 a.u.(mean±std: 1.7 ± 1.19 a.u.). Real-time PCR showed DNA amplification in 13/13 individuals (Ct mean ± std:28.03 ± 14.09). POC-CCA was reactive in 9/13 individuals (mostly weak reactivity or trace). After PZQ treatment, cure rates determined by KK/PCR and POC-CCA were 100% and 61.53%, respectively. KK/PCR combined and POC-CCA is a reliable diagnostic strategy to detect active *Schistosoma* infection in nonendemic areas. However, use of POC-CCA as a marker of drug response is still debatable.

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SCHISTOSOMIASIS IN SUB-SAHARAN AFRICA: SUCCESSES AND BARRIERS TO COMPLETE ERADICATION

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From the narrative of the German explorer of Central and West Africa, Gustav Nachtigal, Schistosomiasis, a neglected tropical disease of poverty has plagued various nations in sub-Saharan Africa since 1881. Schistosomiasis exert great health, social and financial burden on the economies of the region having profound negative effects on child development, outcome of pregnancy, and agricultural productivity. Sixty-seven percent (11.7 million) of people treated in 2008 for schistosomiasis, were from sub-Saharan Africa with Nigeria being the most endemic country for schistosomiasis, burdened by approximately 20 million people mostly children needing treatment. While countries such as Japan, Tunisia and some Caribbean Island countries have made significant progress on the control and management of this disease, sub Saharan countries are still groaning under the burden of this impoverishing disease. This review focuses on the history, epidemiology, successes, and barriers impeding the complete eradication despite significant re-awakening efforts by such organizations as, WHO, State Ministries of Health and the Carter Center to end the anguish of this silent disease.

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THE ASSOCIATION OF RESISTANCE TO *SCHISTOSOMA MANSONI* REINFECTION AND HOST IMMUNITY IN MBITA KENYA COHORT

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Schistosomiasis has been a threat for inhabitant living in endemic area where it is inevitable to contact with the schistosome cercaria infested water in daily activities. Without treatment, schistosomiasis can persist for years and develop to chronic infection with egg-induced pathologic complications including liver fibrosis, bladder fibrosis and cancer. Identification of protective immunity against schistosomiasis is really critical for strategy of vaccine development. In Mbita, Kenya, a *Schistosoma*

mansoni endemic area, we enrolled 160 preschool- and school-age children aged 4-15 years and monitored them by Kato-Katz examination and circulating cathodic antigen (CCA) tests. The children with egg positive stools or CCA positive urine were cured twice with Praziquantel and 2-month interval. Three months after the last treatment, the examination showed that 20% (32 cases) were re-infected (susceptible) with egg positive stools, 55% (88 cases) non-infected (resistant) with egg negative stools, 7% (11) with natural non-infection and 18% (29) did not supply stool samples. Immunological investigation indicated that plasma levels of SWA-specific IgG1 was higher in resistant- than susceptible-children (absorbance of 450nm: 0.271 vs 0.135, $p < 0.05$, respectively) whereas there was no difference in plasma levels of SWA-specific IgG4. Production of IFN-γand IL-13 from blood cells under stimulation of SWA were higher in resistant- than susceptible- children (793 vs 15.6 pg/ml; 272 vs 68 pg/ml, respectively, $p < 0.05$). Meanwhile, IL-10 production was similar in these two groups of children. Under non-specific stimulation, resistant children possess higher proportions of IFN-γproducing CD4+ T and non-CD4 non-CD8 T cell than susceptible children (2.2% vs 1.19 %; 0.76% vs 0.34%, respectively, $p < 0.05$), whereas there was no difference in proportion of IL-13 producing CD4+T cells in these two groups (1.58% vs 1.78%, respectively). In conclusion, anti-SWA IgG1 antibody, CD4+ and non-CD4 non-CD8 cell derived IFN-γ and IL-13 could be protective factors against reinfection of S.mansoni.

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DEMOGRAPHIC COVARIATES OF CHOLERA RISK IN CAMEROON

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Since cholera's arrival in Cameroon in 1971, the country has experienced multiple outbreaks with thousands of reported cases. The outbreak in 2010-2011 has been Cameroon's largest, with over 27,000 people infected. During the 2010-2011 outbreak, the cholera attack rates among Cameroon's 179 health districts varied widely, ranging from 0 to 1,139 cases per 100,000 people. Using data compiled in the Demographic and Health Surveys and national surveillance data reported by the Ministry of Health, this study examines the relationship between demographic covariates and cholera risk. In a country-wide analysis of all health districts, we found the number of children under five living in a home and family size to be positively associated with attack rate. When analyzing health districts outside of the southwestern part of the country, higher education, access to improved sanitation, and higher SES were all negatively associated with cholera attack rates. Access to improved water and cholera attack rates were not associated. In the southwest part of the country, the covariates were no longer sensible predictors of cholera risk. Different environmental conditions in the southwestern part of Cameroon may be driving these results, for example as a consequence of the construction of the Bamendjin dam. The results suggest that basic demographic variables may serve as useful predictors of cholera risk and, in conjunction with environmental variables, may inform policy including the deployment of the oral cholera vaccine stockpile.

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SHARED SANITATION FACILITIES AND TWO PATHWAYS OF DIARRHEAL DISEASE TRANSMISSION: A MODELING STUDY

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In many low and lower-middle income countries, access to sanitation is limited for many individuals. Open defecation contaminates the environment and facilitates the transmission of diarrheal pathogens that are transmitted via the environment. The provision of sanitation facilities that are shared by many individuals, such as shared latrines in slums, are considered by many public health officials as the only short-term solution to this problem, especially in urban slums in Asia and Africa. However, epidemiological evidences have shown that shared sanitation may actually increase the prevalence of diarrheal diseases. One hypothesis that may explain this phenomenon is that many different pathogens may cause diarrhea. While sanitation facilities reduce the contamination of the environment by human defecation, unhygienic sanitation facilities are actually fomites for the transmission of other diarrheal pathogens that are transmitted directly between humans. We propose a mathematical model that seeks to explain how the alleviation of environmental transmission of pathogens such as cholera via shared sanitation can lead to an amplification of direct transmission caused by other diarrheal diseases such as norovirus. The model is an ordinary differential equation (ODE) model that runs parameters chosen from a Latin hypercube sampling. After sieving through the parameter space to select parameter sets yielding outbreaks within a specified threshold, varying levels of shared sanitation coverage are implemented on these sets. Changes in disease prevalence are then plotted with respect to these varying levels of shared sanitation intervention. Results give quantifiable evidence that in the presence of an environmental and a person-to-person spread pathogen, total disease prevalence can increase. Under certain conditions the model shows an optimal level of shared sanitation intervention that can decrease environmental disease prevalence while not increasing person-to-person transmission too much. In any case, shared sanitation is most effective when viewed as a long term strategy.

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NOT IN MY BACKYARD: AN INDIVIDUAL-LEVEL META-ANALYSIS OF THE ASSOCIATION BETWEEN COMMUNITY OPEN DEFECATION AND STUNTING

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Approximately 1 billion people live without access to proper toilets and practice open defecation, a practice which the Sustainable Development Goals wishes to end by 2030. Open defecation facilitates the transmission of various diarrheal diseases as well as soil-transmitted helminthes. Access to sanitation decreases the risk of these diseases and any subsequent issues with development thereafter, including stunting. We conducted an individual-level meta-analysis of 177 publicly available, nationally representative household surveys to measure the impact that living in an open defecation free community has on child growth stunting among children aged 12-59 months. To account for selection bias we first matched children on the following parameters: community-level wealth, individual-level wealth, community-level water access, community-level health care access, and mother's education. Second we stratified the analysis among children living in households with and without a latrine. We then adjusted for a variety of factors known to be associated with child stunting (i.e. breastfeeding, immunizations, birth order, wealth, drinking water) and measured the incremental impact of community-level latrine ownership among children with and without latrines. Among children living in households with latrines, living in open defecation free communities (all households have latrines) was associated with decreased odds of stunting (odds ratio [OR] = 0.95, 95% confidence interval [CI] = 0.92 - 0.99). Among children living in households without latrines, living in communities with less open defecation was associated with decreased odds of stunting (OR = 0.96, CI = 0.93 - 0.99). The elimination of open defecation is an important sustainable development goal and as the results of our study suggests can subsequently have important benefits in health as indicated by child stunting.

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COMMUNITY BASED METHODS FOR SCHISTOSOMIASIS PREDICTION AND SUSTAINABLE CONTROL

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Schistosoma haematobium transmission is influenced by environmental conditions that determine the suitability of the parasite and intermediate host snail habitats, as well as by socioeconomic conditions, access to water and sanitation infrastructure, and human behaviors. We present a mixed-methods approach that builds on the remotely sensed ecological variables by exploring water and sanitation related community characteristics as independent risk factors of schistosomiasis transmission. The study area includes 74 rural communities in the Eastern Region of Ghana. Environmental conditions relevant for disease transmission such as stagnant or slow moving water bodies, riparian vegetation and water surface temperatures have been derived using remote sensing data from the Landsat 8 and Sentinel 2A satellites, as well as the 30m Advanced Spaceborne Thermal Emission and Reflection Radiometer Global Digital Elevation Model (ASTER GDEM) and integrated into a habitat suitability index (HSI). Additionally, for each study town, GPS coordinates and basic field survey data were available for all public water sources including improved water infrastructure according to the Joint Monitoring Program definition, and surface water access points. We calculated improved water coverage expressed as % of population with access to an improved water source within 100-500 meters of residence and groundwater quality score related to iron, salinity and hardness of the water as well as a recreation potential score. The HSI was complemented with community specific variables to predict schistosomiasis risk based on the hypothesis that in a small geographical area with minimal variability in environmental conditions, these potential community level drivers of surface water contact increase schistosomiasis risk. We validated the model using schistosomiasis prevalence data from a field survey.

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THE ROLE OF ENVIRONMENTAL PROCESSES IN INFECTIOUS DISEASE DYNAMICS

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For many infectious pathogens, the environment plays an important role in transmission from one host to another. This environmental mediation may occur through a number of media—including air, food, and fomites—but water is especially of interest in the transmission, fate, and transport of enteric pathogens. Mitigation, therefore, often involves environmental WASH interventions designed to reduce one's exposure to pathogens. Mathematical modeling can be used as a tool to investigate and assess potential interventions, allowing for more effective intervention design and allocation of resources, and has been used in the past to study waterborne outbreaks, notably in the recent cholera epidemic in Haiti. We leverage modeling of dose-response relationships and pathogen persistence in the environment to provide an improved mechanistic understanding of interventions. We conduct a comparative analysis to assess interventions that are designed explicitly to reduce per-contact pathogen load—such as water filtration devices—and those designed to reduce the frequency of contact with pathogens in the environment—such as sanitation interventions.

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INTEGRATING WATER SANITATION AND HYGIENE PRACTICES AND NEGLECTED TROPICAL DISEASE INTERVENTIONS: EXPERIENCE FROM SOUTHERN TANZANIA

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Tanzania is endemic for NTDs particularly the 5 PCT NTD,Trachoma being one of them. Efforts for control and elimination of The NTDs are underway since 1990s,they include MDA, Health education and Promotion and Morbidity Control. Following mapping, Trachoma was found to be endemic in 54 districts in year 2004 -2006 surveys and Mass treatment started in a phased approach. As per WHO a comprehensive SAFE strategy is paramount for Trachoma elimination but there has been some limitations due to several factors. Moreover a clear assessment of Integration of Water Sanitation and Hygiene (WASH) practices has not been well assessed. In 2014, The programme designed a project to assess and operationalize Integration between WASH and NTDs Interventions in two districts of Tunduru and Namtumbo of Ruvuma region, Southern Tanzania. Baseline sanitation data was collected through house to house visits using a questionnaires as well as from sanitation registers. Trachoma prevalence for Tunduru was from the surveys and most updated data shows a TF 7.20%, TT 1.2% while Namtumbo TF of 2.20%, TT 1.10% in 2014. Local artisan Training was conducted to all communities of the two councils and 20 artisans were trained. At each level a combination of two people was made in each visit an NTD personnel and WASH personnel. Assessments done after 1 year indicates both ; Increased number of Households with improved latrines from 603 to 2015 households out of 8063 total of households in Namtumbo and Tunduru. Moreover, households with fixed hand wash facilities increased from 57 to 1698 household in the period of March November 2015.For Trachoma Prevalence Namtumbo district has passed the threshold of <5% TF prevalence and thus do not require MDA for zithromax. These results signifies good cooperation/collaboration between government officials, partners and councils at all levels and the 2 districts can meet Trachoma elimination targets if integration of sanitation and hygiene with NTD interventions is strengthen.

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CHOLERA AND ENVIRONMENTAL DYNAMICS IN AN ECUADOREAN ESTUARINE SYSTEM

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The emergence of waterborne diseases such as cholera, whose causative agent is pathogenic strains of *Vibrio,* is strongly linked to the local environmental and ecological context. Machala is a port city of 250,000 people in El Oro province, on the southern coast of Ecuador, near the Peruvian border. The 1991-2004 cholera pandemic emerged in Peru and spread north into El Oro, making it a key sentinel site for understanding dynamics in the ongoing 7th pandemic. In Machala, many peoples' livelihoods depend on the estuarine system, from fishing for subsistence and trade, to domestic water use, making the coupled human-estuarine system an important component of public health management. We sampled five estuarine locations twice weekly over a 10-month span, within a gradient of human use, and over a geographic range from inland to ocean, to measure water-specific environmental variables such

as pH, temperature, salinity, conductance, and algal concentration, and conducted PCR testing for *Vibrio spp.*, including pathogenic strains, across 5 months. Our sites exhibited considerable seasonal and spatial heterogeneity in environmental variables, with clear peaks during specific months. We found evidence of an environmental reservoir for *Vibrio spp.*, including pandemic strains O1 and O139, but did not confirm ongoing toxigenic presence. We found that the timing of positive PCR results was coupled to the environment. This study was conducted in a moderately normal climate year, providing a preliminary framework for monitoring coupled *Vibrio* – estuarine dynamics for potential emergence of cholera outbreaks in the region.

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GLOBAL TRACHOMA MAPPING PROJECT: SANITATION COVERAGE THRESHOLD LEVELS AND PROTECTION AGAINST TRACHOMA

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Improved sanitation is thought to reduce trachoma by reducing the number of fly breeding sites. No study has attempted to characterize the thresholds of sanitation for trachoma; particularly, if there is a lower sanitation coverage threshold required to reduce trachoma, and also if there is some upper threshold at which sanitation might reduce trachoma even for those who don't use latrines through herd protection. We used data from the Global Trachoma Mapping Project (GTMP), collected between December 2012 and January 2016. To date, we have included data from 325,315 participants from 5 countries; additional data will soon be available from 17 countries in sub-Saharan Africa, Oceana, Asia, and Central and South America. Data cover all endemic districts in these countries using cluster sampling. Participants were surveyed about WASH access and practices and both eyes were examined for trachomatous inflammation - follicular (TF), trachomatous inflammation - intense (TI), and trachomatous trichiasis. Our outcome was combined TF/TI in either eye (binary), and the primary exposures of interest were household-level access to improved sanitation, and community-level prevalence of improved sanitation. Community-level access was defined as the prevalence of sanitation aggregated at the cluster-level. We employed multivariable mixed-effects modified Poisson regression models to jointly assess the relationship between household and community toilet coverage on TF/TI prevalence. We graphically show and present the relationship between the trachoma outcomes and individual and community sanitation coverage. The trachoma prevalence from adjusted models was generally lowest among latrine owners who were also are in the top latrine coverage quartile (prevalence = 1.5%; 95% CI: %1.3-%1.7). Our study provides insights into the sanitation thresholds required to reduce trachoma and our findings will have considerable public health and policy implications for achievement of elimination of blinding trachoma by 2020.

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USE OF MULTI-PARALLEL QUANTITATIVE REAL-TIME PCR FOR GASTROINTESTINAL PARASITES IN RURAL MOZAMBIQUE: CORRELATION OF INFECTION INTENSITY TO WATER ACCESS, SANITATION AND HYGIENE (WASH)

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Sub-Sahara Africa has the highest rates of intestinal parasites worldwide. More than 30% of children in these regions have a parasitic infection. These infections have the potential to cause morbidity and are related to environmental conditions. Our objectives were to deploy the first time use of multi-parallel quantitative real-time PCR (qPCR) to describe epidemiology of common soil-transmitted helminths and protozoa, and association with living conditions. This study involved 250 children (ages 1 to 10 years old, Geomean = 4.98 years old) residing in rural areas in Manhiça, Mozambique. Participants presented for routine care, stool samples obtained, and extensive questionnaire on water access, sanitation and hygiene (WASH), clinical and laboratory data were obtained for all enrollees. DNA extraction of stool and qPCR was performed at the Centro de Investigação em Saúde de Manhiça (CISM). qPCR detected 65% of children with 1 or more parasites, these include *Giardia lamblia* 61%, *Ascaris lumbricoides* 10.2%, *Strongyloides stercoralis* 8.6%, *Cryptosporidium* 4%, *Necator americanus* 2.8%, *Ancylostoma duodenale* and *Entamoeba histolytica* were not detected, *Trichuris trichiura* results are pending. Concentration of *Ascaris* DNA was converted to eggs per gram (EPG) via previous correlation studies. Greater than 60% had heavy *Ascaris* EPG burdens. Heavy *Ascaris* EPG burden correlated with increased *Giardia* DNA burden in co-infected children (p = 0.0177). Preliminary data points to a higher prevalence of helminth and protozoal infections than previous known. Ongoing analysis will correlate WASH data to qPCR prevalence and co-infections, associating the lack of sanitation to higher rates and intensities of infections. These studies improve our understanding of the interaction between sanitation and parasitic infections, and build capacity for ongoing public-health initiatives in endemic regions.

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EFFECT OF A COMBINED HARDWARE AND BEHAVIOR CHANGE INTERVENTION ON HANDWASHING BEHAVIORS IN PRIMARY SCHOOL CHILDREN: THE POVU POA SCHOOL PILOT STUDY

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Kenyan schools often lack bar soap for handwashing and experience water scarcity. Soapy water can deter soap loss and is inexpensive. A behavior change intervention based on disgust and promoting social norm change increased handwashing in a community setting, but effectiveness in schools has not been assessed. In Kenyan public schools, we tested Povu Poa: a handwashing station with a dispenser that produces foam from soapy water along with a behavior change intervention for schoolchildren with disgust-based triggers and social norm change components. In a stepped-wedge cluster-randomized trial, we assessed effects of the intervention on 1) availability of handwashing materials, and 2) handwashing behavior after toilet use among schoolchildren. We randomly selected 30 schools in Kisumu and divided them into 3 groups of 10 schools. After baseline data collection, we delivered the intervention sequentially (Group 1: 3-5 weeks post-baseline; Group 2: 6-8 weeks; Group 3: 19-24 weeks). We observed outcomes at baseline and at Round 1: 3-5 weeks after baseline, Round 2: 9-12 weeks, and Round 3: 20-28 weeks. We compared outcomes at schools prior to intervention (Comparison Group) to outcomes at schools after intervention (Intervention Group). Water and soap / soapy water or foam were available at <1% of handwashing places in the Comparison Group, and at 50% of handwashing stations in the Intervention Group. In the Comparison Group, we observed handwashing with water after 13% of toilet use events; we did not observe any handwashing with soap. In the Intervention Group, we observed handwashing with water after 36% of toilet use events (RR = 5.14, 95% CI = 2.55, 10.34) and handwashing with foaming soap after 32% of the events (RR incalculable because there was no handwashing with soap in the Comparison Group). The Povu-Poa intervention increased handwashing in schoolchildren, although a sizable proportion of toilet use events were not followed by handwashing with soap. Investigation of barriers to both maintenance of the soap foamer and adherence to handwashing with foaming soapy water after toilet use would inform improvements in intervention design.

1801

EVALUATING THE IMPACT OF SCHOOL WATER, SANITATION AND HYGIENE IMPROVEMENTS USING THE PRESENCE OF SERUM ANTIBODIES FOR ENTERIC AND NEGLECTED TROPICAL DISEASES AMONG SCHOOL CHILDREN IN MALI

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The purpose of this study was to evaluate the biologic impact of school WASH improvements on enteric and NTD incidence among pupils in Mali. We piloted the use of dried blood spots (DBS) from school-aged children (SAC) to detect blood serum antibodies for 32 analytes of enteric and NTDs using a Luminex multiplexing assay. This method has yet to be employed among SAC, and has had limited employment in evaluation of WASH trials. We randomly selected 21 beneficiary schools participating in an evaluation of a comprehensive school-based WASH intervention in Mali, and their 21 matched control schools. In each school, 20 pupils were randomly selected and interviewed about their household WASH access, school absence, and recent illness. Capillary whole blood in the form of DBS was collected from each student. DBS were analyzed for blood antibodies for E. histolytica, Giardia, *Cryptosporidium*, *P. falciparum*, *P. vivax*, dengue, Chikungunya virus, *E. coli*, cholera, Salmonella, norovirus, *Campylobacter*, filariasis, Strongyloides, trachoma, cysticercosis, and schistosomiasis using a Luminex multiplexing assay. Data were analyzed using generalized linear latent and mixed models (GLLAMM). Factor analysis identified three distinct latent variables representing vector-transmitted disease, food- and water-transmitted disease, and person-to-person transmitted disease. The GLLAMM modeling framework consisted of a measurement model of these three latent variables, clustered at the school level and controlling for pupil age, and a structural model of the regression of intervention and household WASH access on the latent variables. Food/water transmitted disease and person-to-person transmitted disease was lower among pupils attending intervention schools. Vector transmitted disease was higher among pupils attending intervention schools. Results from this pilot support findings from the impact evaluation of the larger trial, which found reduced incidence of diarrhea among pupils attending beneficiary schools. Analysis of DBS is promising method to objectively evaluate WASH impacts in low-resource field settings.

1802

PREVALENCE OF ANTIBIOTIC-RESISTANT BACTERIA AND THEIR RESISTANCE GENES IN SURFACE WATERS IN A RURAL COMMUNITY OF BRAZIL

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Brazil uses less antibiotics for humans than the USA, but approximately the same amount in agriculture. We therefore studied the antibiotic resistance profile of a river system in a rural community of Bahia, Brazil. In this population of ~500, all homes have a flush toilet, but 50% of these flush to the river instead of a septic tank. Agriculture is the principle occupation, and cattle, pigs and chickens are kept within and around the community. In contrast to the USA, quinolones are not added to feed, except for treatments. River water was collected from 10 points where the population commonly has water contact as well as 2 samples of the local piped water. Monthly records of water quality and climate were made for all sites including physical characteristics, coliform count and qPCR for fecal microbial source tracking (MST). Bacterial susceptibility to ciprofloxacin, cefotaxime and meropenem was tested by disk diffusion. DNA was extracted from filter retentate of 500 ml collected from each site to identify known quinolone, cephalosporin and carbapenam resistance genes by PCR. Coliforms and *E. coli* were found at high concentrations at all sites. By contrast, MST indicated that the highest concentration of human fecal waste was downstream from the population center, and diminished 500 m downstream of the last home. In July of 2015, all sampled points showed at least one bacterial colony resistant to 1 of the 3 antibiotics. Four Enterobacteriaceae with resistance to at least 1 antibiotic were isolated, including one from the drinking water supply. Citrobacter freundii which proved to be positive for an extended beta-lactamase (ESBL) was found in one of the points. PCR assays for 4 common quinolone resistance genes, a cephalosporinase gene and 6 carbapenamase genes were negative at all sites. These results indicate that MST may provide a more reliable estimate of human fecal contamination; that surface waters in this rural community do not meet national standards for human contact and the drinking water was doubtful for consumption. Yet the limited use of quinolones in veterinary practice may explain the absence of resistance genes in these waters.

1803

FECAL FINGERPRINTS: THE LANDSCAPE OF ENTERIC PATHOGEN CONTAMINATION IN LOW-INCOME, URBAN NEIGHBORHOODS OF KISUMU, KENYA

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Children in developing countries are infected with a variety of enteropathogens in the first years of life, suggesting they experience complex environmental exposure risks. Little is known about how different fecal transmission pathways contribute to enteric infection patterns. During an exposure assessment, the Social Microbes study characterized the spatial distribution and correlative relationships for twenty-two types of enteropathogens in three low-income, urban neighborhoods of Kisumu, Kenya. Landscape features, condition of latrines, evidence of open defecation, zoonotic vectors, and number of children and their behaviors were recorded at sixty randomly-selected sites in each neighborhood (N=180). Soil and surface water from each site was analyzed using enterococci indicator assays. A microfluoridic qPCR tool was used to detect twenty-two enteric viruses, bacteria, or parasites in nucleic acid extracted from 0.5 grams of soil and 10 mls of surface water. Enterococci were detected in 100% of surface water samples (N=26) and 73% of soil samples (N=114) in two neighborhoods. Enteropathogens were detected in 92% of water samples (5.1 enteropathogens/sample) and 84% of soil samples (1.2 enteropathogens/sample). The five most common pathogens in water were *Cryptosporidium* spp. (89%), Enteroaggregative *E. coli* (EAEC) (77%), Enterotoxigenic *E. coli* (ETEC) ST/LT (54%), human adenovirus (50%), and *Giardia lamblia* (42%). Fourteen other enteropathogens were detected at lower frequencies. The five most common pathogens in soil were *Cryptosporidium* spp. (70%), *G. lamblia* (16%), EAEC (8%), human adenovirus (7%), and ETEC-LT (6%). Twelve other enteropathogens were detected at lower frequencies. Multidimensional correlation between enteropathogens was observed in water, but not soil. The variety and prevalence of specific enteropathogens detected in environmental fomites in Kisumu provides powerful clues for explaining the etiological complexity of pediatric enteric infection in developing countries, and highlights the need for improved exposure assessment methods for identifying fecal transmission pathways.

1804

MULTI-PARALLEL QUANTITATIVE REAL-TIME PCR FOR GASTROINTESTINAL PARASITES AND INFECTION BURDEN IN DISTINCT COLOMBIAN COMMUNITIES

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Gastrointestinal (GI) parasites are globally widespread infectious agents disproportionately affecting children in resource-deprived areas with associated morbidity that is poorly understood. Environmental surroundings influence exposure to these parasites as does the differences of water access, sanitation, and hygiene (WASH) between different community settings (urban, peri-urban, rural). Stool samples from 194 children in a urban slum (n = 72, mean age = 2.5 yrs), peri-urban (n = 50, mean age = 6 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, Cryptosporidium, Entamoeba histolytica*, and *Giardia lamblia*. Prevalence was 62.5% *Giardia*, 23.6% *Cryptosporidium*, 19.4% *Ascaris*, and 5.5% *Trichuris* in urban slum; 26.4% *Giardia* and 2.7% *Ascaris* in peri-urban area; and 68% *Giardia*, 20% *Entamoeba*, 50% *Ascaris*, 46% *Trichuris*, and 2% *Strongyloides* in rural area. Children infected with polyparasitism (2 or more parasites) correlated to living in rural areas compared to urban and peri-urban (59%, 19%, 0%, p = 0.001). Prevalence was lowest in peri-urban area, likely due to less exposures in older age group. Higher *Giardia* DNA burden correlated to living in urban slums (p = 0.008); potentially due to crowding and sharing contaminated water. Helminth burden was correlated to eggs per gram (EPG) with higher *Trichuris* burden found in rural areas compared to urban slum (9953 versus 325 EPG, p = 0.0023). Over 40% of helminth infections in rural area are classified as heavy burdens by WHO classes of intensity. Our data is useful for morbidity studies and public health interventions in highlighting need for improvements in WASH infrastructure. Ongoing work will contribute *Giardia* and *Cryptosporidium* spiking studies correlating trophozoite/cyst to burden. Future work will correlate parasite DNA to clinical outcomes and explore associations with childhood morbidity.

1805

ANTIBIOTIC STEWARDSHIP AND SANITATION: A MISSING PARTNERSHIP

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Antimicrobial resistance is a major global public health problem by limiting treatment of patients infected with multi-resistant bacteria. We evaluated the presence of resistance genes for quinolones (qnrA, qnrS, aac(6'), oqxA), cephalosporins (blaCTX-M) and carbapenems (blaOXA-48, blaVIM-2, blaNDM-1, blaKPC, and blaSPM) from a lake (DC) in a major urban center in Brazil (Salvador, Bahia) compared with a river system in a rural community in the state of Bahia, Brazil, a Lake (SL) in Cleveland, Ohio and the Cleveland sewer system. Water was sampled from DC in 2013 and 2015. All other sites were sampled in 2015. The 500 ml samples were filtered through a 0.22 µm pore nitrocellulose filter and DNA extracted with phenol-chloroform. Standard PCR assays were used to identify antibiotic resistance genes. Bacterial source tracking in DC showed high human fecal contamination similar to Cleveland sewage. For the DC in 2013, 2/15 samples were positive for OXA-48 and 7/10 in 2015. Of the 7 sites positive for OXA-48, 3 were also KPC positive. VIM-2 was identified at 2 sites. Quinolone resistance genes were found at multiple sites in 2013 for DC, but this analysis is pending for 2015. The sample taken from the sewer system of Cleveland was positive for only VIM-2. Few or no resistance genes were identified in river samples from the rural community in Brazil and the SL in the USA. From the latter, only 1 bacterial isolate was resistant to any antibiotics tested in contrast to all other locations where there were numerous resistant isolates. The earliest report of the OXA-48 gene family in Enterobacteriaciae in Brazil was from 3000 km south of Salvador in a hospital in Porto Alegre in 2013. This gene had, however, clearly entered the country earlier and was already widely disseminated in the environment in 2013. The frequency and number of antibiotic resistance genes in DC is alarming but not unusual for urban surface water that is used by the community for recreation and fishing. The presence of poor sanitation and feces in urban surface water is likely an important factor contributing to the spread of these genes to many bacterial species and back to humans.

1806

WHO INFLUENCES YOU? THE ROLE OF WOMEN IN INFORMATION DIFFUSION OF SANITATION AND WATER PRACTICES IN 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. Aside from vaccination, well known measures to prevent diarrheal infection include good water, sanitation, and hygiene practices. These behavioral practices, however, are influenced by a multitude of factors, including community-level social cohesion. Women, in particular, experience a continual tradeoff in daily tasks, including water-associated behaviors and child care, and likely play a role in influencing information diffusion in societies with high social cohesion. Previous studies conducted on coastal Ecuadorian populations have shown that a greater density of social ties between individuals in remote communities may lead to the spread of sanitation and water practices, both individual and collective, that help reduce the transmission of diarrheal disease. The role of women and the effect across time were not examined. We aim to examine the influential role women play on information diffusion, as defined by adopting improved sanitation and water practices, and diarrheal disease reduction in coastal Ecuador over the course of ten years. Using longitudinal social network data collected from villages in northern coastal Ecuador at multiple intervals from 2003 to 2013, we first defined communities with high and low social cohesion by measures of network density and clustering. We then measured node centrality, including average degree, closeness, and betweenness, by gender in networks of high and low social cohesion to examine the presence of influential nodes. We also assessed the presence of strong and weak ties. We conducted Markov-chain Monte Carlo models to determine the influence of women on the effect of high social cohesion on changes in sanitation and water practices and diarrheal disease over time. Qualitative data was used to describe difference in the role of women in communities of low and high social cohesion. By understanding who the influential persons are in social networks, we can better understand how to leverage social learning to reduce diarrheal disease transmission.

1807

CLINICAL PREDICTION RULE OPERATED BY MOBILE PHONES FOR EARLY DETECTION AND REFERRAL OF CUTANEOUS LEISHMANIASIS IN RURAL AREAS OF COLOMBIA

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Detection and diagnosis of cutaneous leishmaniasis (CL) in rural populations is a public health challenge. A Clinical Prediction Rule (CPR) previously validated in Tumaco could provide presumptive diagnosis of CL using 6 readily obtained variables. We sought to adapt the CPR as mobile phone app to facilitate case detection in rural areas and evaluate users performance and acceptability. 6 community volunteers and 3 health technicians were trained in the use of "Leishmaniasis App". During Feb 2015 to Mar 2016 patients with skin lesions were evaluated with the CPR and received parasitological tests. Number of confirmed cases and time from symptoms onset to diagnosis during were compared with data reported by the national surveillance system during 2012-2014. Agreement between community volunteers and health technicians with an experienced physician was estimated. Semi-structured interviews and focus group were used to evaluate users' acceptance and usability of the app. A total of 115 patients were evaluated, 83.5% had parasitological confirmation and 16.5% other dermatologic conditions. Confirmed cases increased 27% during the study period compared to years 2012-2014 (213 vs 167) and average time from symptoms onset to confirmed diagnosis decreased 53.8%, from 30.4 to 11 days. Overall agreement between the experienced physician and community volunteers was 93.8% (Kappa:0.68) and 96.8% (Kappa:0.72) for health technicians. Variables referred by patients (i.e. risk activities, vector contact and trauma) had ≥ 87.5% agreement. Presence of clustered lesions had agreement ranging 56.3% to 100%. Ninety percent of users fully agreed with usefulness of the app and 72% considered use of mobile phones easy and relevant. Main perceived barriers were cultural differences of indigenous communities and armed groups. Mobile phone use was facilitated by familiarity with technology and relevance of having an appropriate tool for CL detection. The use of a mobile app adapting a validated CPR by community volunteers and health technicians evidences the utility and acceptability of an m-health tool for presumptive diagnosis of CL in rural communities.

1808

THE EFFECT OF TEXT MESSAGE REMINDERS TO HEALTH WORKERS ON QUALITY OF CARE FOR MALARIA, PNEUMONIA, AND DIARRHEA IN MALAWI: A RANDOMIZED CONTROLLED TRIAL

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Mobile (mHealth) technologies hold promise as innovative ways to improve health worker (HW) performance in low-resource settings. We conducted a cluster-randomized controlled trial to evaluate the effect of text message reminders to HWs in outpatient health facilities (HFs) on the quality of care for malaria, pneumonia, and diarrhea in Malawi. After a baseline HF survey in January 2015 with patient interviews, HF assessments, and HW interviews, 105 HFs were randomized, stratified by baseline quality of care, to three arms: 1) text messages to HWs on malaria case management; 2) text messages to HWs on malaria, pneumonia, and diarrhea (latter two for children <5 years); and 3) control arm (no messages). Messages were sent twice a day for six months, followed by an end-line HF survey in November 2015. Difference-of-differences logistic regression analyses, accounting for clustering at facility level, were performed. We interviewed 2,360 patients at baseline and 2,536 at end-line. The proportion of patients with suspected uncomplicated malaria managed correctly increased from 40.3% to 52.8% in the control arm, from 41.4% to 55.6% in arm 1 (effect size 1.7%-points, p=0.84), and from 32.9% to 53.5% in arm 2 (effect size 8.1%-points, p=0.34). Prescription of first-line antibiotics to children <5 years with clinically-defined pneumonia increased from 69.1% to 70.6% in the control arm, from 68.9% to 71.3% in arm 1 (effect size 0.9%-points, p=0.95), and from 69.6% to 76.5% in arm 2 (effect size 5.4%-points, p=0.68). Prescription of oral rehydration solution to children with diarrhea declined slightly in all arms from baseline to end-line. Per-protocol analyses limited to patients seen by HWs in arms 1 and 2 who reported receiving messages (39.5% and 45.5%, respectively) yielded similar results. We found no significant improvements in malaria, pneumonia, or diarrhea treatment practices after six months of twice-daily text message reminders to HWs, illustrating the importance of rigorously testing new interventions before adoption and understanding why interventions work well in some settings, but poorly in others.

1809

MULTIMEDIA TOOL FOR OBTAINING INFORMED CONSENT IN THE GAMBIA: A MIXED METHOD STUDY

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Communicating crucial research information to low literacy research participants in Africa is highly challenging in the context of several factors which make the participants vulnerable to poor comprehension of consent information. We previously developed and validated a multimedia consent tool and a digitized audio comprehension questionnaire. This study was undertaken to evaluate the effectiveness of the multimedia consent tool amongst adults participating in a clinical trial in The Gambia. Adults eligible for inclusion in a malaria treatment trial (n = 311) were randomized to receive information needed for informed consent using either a multimedia tool (intervention arm) or a standard procedure (control arm). A computerized, audio questionnaire was used to assess participants' comprehension of informed consent. This was done immediately after consent had been obtained (at day 0) and at subsequent follow-up visits (days 7, 14, 21 and 28). The acceptability and ease of use of the multimedia tool were assessed in focus groups. On day 0, the median comprehension score in the intervention arm was 64% compared with 40% in the control arm (P = 0.042). The difference remained significant at all follow-up visits. Poorer comprehension was independently associated with female sex (odds ratio, OR: 0.29; 95% confidence interval, CI: 0.12-0.70) and residing in Jahaly rather than Basse province (OR: 0.33; 95% CI: 0.13-0.82). There was no significant independent association with educational level. The risk that a participant's comprehension score would drop to half of the initial value was lower in the intervention arm (hazard ratio 0.22, 95% CI: 0.16-0.31). Overall, 70% (42/60) of focus group participants from the intervention arm found the multimedia tool clear and easy to understand. In conclusion, a multimedia tool significantly improved comprehension and retention of consent information by research participants with low levels of literacy in The Gambia. Further evaluation of the tool is warrranted in similar settings.

1810

FIRST ORAL CHOLERA VACCINATION CAMPAIGN IN IRAQ DURING AN OUTBREAK AND HUMANITARIAN CRISIS: FINDINGS FROM THE COVERAGE SURVEY, 2015

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As part of the 2015 cholera outbreak response in Iraq, the Iraqi Ministry of Health (MOH) targeted approximately 255,000 persons aged ≥1 year living in selected refugee camps, internally displaced persons (IDP) camps, and collective centers with two doses of oral cholera vaccine (OCV) during November-December 2015. This was the first use of the OCV in Iraq and the largest global OCV stockpile deployment to date. We conducted a multi-stage cluster survey to obtain OCV coverage estimates in 10 governorates that were targeted during the 2015 campaign. Within each governorate we proportionally allocated our sample based on the estimated population size of each refugee/IDP camp or collective center; approximately 120 households were systematically sampled in each governorate. In each selected household, all persons aged ≥1 year were interviewed. In total, 1,226 household and 5,007 individual interviews were conducted. Overall, two-dose OCV coverage in the targeted camps was 87% (95% CI: 85%, 89%). Coverage was similar across age groups; 85% (95% CI: 81%, 88%) among children 1-4 years old, 89% (95% CI: 85%, 92%) among children 5-14 years old, and 87% (95% CI: 84%, 90%) among persons aged ≥15 years. Two-dose OCV coverage was higher in the three Northern governorates at 91% (95% CI: 89%, 93%) (range: 89% (Dahuk) to 93% (Erbil and Sulaymaniya)) compared with the seven South and Central (S/C) governorates at 80% (95% CI: 77%, 82%), where greater variation between governorates was noted (range: 21% (Babil) to 98% (Anbar)). Lower two-dose coverage in S/C governorates were likely due to civil strife, heavy rains, and challenges in program management. One-dose only coverage was higher (10%; 95% CI: 8%, 12%) among the S/C governorates compared to the Northern governorates (6%; 95% CI: 4%, 9%). The most common reasons for not receiving OCV was being absent during the campaign or teams not visiting their homes. No serious adverse events following immunization were reported. The Iraq experience demonstrates that OCV campaigns can be successfully implemented as part of a comprehensive response to cholera outbreaks among high-risk populations in conflict settings.

1811

TIMELINESS OF VACCINATION IN AN URBAN SLUM IN NAIROBI, KENYA

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Although routine infant immunization programs achieve high coverage in the first year of life in many low-resource settings, delays in vaccine receipt can leave very young children at risk for preventable diseases, precisely when they are at greatest risk for severe infection. Children living in urban slums are vulnerable to many diseases because of precarious living conditions and high population density. We assessed timeliness of vaccination among a cohort of fully vaccinated children within a population-based surveillance platform in Kibera, an urban slum in Nairobi, Kenya. Surveillance participants (~25,000) were visited at home biweekly and received free care for acute illness at a designated clinic. At each visit parents were queried about vaccines children had received since the prior visit; reported doses were verified using the child's immunization card. We identified all children <5 years old with 3 card-confirmed doses of pentavalent (diphtheria-tetanus-pertussis-Haemophilus influenzae type b-hepatitis B) vaccine, which is given at 6, 10, and 14 weeks in Kenya. We used inverse Kaplan Meier curves and Cox proportional hazards models to identify factors associated with timely receipt of the 3rd dose. From December 2009 to December 2014, 1,874 children received 3 pentavalent doses. The mean and median age at receipt of the 3rd dose was 17 and 15 weeks. The proportion with 3 doses by 4, 6, 12, and 24 months was 76.1%, 95.3%, 98.2% and 99.6% respectively. Timeliness of vaccination was not significantly associated with sex, birth in rainy season, or household size. Residence in a geographic zone close to the clinic was associated with delayed vaccination (HR=0.87 95%CI: 0.77-0.99) and birth in December was associated with timely vaccination (HR=1.24; 95%CI: 1.04-1.48). We found receipt of pentavalent vaccine to be quite timely among children who eventually received 3 doses. Although nearly a quarter were missing at least one dose at the age of 4 months, by 6 months >95% had received all 3 doses. The relevance of identified factors associated with timeliness is unclear, particularly given the small numbers of children with substantial delay.

1812

COST-EFFECTIVENESS OF DENGUE VACCINATION IN FIVE LATIN AMERICAN COUNTRIES

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In 2015, the first dengue vaccine was licensed in several Latin American countries, providing a promising tool against an expanding disease. However, decisions about the vaccine's use depend on quantifying its health benefits, costs, and cost-effectiveness. To inform policy discussions in Latin America, we used a transmission model calibrated with data from Phase III efficacy trials. Costs of vaccine administration, procurement, and dengue treatment were based on publications and reports. Each vaccine dose was projected to cost $2 for vaccine delivery plus $20 for vaccine procurement. Our base case assumed that a 3-dose vaccination program would be offered to all 9 year-old children each year, plus a 4-cohort initial catch up (10-13 year-olds), phased over 3 years and achieving 80% coverage. Our base case expressed costs in 2013 US dollars from a health system perspective, conducted 100 simulations with a 30-year horizon to account for variability in dengue transmission and uncertainty on vaccine efficacy, measured health impacts in disability-adjusted life years (DALYs), and assessed cost effectiveness as $/DALY averted. Our base case results found that vaccination would save from $0.19 (Honduras) to $1.91 (Puerto Rico) in annual per capita dengue treatment costs and would reduce dengue-related DALYs by 27.4% (Mexico) to 32.2% (Honduras). Cost-effectiveness ratios, expressed as multiples of each country's per capita gross domestic product (GDP), were: Brazil (0.74), Colombia (0.27), Honduras (3.58), Mexico (0.18), and Puerto Rico (-0.15). In the base case, the vaccine is cost saving in Puerto Rico. Using WHO benchmarks of 1 and 3 times per capita GDP, the vaccine is highly cost effective in Brazil, Columbia and Mexico (under the most stringent benchmark), but not cost effective in Honduras from a health system perspective. Cost-effectiveness results were similar for other programs (0 to 8 catch up cohorts) and coverage rates (50% to 80%). The consideration of a societal perspective, increasing dengue incidence, dengue's adverse impacts on tourism, and rising real incomes and health care costs further strengthen the case for vaccination.

1813

SNAKEBITE: STRATEGIES TO REVERSE THE PUBLIC HEALTH NEGLECT OF TROPICAL SNAKEBITE VICTIMS

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Globally, snakebite kills one fifth the number of people that die from malaria. In India, half the number of people dying from HIV are killed by snakebite every year. In Africa, snakebite causes nearly twice the number of deaths, every year, than the recent Ebola epidemic - and imposes a disease burden (319,874 DALYs; 16 countries) equal or exceeding that of regional NTDs such as Buruli ulcer, Echinococcosis, Leishmaniasis, Trachoma and Trypanosomiasis. Surviving snakebite victims suffer substantial psychological morbidity that is typically unrecognised and untreated. The support and investment provided by International Health Agencies and tropical governments to greatly reduce the disease burden of malaria, HIV, Ebola and the NTDs is typically denied to tropical, and particularly to sub-Saharan African, snakebite victims - despite the high mortality rate and the physical, psychological and socio-economic burden of tropical snakebite. In an effort to reverse this public health neglect of tropical snakebite victims, the authors organised (September 2015) a Wellcome Trust-funded workshop to identify key interventions (i) reduce snakebite incidence, (ii) improve access to hospital care, (iii) improve clinical management of hospitalised snakebite victims and (iv) improve post-hospital management of snakebite victims. We will report that progress since then includes (i) the announcement by the World Health Organisation of an 'African antivenom prequalification' program designed to prevent the distribution in Africa of ineffective antivenoms, (ii) that the NGO, Health Action International, has assumed the secretariat and advocacy roles for the Global Snakebite Initiative, (iii) that, with the advocacy support of over 13 tropical MoHs and NGOs (eg, MSF, HAI, DNDi), the Global Snakebite Initiative acquired a side briefing at the World Health Assembly in May, 2016, (iv) that a motorcycle ambulance/smart phone app-coordinated Snakebite Emergency Response System will be trialled in Kenya as an affordable, rapid means of delivering rural snakebite victims to effective treatment.

1814

A NOVEL FAMILY OF KUNITZ-TYPE INHIBITORS FROM *FASCIOLA HEPATICA* - POTENT INHIBITION OF VIRULENCE-ASSOCIATED CYSTEINE PROTEASES

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Fasciola hepatica is a zoonotic food-borne helminth parasite of global veterinary and medical importance. The parasite expresses a family of seven Kunitz-type (KT) protease inhibitors that are highly regulated during the parasites migration and development in the mammalian host. Phylogenetic analysis demonstrates they separate into five subgroups (FhKT1 – 5), although transcriptomic data shows that the FhKT1 group has the highest expression over the course of infection and is upregulated in the infectious newly excysted juveniles (NEJs) and in adults. To date, KT inhibitors are ubiquitously expressed in eukaryotics and are classical described as inhibitors of serine proteases. Unexpectedly, we discovered that the FhKT1 inhibitors do not inhibit serine proteases but exhibit potent inhibition towards cysteine proteases. Recombinant FhKT1 is a potent inhibitor of the major secreted virulence-associated cathepsin L cysteine proteases of *F. hepatica*, FhCL1, FhCL2 and FhCL3, and of human cathepsins L and K (K_i = 0.2474 nM – 24.607 nM). FhKT1 also prevented the auto-catalytic activation of FhCLs and formed stable complexes with the mature enzymes. Pull-down experiments showed that rFhKT1 interacts specifically with native secreted adult FhCL1, FhCL2 and FhCL5. Substitution of an unusual P1 Leu^{15} within the exposed reactive loop of FhKT1 for the more commonly found Arg^{15} (FhKT1Leu^{15}/Arg^{15}) had modest adverse affects on cysteine protease inhibition but conferred potent activity against the serine protease trypsin (K_i = 2.28 nM). Computational docking and sequence analysis provided molecular explanations for the exclusive binding of FhKT1 to cysteine proteases, suggested a pivotal role for the P1 Leu^{15} in anchoring the inhibitor into the S2 active site pocket, and helped explain the selectivity towards cathepsin L-like proteases. FhKT1 represents a novel evolutionary adaptation of KT protease inhibitors by *F. hepatica*, with its prime purpose likely in the regulation of the major parasite-secreted proteases and/or host proteases during infection, making this a novel vaccine and drug target.

1815

DRAFT GENOMES OF FOUR SPECIES OF THE LUNG FLUKE *PARAGONIMUS*

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Paragonimus spp, the lung fluke, is among the most injurious of the food-borne helminths, infecting ~20 million people worldwide, with an estimated 293 million people at risk for infection. Paragonimiasis is acquired by consuming raw or undercooked crustaceans containing *Paragonimus* metacercariae, and primarily affects the lungs, but often causes lesions elsewhere in the body, including the brain. The disease is a major public health concern in parts of Southeast Asia, West Africa, South and Central America, and Northeast India, where it is frequently mistaken for tuberculosis due to its similar respiratory symptoms. To substantially improve our understanding of pathogens across this genus at the molecular level, we have assembled, annotated and compared draft genomes of three *Paragonimus* species from Asia (*P. miyazaki*, *P. westermani*, *P. heterotremus*) and one from North America (*P. kellicotti*). The genomes range in size from 697 to 923 Mb, contain between 11,761 and 12,762 genes, and are estimated to be between 80% and 91% complete. Comparative orthologous protein family (OPF) analysis spanning 19 species (4 *Paragonimus* species, 3 other foodborne trematodes, 3 schistosomes, 4 other platyhelminths, 4 hosts and an outgroup) identified proteins and functions of phylogenetic interest, including 364 OPFs conserved across and specific to the four *Paragonimus* species, which were enriched for proteins responsible for transcription factor activity, iron homeostasis, and serine endopeptidase activity. Transcriptomic analysis identified gene sets with conserved expression across *Paragonimus* species, as well as genes overexpressed during host parasitic stages, including 179 *P. miyazaki* genes overexpressed in peritoneal and pleural cavities compared to liver and lung tissues, which were enriched for cysteine endopeptidase activity and microtubule processes. This study provides a foundation for future studies of *Paragonimus* and other food-borne trematode pathogens, and represents a major contribution to ongoing trematode genome sequencing efforts.

1816

COMPLEMENTATION OF CELLULAR PROLIFERATION DRIVEN BY GRANULIN BY LIVER FLUKE GRANULIN IN A CHOLANGIOCYTE LINE AFTER GENOME EDITING TO MUTATE HGRN

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The highest incidence of cholangiocarcinoma (CCA), bile duct cancer, has been reported in northeastern Thailand, a region where infection with the fish-borne liver fluke, *Opisthorchis viverrini* is endemic. Infection with *O. viverrini* is a Group 1 biological carcinogen that induces CCA. How opisthorchiasis causes CCA is not yet clear, but likely is a multi-factorial process. Among other factors, *O. viverrini* secretes a mitogen termed granulin (Ov-GRN-1) that stimulates proliferation of cholangiocytes, and we have postulated Ov-GRN-1 released from the parasites contributes to opisthorchiasis-induced CCA. Human orthologue, hGRN (human granulin) is a growth factor with multiple functions in inflammation, would repair, and tumorigenesis. To investigate these phenomena, we undertook complementation of native hGRN with Ov-GRN-1 in cultures of a cholangiocyte cell line named H69 where the encoding hGRN gene had been gene-edited out by CRISPR/Cas9. In real time growth assays (xCELLigence), mutant (hGRN knockout) cells exhibited reduced growth proliferation compared to wild-type H69 cells, a deficit that was relieved by addition of Ov-GRN-1. Thereafter, transcripts recovered from exosomes of H69 cells were evaluated; annexin, c-Myc, C-met were up-regulated. Moreover, perioxiredoxin, Prx I, an antioxidant involved with cellular homeostasis and which can promote tumorigenesis through activities driven via mTOR exhibited marked induction in the mutant H69 cells. In ongoing studies, intercellular functions of endogenous and exogenous granulin in cellular proliferation and/or tumorigenesis upstream of the mTOR pathway are under investigation.

1817

TISSUE SPECIFIC LOCALIZATION OF *NEORICKETTSIA* ENDOSYMBIONTS IN THE INTESTINAL TREMATODE *PLAGIORCHIS ELEGANS* AND THE LIVER FLUKE *FASCIOLA HEPATICA* SHOW SIMILAR DISTRIBUTION PATTERNS

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Neorickettsia are α Proteobacteria that can cause serious diseases in livestock animals and humans. These intracellular bacteria are transmitted by digenean trematodes but little is known about their relationship. *Neorickettsia risticii* has been isolated from infected horses, cultured in the laboratory, and described by transmission electron microscopy (TEM). However, ultrastructure and tissue localization of Neorickettsia in digeneans is largely unknown. We expressed a surface protein of Neorickettsia of *Plagiorchis elegans* (PeNsp-3) from experimentally infected hamsters, and raised antibodies to it for immunolocalization. TEM studies of *P. elegans* revealed pleomorphic bacteria with a median size of 600 x 400 nm and with characteristic double membranes. Bacteria secreted polymorphic vesicles into the host cell or cell syncytium. We used the PeNsp-3 antibody for comparative detection of Neorickettsia in adults of *P. elegans* (North Dakota) and *Fasciola hepatica* (Oregon). Neorickettsia from *P. elegans* and *F. hepatica* are closely related to each other and to N. risticii (Illinois). On the amino acid level PeNsp-3 is 98% identical to its ortholog of Neorickettsia from *F. hepatica*. Neorickettsia showed similar localization pattern in both trematode species. Endosymbionts were unevenly localized as single cells, or as small morula-like clusters in tegument, ovaries, vitelline glands, uterus, eggs, testis, seminal receptacle, intestine and oral and ventral sucker. Large numbers were present in the Mehlis' gland. Examination of hamster small intestine infected with *P. elegans* showed bacteria at the host-parasite interface of the oral and ventral sucker. We conclude that in *P. elegans* and *F. hepatica* large numbers of Neorickettsia in the Mehlis' gland and adjacent tissues involved in egg assembly participate in vertical transmission. Their presence in suckers and intestinal tissues may facilitate horizontal transmission to the host of the trematode. This first localization of Neorickettsia endsymbionts in adult trematodes of medical and veterinary importance provides important clues about their transmission modes.

1818

MATHEMATICAL MODELING OF THE TRANSMISSION DYNAMICS OF *OPISTHORCHIS VIVERRINI* IN LAO PDR

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The trematode liver fluke, *Opisthorchis viverrini*, which causes the chronic hepatobiliary disease, opisthorchiasis, is prevalent is southeast Asia. We develop a mathematical model of the transmission dynamics of *O. viverrini* through its life cycle in snails, fish, and humans; and a second model that includes potential transmission from reservoir hosts such as domestic cats and dogs. We calibrate these models to data collected from two communities in Khong Island in Southern Lao PDR. Analysis of the model assuming no reservoir hosts, shows that interventions such as behavioral changes in dietary habits (reducing transmission from fish to humans) and improved sanitation (reducing transmission from humans to snails) are most effective in reducing transmission potential and the mean burden of worms in humans. However, in the presence of reservoir hosts, snail control, if feasible, is the most effective intervention for reducing transmission potential, but behavioral changes in dietary habits remains the most effective intervention for reducing the worm burden in humans. Additionally, the model suggests that for the observed prevalence of infection in dogs and cats in Khong Island, these reservoir hosts are capable of maintaining transmission in the population, even if perfect sanitation were to be achieved for all humans. Therefore, although improved sanitation and mass drug administration substantially reduce the mean worm burden in humans, additional strategies, such as behavioral changes in the feeding practices of domestic pets, safe fish production and/or snail control, would be necessary to eliminate *O. viverrini* transmission in Khong Island.

1819

OUTCOME OF TWO PHASE I RELATIVE BIOAVAILABILITY STUDIES IN HEALTHY VOLUNTEERS AFTER ADMINISTRATION OF THE NEW PEDIATRIC ODT FORMULATIONS OF RACEMATE PRAZIQUANTEL (RAC-PZQ) AND OF THE ACTIVE ENANTIOMER OF PRAZIQUANTEL (L-PZQ)

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Praziquantel (PZQ) was developed in the 1970s to treat schistosomiasis. PZQ tablets are available to treat adults and school aged children, but there is a pressing need to develop a suitable pediatric formulation for treating preschool children. New pediatric oral disintegrating tablets (ODTs) of racemic Praziquantel (rac-PZQ), as well as of the active L-enantiomer of PZQ (L-PZQ), are under development by the Pediatric Praziquantel Consortium. These ODT formulations were assessed for their relative bioavailability against the reference PZQ tablets (Cysticide) in 2 randomized cross-over studies in healthy males. Each study included resp. 32 and 36 subjects, who received single oral doses dispersed in water (ODT formulation) or as tablets (Cysticide), with a wash-out of 7 days in between. Treatments were resp. rac-PZQ ODT at oral doses of 20, 40 and 60 mg/kg and L-PZQ ODT at doses of 10, 20 or 30 mg/kg under fed conditions, and either 40 (rac-PZQ) or 20 (L-PZQ) mg/kg ODT under fasting conditions, and 40 mg/kg PZQ (Cysticide) under fed conditions. Plasma samples for PK were taken at pre-specified time-points up to 24 hours and concentrations of L- and D-PZQ were measured with a validated enantioselective LC-MS/MS method. PK parameters Cmax and area under the curve (AUC) were calculated. After administration of L-PZQ no conversion to the D-PZQ enantiomer was seen. PK profiles after administration of all formulations were quite variable, showing a food effect and supra-proportionality not allowing to build a compartmental model describing the PK profiles. Instead, a linear mixed effects model was built to describe the PK parameters Cmax and AUC and used to predict the dose-exposure relationship in children. Exposure to L-PZQ administered as rac-PZQ ODT or as Cysticide tablets was comparable, but both were higher than after administration of equivalent doses of L-PZQ as ODT. The low exposure (40%) of L-PZQ after administration of L-PZQ ODT compared to administration of equivalent amounts of racemic-PZQ tablets indicates the need for higher dosages of L-PZQ ODT to be administered to achieve therapeutic effects.

1820

COMPARATIVE EFFICIENCY OF *BIOMPHALARIA PFEIFFERI* AND B. *SUDANICA* AS INTERMEDIATE HOST SNAILS FOR *SCHISTOSOMA MANSONI* AND ITS IMPLICATIONS FOR TRANSMISSION OF SCHISTOSOMIASIS IN KENYA

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In Kenya, schistosomiasis infects an estimated 6 million people with >30 million people at risk of infection. *Schistosoma mansoni* is commonly transmitted by *Biomphalaria pfeifferi*, an inhabitant of streams and small water bodies, and *B. sudanica*, which is mostly found along shores of Lake Victoria. Recent studies have accentuated the role of infected snails in maintaining transmission as some snails can survive for over a year shedding cercariae daily. We sought to determine if these two snail species may differ with respect to the efficiency with which they support *S. mansoni* infections. We exposed field-derived *B. pfeifferi* (Kirinyaga, central Kenya) and *B. sudanica* (Kisumu, western Kenya) to *S. mansoni* derived from human subjects from Kirinyaga or Kisumu. The reciprocal cross infection design allowed us to ascertain if local adaptation effects might influence infection outcomes. Juvenile (<6 mm shell diameter), young adult (6-9 mm) and adult snails (> 9 mm) were exposed, all to one miracidium/snail. Overall, *B. pfeifferi* consistently had higher infection rates than *B. sudanica* (39.6 - 80.7% vs. 2.4 - 21.5%), regardless of the source of *S. mansoni* or the size of the snails used. Allopatric *B. pfeifferi* - *S. mansoni* combinations had higher infection rates than sympatric combinations while *B. sudanica* showed the opposite trend. Infection rates were inversely proportional to snail size. Mean daily cercariae production was greater for *B. pfeifferi* exposed to sympatric than allopatric *S. mansoni* (62 –2465 and 100 – 1232, respectively), and this trend increased with snail size. Overall mean daily cercariae production amongst all *B. sudanica* was low (50–590) with no significant differences between sympatric or allopatric combinations, or among the different snail sizes ($p < 0.05$). In conclusion *B. pfeifferi* is more likely to become infected and to shed more cercariae than *B. sudanica,* suggesting that the per snail risk of perpetuating transmission in Kenyan streams and lacustrine habitats may differ considerably with noteworthy implications for understanding transmission dynamics and planning control efforts.

1821

DEVELOPMENT OF A NONHUMAN PRIMATE MODEL OF ZIKA VIRUS INFECTION IN PREGNANT AND NON-PREGNANT RHESUS MACAQUES

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Zika virus has recently been identified as the cause of clinically significant disease with outcomes including fetal abnormalities in the Americas. However, little is known about the natural history of Zika virus, nor the full spectrum of associated diseases. To investigate virus dynamics and immune responses *in vivo*, we developed a rhesus macaque model for Zika virus infection. We also examined the effects of maternal Zika virus infection on fetal development at different stages of pregnancy. We subcutaneously inoculated non-pregnant and pregnant animals with Asian or African lineage Zika virus. Viral RNA was detected in plasma one day post-infection (dpi) in all animals, with peak viral loads reaching above 1 x 10^5 viral RNA copies/mL. Viral RNA was also present in saliva, urine, and cerebrospinal fluid, consistent with case reports from infected humans. Two of four pregnant animals remained viremic for longer periods than non-pregnant animals. Viral RNA was detected in amniotic fluid in one pregnant animal infected during the third trimester. In all animals, infection was associated with transient increases in proliferating natural killer cells, CD8+ T cells, CD4+ T cells, and plasmablasts. Neutralizing antibodies were detected in all animals by 21 dpi. Rechallenge of non-pregnant animals with the Asian lineage Zika virus resulted in no detectable virus replication, suggesting that primary Zika virus infection elicits protective immunity against homologous and heterologous virus strains. Measurements of fetal growth by ultrasonography, examination of fetal brain abnormalities by magnetic resonance imaging, and tissue tropism studies in fetuses are ongoing. These studies establish that Asian lineage Zika virus infection of rhesus macaques provides a relevant animal model for studying natural history and pathogenesis in pregnant and non-pregnant nonhuman primates.

1822

ZIKA VIRUS INFECTION OF HUMAN PLACENTAL CELLS AND EXPLANTS: THE ROLE OF ZIKV RECEPTORS AND ANTI-FLAVIVIRUS ANTIBODIES

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The Zika epidemic that began in Brazil and spread throughout the Americas has been reported as definitively linked to severe birth defects - microcephaly, miscarriage and stillbirth. Detection of ZIKV RNA in the placenta and fetus as well as intrauterine growth restriction suggests extensive infection of the placenta leading to substantial virus-induced pathology. Our studies in placental explants and primary cells isolated from human placenta reveal that prototype and recently isolated Nicaraguan ZIKV 2016 strains infect cells that express AXL, Tyro3 and TIM1 tyrosine kinase receptors, which mediate infection by ZIKV and the closely related dengue virus (DENV) in skin. Infected placental cells, including fetal amniotic epithelial cells, placental fibroblasts, umbilical vein endothelial cells and trophoblast progenitor cells (TBPC), developed cytopathology and expressed ZIKV envelope and nonstructural NS3 proteins, and virus titers released depended on receptors expressed and gestational age. Indicative of infection route, AXL was detected in decidua (uterine decidual cells, invasive cytotrophoblasts), chorionic villi (placental fibroblasts, Hofbauer cells, blood vessels) and fetal membranes (amniotic epithelial cells, TBPC). ZIKV-infected cells downregulated AXL, which was strongly induced in neighboring cells, suggesting a contribution to infection. Differential expression of receptors suggests how ZIKV could infect the decidua and spread to the placenta, fetus and amnion-chorionic membranes. Further, in endemic regions, cross-reactive pre-existing antibodies to DENV could play a critical role in protection or pathogenesis of ZIKV in placenta tissues during pregnancy. Thus, we are studying the neutralizing and potentially enhancing role of ZIKV-specific and DENV-cross-reactive antibodies on infection in primary placental cells and explants. These studies reveal molecular mechanisms of ZIKV infection and routes of virus transmission to the fetus, and we are using the model to assess the therapeutic potential of antibodies and small molecule inhibitors to block infection and prevent congenital disease.

1823

THE CRYO-EM STRUCTURE OF ZIKA VIRUS

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Zika virus (ZIKV) has recently attracted global notoriety due to its explosive spread through the Pacific Islands and Latin/Central America and its link to neurological complications such as congenital microcephaly and Guillain Barré syndrome. It poses a looming threat to many countries (including USA) infested with the *Aedes* species of mosquitoes that transmit the virus. ZIKV can also be sexually and vertically transmitted which expands the geographical reach of the virus. The World Health Organization therefore declared Zika epidemic as 'a public health emergency of international concern'. ZIKV is a positive sense RNA virus belonging to the Flaviviridae family that includes other pathogenic viruses such as dengue virus, West Nile virus, yellow fever virus and tick-borne encephalitis virus. An understanding of the biology of the virus and the mechanism of disease are required to provide appropriate recommendations to tackle the virus and for timely development of diagnostic kits, vaccines and antivirals. We have determined the structure of mature ZIKV at 3.8Å resolution using cryo-electron microscopy The structure of ZIKV is similar to other known flavivirus structures except for the ~10 amino acids that surround the Asn154 glycosylation site found in each of the 180 surface envelope glycoproteins that make up the icosahedral shell. The carbohydrate moiety associated with this residue may function as an attachment site of the virus to host cells. This region varies not only among ZIKV strains but also in other flaviviruses and suggests that changes in this region could influence virus transmission and disease. The atomic structure of ZIKV and its comparison with other flaviviruses will be discussed.

1824

BOOSTING ALTERS THE CROSS-NEUTRALIZING CAPACITY OF ANTIBODY-RESPONSE FOLLOWING ZIKA EXPOSURE IN C57BL/6 MICE

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Zika virus (ZIKV) has recently emerged in the Americas, in areas where the related Flavivirus, dengue virus (DENV), is already endemic. Cross-neutralization of antibodies among Flaviviruses has previously been demonstrated, and reports from diagnostic serological tests have suggested this cross-reactivity occurs among DENV1-4 and ZIKV. In this study, we investigate the cross-neutralizing capacity of the antibody population after subcutaneous exposure to ZIKV in C57BL/6 mice using plaque reduction neutralization tests (PRNTs). Exposed mice initially produced very highly neutralizing antibody to ZIKV (PRNT80) that also highly cross-neutralized DENV2 (PRNT50). After a regimen of homologous boosting with ZIKV, the antibody neutralization capacity remained very high to ZIKV (PRNT80). However, the cross-neutralization to DENV2 decreased to almost nothing. These results indicate that C57BL/6 mice produce a strong antibody response in the absence of robust viremia, suggesting its utility as a model for investigating antibody responses to ZIKV and cross-neutralization to at least DENV2. These results further suggest that homologous boosting with ZIKV contributes to the evolving specificity of the antibody population toward ZIKV in these mice, as it decreases cross-neutralization of heterologous Flaviviruses.

1825

VECTOR COMPETENCE OF AMERICAN MOSQUITOES FOR MULTIPLE STRAINS OF ZIKV REPRESENTING EACH GENETIC CLADE

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In 2015 Zika virus (ZIKV; Flaviviridae. Flavivirus) emerged in the Americas, causing millions of infections in dozens of countries from Brazil to Mexico. The rapid spread of the virus and the association with concerning disease outcomes such as Guillain-Barré syndrome and microcephaly make understanding transmission dynamics essential. Currently, there are no reports of vector competence (VC) of American mosquitoes for ZIKV isolates from the Americas. Further, it is not clear whether locally circulating strains display enhanced transmissibility by local mosquitoes. First, we determined if/whether freezing ZIKV prior to experiments impacts VC estimates as has been shown for dengue virus. Mexican *Aedes aegypti* mosquitoes were given an infectious bloodmeal with either fresh or frozen ZIKV that was originally isolated from an infected human in Puerto Rico (Strain PRVABC59, Asian clade). While infection and transmission rates were significantly higher in mosquitoes fed fresh virus on day 7 post-exposure, no differences were observed in infection, dissemination or transmission rates by day 14, and high infection rates were observed, indicating that previously frozen virus could be used for ZIKV vector competence studies. *Ae. aegypti* mosquitoes were then infected with viruses from the other two recognized ZIKV clades, strain 41525 from the West African clade and strain MR766 from the East African clade along with the aforementioned PRVABC59 strain. Studies showed that while

mosquito infection and dissemination rates were different between the three strains, all viruses were able to infect, disseminate, and were found in saliva (all groups greater than 60% transmission rate by 14 days post-exposure) in the American mosquitoes tested, indicating transmission potential. These data demonstrate that American mosquitoes are highly competent for ZIKV from all three viral clades and that emergence of viral strains from Africa in addition to the currently circulating Asian lineage strain should be monitored.

1826

MAPPING ZIKA VIRUS CROSS-NEUTRALIZING EPITOPES

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The recent emergence and spread of Zika virus (ZIKV) in the Americas has presented a global Public Health emergency and new therapeutic strategies are needed to protect against severe life threatening infections. Moreover, several groups have identified a strong causal relationship between prenatal ZIKV infection and microcephaly in the developing fetus, as well as an association with other serious brain abnormalities. While the ZIKV adult pathologies are less severe than those caused by other flaviviruses, immunity in pregnant females may offer protection from the more devastating outcomes in infected fetuses. ZIKV is closely related to dengue viruses. Moreover, the close phylogenetic relationship between ZIKV to DENV provides an opportunity study the antigenic relationships between these two flavivirus strains. Using a panel of human and mouse monoclonal antibodies generated against various dengue virus (DENV) strains, we have identified some with pan-flavivirus binding reactivity. Using an *in vitro* neutralization assay, a binding assay, and an *in vivo* protection study, we have shown that many DENV antibodies bind to ZIKV, but only a few were broadly neutralizing and mapped onto the E glycoprotein dimer. As structural studies indicate that ZIKV is very mature, it was also not surprising that the prM targeting antibodies did not bind ZIKV. These experiments have revealed a set of monoclonal antibodies targeting a highly conserved neutralizing epitope in DENV and ZIKV. Using an *in vivo* mouse model, we are currently testing the ability of these broadly cross neutralizing antibodies to protect against ZIKV virus infection, potentially identifying a therapeutic antibody for human use.

1827

DEVELOPMENT AND CHARACTERIZATION OF LIVE ATTENUATED VACCINE CANDIDATES FOR ZIKA VIRUS

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The goal of the NIH Laboratory of Infectious Diseases (LID) vaccine program is the development of market-sustainable, live-attenuated vaccines for several medically- important flaviviruses. In the past, the focus has been on the development of the tetravalent dengue virus (DENV) vaccine, which is currently undergoing Phase III evaluation by the Butantan Institute in Brazil. With the recent outbreak of Zika virus (ZIKV) in Latin America, attention has been turned to leveraging the dengue vaccine platform for the creation of vaccine candidates against ZIKV. The live vaccine candidates should be minimally reactogenic, highly immunogenic across all age-groups, cost-effective, and safe for the community. In addition, it would be ideal if they were compatible with the existing tetravalent dengue formulation to allow for inclusion into a pentavalent dengue/Zika formulation for use in regions where these viruses co-circulate. Recombinant chimeric viruses expressing the structural proteins of ZIKV in the background of different DENV serotypes containing the delta-30 deletion have been generated. In addition, full-length ZIKV cDNA molecules containing altered 3' untranslated regions are also under construction. Preclinical trials in mice and rhesus monkeys are being used to demonstrate the attenuation phenotype of these ZIKV candidates and to down-select suitable strains. Phase I trials will evaluate the safety and immunogenicity of both monovalent ZIKV vaccine candidates and combinations with the tetravalent DENV vaccine. The LID is also developing a human challenge model for ZIKV using cGMP isolates or recombinant-derived strains. Such a model can be used for investigating vaccine-induced protection, establishing immune correlates of protection, and to accelerate a possible regulatory pathway toward licensure in the face of decreased transmission at the time of future Phase III efficacy trials. The challenge model will also be used to facilitate studies of ZIKV viremia in populations with well-defined pre-existing antibody profiles and to quantify the duration and level of ZIKV shedding in body fluids other than serum.

1828

THE ROLE OF ECDYSONE RECEPTOR IN *ANOPHELES GAMBIAE* MOSQUITO POST-MATING BIOLOGY

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The *Anopheles gambiae* mosquito is responsible for infecting millions of people with malaria each year throughout Africa. Female Anophelines mate a single time in their life making reproduction a crucial point in their life cycle, and a potential target for vector control. The critical insect steroid hormone, 20-hydroxyecdysone (20E), is essential for regulating larval development and egg production in numerous insect species. Recently, our lab identified multiple novel roles for 20E in *An. gambiae* reproduction. We demonstrated that sexual transfer of 20E during copulation is necessary and sufficient to induce two key female post-mating phenotypes: oviposition and refractoriness to multiple matings. Here we show that male-transferred 20E induces these phenotypes by initiating signaling cascades following its interaction with specific receptors localized to the female reproductive tissues. Ecdysone Receptor (EcR) is known to be an ecdysone-responsive nuclear receptor regulating 20E signaling during larval development, metamorphosis, and adult female vitellogenesis. Our findings suggest that EcR in *An. gambiae* is responsible for regulating 20E-induced oviposition, while female mating refractoriness is induced through a novel, yet unidentified, 20E receptor. We have also discovered that in an EcR depleted background, females fail to store sperm. This sensitized genetic background can be used to provide important biological insights into the mechanism of sperm storage - a critical process for the female's lifelong fertility. Overall, understanding the mechanisms through which male-transferred 20E induces vast transcriptional and physiological changes in the female *An. gambiae* mosquito can not only advance knowledge of unique vector reproductive biology, but it can also reveal novel biological targets for mosquito control.

1829

PLASMODIUM FALCIPARUM PFS47 GENETIC DIVERSITY IN FIELD COLLECTED *ANOPHELES GAMBIAE* AND *ANOPHELES COLUZZI* FROM MALI, AFRICA

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The anopheline immune system has the capacity to mount effective antiplasmodial responses. We have shown previously that Pfs47 is required by *Plasmodium falciparum* to evade the *Anopheles* immune system, which can be an important barrier for the adaptation of the parasite to different vectors. Adaptation of *P. falciparum* to evolutionary distant anophelines appears to involve natural selection of compatible Pfs47 haplotypes. Pfs47 presents high genetic diversity in Africa and strong geographic structure at

continental level worldwide, consistent with natural selection of the gene by different anophelines. Here we test whether Pfs47 may be differentially selected by sympatric African malaria vectors. We studied Pfs47 genetic diversity being transmitted by *An. gambiae* and *An. coluzzi* in a small region of Mali. Pfs47 was genotyped in a total of 150 sporozoite-infected mosquitoes collected throughout the year. Multiple Pfs47 haplotypes were detected in 26% of the mosquitoes. A high diversity of Pfs47 was detected (11 haplotypes) with 2 haplotypes accounting for 73% of the *P. falciparum* infected mosquitoes. Temporal analysis of haplotype distribution showed Pfs47 haplotype present diversity throughout the year in both species of mosquitoes. The most frequent Pfs47 haplotypes were present in both *An. gambiae* and *An. coluzzi*, but there were some differences in the frequency between both species. *An. gambiae* and *An. coluzzi* don't appear to be genetically distant to cause major differential selection of Pfs47 haplotypes.

1830

HYBRID ALLELIC IMBALANCE AND GENE EXPRESSION EVOLUTION IN THE *ANOPHELES GAMBIAE* SPECIES COMPLEX

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The accumulation of genetic incompatibilities that cause hybrid sterility and/or inviability between diverged populations is an important step in the formation and maintenance of species boundaries in the face of hybridization. In the *Anopheles gambiae* species complex, F1 hybrid males are sterile, while females are fully fertile and can backcross to either parental species. Thus, F1 hybrid females facilitate the introgression of genomic regions between species (e.g. chromosomal inversions) that may be adaptive. While some chromosomal incompatibilities that cause hybrid sterility and inviability between *An. gambiae* and *An. arabiensis* have been identified, little is understood about the nature of these incompatibilities. Divergence in gene expression, rather than genetic differences, is thought to account for a large proportion of phenotypic differences between species, and may also play a role in hybrid sterility and inviability. We analyzed gene expression in F1 hybrid male and female pupae and compared it to parental males and females in bi-directional crosses between *An. gambiae, An. arabiensis,* and *An. quadriannulatus*. By analyzing genome-wide, allele-specific gene expression, we explored the roles of *cis* and *trans* regulatory divergence between species and in male and female hybrid phenotypes. The relationship between allele-specific expression, patterns of sequence evolution, and known hybrid sterility/inviability QTL was also explored. Our analysis provides insight into gene expression divergence and evolution in the *An. gambiae* species complex.

1831

HEMOCYTE-SPECIFIC MANIPULATION OF THE IMD PATHWAY AFFECTS *PLASMODIUM* INFECTION IN *ANOPHELES STEPHENSI*

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Using a newly developed hemocyte specific *Gal4*/UAS-based expression system in *Anopheles stephensi* we have begun to investigate the role of the IMD pathway in the hemocyte's ability to respond to a *Plasmodium* infection. The IMD pathway has been shown to contribute to the mosquito's ability to fight a *Plasmodium* infection through studies using RNAi as a method to perturb post-transcriptional gene expression in the IMD pathway. However, due to the pleotropic effects of dsRNA injections it becomes difficult to ascertain the individual contributions of various tissues to the immune response against a *Plasmodium* infection beyond the midgut stage of parasite development. Using the *Gal4*/UAS system we are able to disrupt the IMD pathway specifically in hemocytes, the cellular component of the mosquito's innate immune system, and analyze the mosquito's response to a parasite challenge. We have found that manipulation of the IMD pathway by knocking down or over expressing *Caspar* leads to altered levels of infection in both midguts and salivary glands in comparison to controls. This suggests the IMD pathway plays an important role in the hemocyte's ability to defend against infection during multiple stages of *Plasmodium* infection, independent of a normal IMD pathway in all other tissues. This approach allows for questions to be answered concerning the parasite-vector interaction during the post-midgut stage of parasites development; a pivotal point in defense against salivary gland invasion by sporozoites.

1832

LANDSCAPE GENETICS OF PYRETHROID RESISTANCE IN *ANOPHELES ARABIENSIS* IN KENYA

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Anopheles arabiensis have become increasingly abundant in Africa and are playing an important role in maintaining residual malaria transmission in sub-Saharan Africa. Overall, insecticide resistance in *An. arabiensis* has remained relatively low with respect to *An. gambiae*. However, recent studies suggest that resistance in *An. arabiensis* is emerging in Africa. Resistance could potentially increase and spread rapidly if gene flow between populations is large. Knowledge of *An. arabiensis* population genetic structure is critical to understanding insecticide resistance spread. We test how various ecological variables affect gene flow (dispersal) using a landscape genetics approach utilizing techniques from population genetics, landscape ecology, and spatial statistics. We genotyped *An. arabiensis* collected from 14 study sites across Kenya at 10 microsatellite loci and at kdr L1014F/S. We created resistance surfaces in ArcGIS for key environmental and landscape variables hypothesized to influence gene flow of *An. arabiensis*. We optimized resistance surfaces using the ResistanceGA package in R which utilizes a genetic algorithm to optimize surfaces based on pairwise genetic distances and CIRCUITSCAPE resistance distances. Lastly, mixed effects models were fit by maximum likelihood in the lme4 package in R. We observed both kdr 1014F and 1014S alleles. Kdr mutation frequencies were 0.023 and 0.103 at two sites in western Kenya and were absent from other sites. We hypothesize that forest cover and elevation provide the greatest barriers to gene flow and population density and roads largely promote gene flow. Understanding the factors promoting gene flow and insecticide resistance spread is critical to informing antimalarial interventions, especially since pyrethroid resistance in *An. arabiensis* is relatively low and patchily distributed.

1833

GENOMIC ANALYSIS OF THE *ANOPHELES GAMBIAE* BAMAKO ECOTYPE

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The Bamako ecotype is a chromosomal form of *Anopheles gambiae* found in southern Mali and northern Guinea in association with rock pools in the Niger River. The ecotype is defined by three fixed chromosomal inversions on arm 2R, *j*, *c*, and *u*, the latter two of which also segregate at appreciable frequencies in sympatric *An. coluzzii* and non-Bamako *An. gambiae*. Previous studies have found evidence for some degree of assortative mating within the Bamako ecotype, but the status of this ecotype as an independently evolving entity remains unclear, as does the genomic basis of differential habitat preferences between Bamako and non-Bamako forms. Clarifying the status of this ecotype offers a chance to explore the genomic basis of habitat adaptation in *An. gambiae*. Using

pooled resequencing (*pool-seq*) of Bamako, non-Bamako *An. gambiae*, and *An. coluzzii* from southern Mali, as well as individual whole-genome resequencing, we present the first whole-genome analysis of the Bamako chromosomal form. Clustering of individual samples provides evidence for Bamako as an independent entity, while genome scans show that differentiation between Bamako and other sympatric populations is concentrated in the inversions that define Bamako. However, the strongest signals of differentiation are found not in the 2R*j* inversion, which is relatively unique to Bamako in this geographic region, but in inversions 2Rc and *u*, which are also found in sympatric populations of non-Bamako *An. gambiae* and *An. coluzzii*. This pattern of differentiation in shared inversions is partially driven by novel, Bamako-specific alleles in genes known to be involved in insecticide resistance, which may be candidate genes for habitat adaptation in this ecotype.

1834

ASSESSMENT OF THE POST-ZYGOTIC REPRODUCTIVE BARRIERS BETWEEN *ANOPHELES GAMBIAE* ET *AN. COLUZZI*I

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Anopheles gambiae and *An. coluzzi*i are two of the most important malaria vector species in sub-Saharan Africa. These recently-diverged sibling species are thought to be separated by strong assortative mating combined with selection against hybrids. At present, little is known about hybridization and the post-zygotic reproductive barriers between these cryptic taxa. Swarm segregation and assortative mating between *An. gambiae* and *An. coluzzi*i were studied in the villages of VK7 and Soumousso, Western Burkina Faso. Natural swarms and pairs in copula were collected and genotyped, the proportion of intra and interspecific matings determined, and interspecific sperm transfer checked genetically. Females were collected resting indoors or as larvae and genotyped or sexed-and-genotyped via a multiplex PCR. Larval development and adult swarming success of hybrids were also estimated and compared to the parental species in semi-field experiment. A total of 3,687 males and 220 females were collected from 109 natural swarms and genotyped. Amongst 187 females captured in copula, 4 *An. gambiae* and one *An. coluzzi*i females were found paired with and inseminated by heterospecific males. The lower overall hybridization rates observed at the larval and adult indoor stages compared to cross-mating rates support post-mating selection processes acting against hybrids. A total of 5,400 first instar larvae were transplanted in 36 cages in rice field with or without predators. Although no statistical difference was found between reciprocal hybrids and parental species in adult wing size, the development sucess varied significantly. In total 6,400 males of *An. gambiae*, *An. coluzzi*i and the two reciprocal hybrids were randomly released in grand cages and swarm activities were daily monitored. A total of 428 males were captured in swarms but the frequencies of *An. coluzzi*i (61.68%) followed by *An. gambiae* (37.62%) were significantecly higher than those of the hybrids (0.70%). These findings are important for our understanding of the process of sympatric speciation in these important vector species.

1835

INTEGRATED PEDIATRIC FEVER MANAGEMENT AND ANTIBIOTIC OVER-TREATMENT IN MALAWI HEALTH FACILITIES: DATA MINING A NATIONAL FACILITY CENSUS

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There are concerns about growing irrational antibiotic prescription practices in the era of test-based malaria case management. This study assessed integrated pediatric fever management using malaria rapid diagnostic tests (RDT) and Integrated Management of Childhood Illness (IMCI) guidelines, including the relationship between RDT-negative results and antibiotic over-treatment in Malawi health facilities in 2013-2014. A Malawi national facility census included 1,981 observed sick children 2-59 months with fever complaints. Weighted frequencies were tabulated for other complaints, assessments, and prescriptions for RDT-confirmed malaria, IMCI-classified pneumonia, and clinical diarrhea. Classification trees using model-based recursive partitioning estimated the association between RDT results and antibiotic over-treatment and learned the influence of 38 other input variables at patient-, provider-, and facility-levels. Among 1,981 clients, 72% were tested or referred for malaria diagnosis and 85% with RDT-confirmed malaria were prescribed first-line anti-malarials. 28% with IMCI-pneumonia were not prescribed antibiotics (under-treatment) and 59% 'without antibiotic need' were prescribed antibiotics (over-treatment). Few clients had respiratory rates counted to identify antibiotic need for IMCI-pneumonia (18%). RDT-negative children had 16.8 (95% CI: 8.6-32.7) times higher antibiotic over-treatment odds compared to RDT-positive cases conditioned by cough or difficult breathing complaints. Integrated pediatric fever management was sub-optimal for completed assessments and antibiotic targeting despite common compliance to malaria treatment guidelines. RDT-negative results were strongly associated with antibiotic over-treatment conditioned by cough or difficult breathing complaints. A shift from malaria-focused 'test and treat' strategies toward 'IMCI with testing' is needed to improve quality fever care and rational use of both anti-malarials and antibiotics in line with recent global commitments to combat resistance.

1836

VALIDATION OF MATERNAL RECALL OF CARE-SEEKING EVENTS FOR CHILDHOOD ILLNESS IN SOUTHERN PROVINCE, ZAMBIA

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Seeking care from an appropriate provider is the first step in accessing correct management of an episode of childhood illness. Accuracy of maternal-reported care-seeking timing and source of care as collected through household surveys has not been validated in sub-Saharan Africa. A 2016 survey compared reported care-seeking against a gold-standard of documented care-seeking events among a random sample of mothers of children <5 years old in Southern Province, Zambia. A total of 1,156 enrolled children were assigned cards with unique barcodes. A total of 75 potential providers of child curative services in the study area participated in care-seeking event tracking. Providers were given smartphones with

a barcode reader and instructed to scan the cards of all children seeking care at the source, generating an electronic record of the care-seeking event. Additionally, providers gave all caregivers accessing care for a child <5 provider-specific tokens used to verify the point of care during the household survey. Reported care-seeking events were ascertained in each household using a questionnaire modeled off the Zambia Demographic and Health Survey (ZDHS). The ZDHS defines childhood illness as fever, cough with rapid breathing, and/or diarrhea in a child under 60 months of age in the two weeks preceding the survey. Recall of care-seeking events for childhood illnesses reported by mothers was compared against the gold-standard documented care-seeking events to estimate the accuracy of maternal recall of care-seeking behavior. Care-seeking data were collected for 537 children in urban areas and 547 children in rural areas. We present findings on the accuracy, sensitivity, and positive predictive value of caregiver report of care-seeking location by key socio-demographic characteristics. This study assesses whether the current standard care-seeking indicator measured through household surveys can produce valid estimates of care-seeking for childhood illness. This will be used to determine whether new methods are needed to estimate care-seeking behavior to measure progress in global investments in child survival interventions.

1837

POLYPHARMACY, TREATMENT SEEKING, AND DIAGNOSTIC TESTING IN A POPULATION-BASED SURVEY OF FEBRILE ILLNESS IN WESTERN KENYA

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Many fever episodes in malaria endemic areas are treated in the informal sector. Minimal access to diagnostic testing, both in the formal and the informal sectors, lends itself to the potential for polypharmacy and other forms of inappropriate use of medicines. We describe the type and the number of medicines consumed for a febrile illness in a community survey conducted as part of a larger study. Data on any laboratory tests done for the fevers were also collected. The study population consisted of household members above one year of age with a history of a febrile illness in the preceding one month. Out of the 2,007 clients reporting a history of fever for which they took an action, 99.1% reported taking medication for the fever, mainly antipyretics/analgesics (86.7%) and antimalarials (76.4%). Use of antibiotics was reported at 29.8%. Forty seven percent of patients who took a medicine took two different drugs; the commonest combination being an antimalarial and an analgesic. Twenty eight percent (28%) took three different medications while nearly 10% reported taking four or more medicines. Only 15.4% reported using a single drug. The majority of those who took an antimalarial (72.7%) reported using an ACT while the rest received either Sulphadoxine Pyremethamine (SP) or quinine. A malaria test was performed on 44.3% of the clients, while in 229 cases (11.4%) a test other than a malaria test was performed, most commonly for typhoid fever (81.7%), and brucellosis (18.8%). This was in addition to a malaria test for 221 (96.5%) of the 229 for whom a non-malaria test was performed. Twelve percent reported being tested for both typhoid fever and brucellosis. Clients above 5 years of age, those who had a laboratory test, and those visiting a drug shop for fever management were more likely to receive more than two drugs ($p<0.001$). Clients under 5 years were more likely to have a malaria test done than those aged > 5 ($p<0.001$). There is a high rate of consumption of multiple drugs for fever which is exacerbated by poor access to diagnostic testing. There is need for strategies to promote evidence-based management of fevers and rational use of drugs in the community.

1838

QUALITY IMPROVEMENT STRATEGIES TO MONITOR CHVS MRDT PERFORMANCE: A CASE OF MALARIA TESTING IN WESTERN KENYA

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Use of Community Health Workers (CHWs) in malaria diagnosis has been recommended as a task-shifting strategy to counter the shortage of health workers in resource-limited settings. However, monitoring their performance is a challenge. We implemented a set of strategies for monitoring CHW performance of malaria rapid diagnostic tests (mRDTs): 1) Post-training evaluation, 2) regular supervision and refresher training visits, and 3) monitoring of error rates. In the context of a larger study, we trained 300 CHVs from 16 Community Units (CUs) in two sub counties in western Kenya on proper use of mRDTs. At the conclusion of training, we measured CHW proficiency with a standardized mRDT checklist and evaluated their interpretation of a set of used mRDTs. CHWs then administered and interpreted mRDTs within their CUs to any patients with symptoms and/or reported history of fever. Clients with a positive mRDT received an antimalarial discount redeemable at selected private medicine outlets when presented along with their positive mRDT cassette. We collected mRDTs interpreted as positive from the outlets, and mRDTs interpreted as negative from the CHWs. We then re-interpreted the mRDTs to confirm results and test quality. In the first 6 months, we convened a total of 10 supervision meetings with each CHW group in the 16 CUs, and provided refresher training as necessary. After 6 months, 10,872 clients had been tested, 2,256 (19.96%) of which had a positive mRDT and 8616 (80.04%) of which had a negative mRDT. The total mRDT errors were 138 tests (1.27%), with 62 (3%) false positive and 76 (1%) false negative. The incidences of false positive and false negative results trended downwards as supervision visits continued over time; month 1-2: 14.5/23, month 3-4: 10.5/11, month 5-6: 6/3.5. The findings suggest that coupling supervision meetings with confirmation of mRDT results can help identify errors and refresher training needs, as well as improve CHW performance in accurate malaria diagnosis.

1839

MISSED OPPORTUNITIES FOR INTERMITTENT PREVENTIVE TREATMENT IN PREGNANCY FOR MALARIA: EVIDENCE FROM THE KENYA DEMOGRAPHIC AND HEALTH SURVEY, 2014

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Malaria in pregnancy (MIP) is associated with maternal anemia, placental parasitemia, low birth weight and increased perinatal mortality. Intermittent preventive treatment in pregnancy (IPTp) with sulphadoxine-pyrimethamine (SP) is recommended to reduce MIP-associated risk in medium-to-high malaria transmission areas. In Kenya, although antenatal care (ANC) clinic attendance is high, the proportion of women receiving at least two doses of IPTp-SP has historically been low. We assessed the factors associated with missed IPTp opportunities during pregnancy. We analyzed data from the 2014 Kenya Demographic and Health Survey, a two-stage cluster sample, cross-sectional survey of 36,430 households. Missed IPTp opportunities were was defined as a woman aged 15–49 years who attended at least four ANC visits and lived in the 14 malaria-endemic counties with IPTp policy but received fewer than two doses of IPTp-SP during their last completed pregnancy. We used logistic

regression to compare missed IPTp-SP opportunities with demographic, socio-economic and geographic factors. Of the 909 women who attended at least four ANC visits in the 14 malaria-endemic counties, 30.5% (n=277) had a missed opportunity for IPTp during pregnancy. In univariate analysis, living in the lake-endemic region (OR=1.7; 95% CI: 1.1–2.4; p=0.008), parity >4 children (OR=1.5; 95% CI: 1.04–2.22; p=0.028) and more than secondary education (OR=3.5; 95% CI: 1.6–7.5; p=0.001) were significantly associated with missed IPTp opportunities. In multivariate analysis, women with more than secondary education had significantly higher odds (OR=3.3; 95% CI: 1.4–7.9, p=0.007) of missed opportunities for IPTp. Despite high ANC attendance, almost one-third of pregnant women had at least one missed IPTp opportunity. Women with higher education were over three times more likely to have missed IPTp opportunities, which might be due to lack of perceived risk by both women and healthcare providers. Studies are needed to identify modifiable factors to increase IPTp uptake among pregnant women.

1840

IMPLEMENTATION OF SEASONAL MALARIA CHEMOPREVENTION IN THE GAMBIA

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Seasonal Malaria Chemoprevention was adopted in the Gambia as a strategy in 2012, included as part of the national policy in 2013, and implemented from 2014. In 2015, an electronic data system was introduced for monitoring delivery, through the ACCESS-SMC project supported by UNITAID. SMC drugs were administered from August to November in four cycles in Upper River Region and Central River Region. SMC was delivered door-to-door. Each child was issued an SMC card bearing a QR code that could be scanned with an android phone each time the child was treated. Information about the child (age, gender and other details) was captured on the phone using iForm, an offline data capture system, and then uploaded to a central database system, eValuate. The system provides information on all the monthly treatments a child has received avoiding the need for registers and allowing timely feedback to the malaria control programme about progress with SMC delivery. At the end of the transmission season, a cluster sample survey was conducted to measure SMC coverage. Communities selected with probability proportional to size, were divided into segments on a sketch map and all the households in one segment, which was chosen at random, were included in the survey. 1174 children under 7 years of age were surveyed, 690 were eligible for 4 SMC cycles and of these, 93% had received an SMC card and at least one SMC treatment. 84% of children had received at least 3 months of SMC treatment. Coverage was lower in the 4th month, which coincided with harvest activities. The main reason for missed doses was being away when the health worker visited. Relatively few children outside the recommended age range were treated, among children 6 to 7 years of age, fewer than 30% had received SMC doses. Door-to-door delivery achieved high coverage of SMC in The Gambia. Outreach strategies may improve coverage at the end of the transmission season.

1841

A CLUSTER RANDOMIZED TRIAL OF TARGETED BEHAVIOR CHANGE COMMUNICATION USING A MOBILE HEALTH PLATFORM TO INCREASE UPTAKE OF LLINS AMONG PREGNANT WOMEN IN TANZANIA: THE HATI-SALAMA PROJECT

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The Hati Salama (HASA) cluster-randomized controlled trial aims to increase malaria awareness among pregnant women using mhealth technology in Tanzania. HASA utilized an electronic system whereby nurses issue vouchers to pregnant women, allowing them to redeem a Long Lasting Insecticidal bednet (LLIN) at a retailer for a highly subsidized cost. A RCT was selected to test efficacy of SMS behaviour change communication messages aimed to increase the uptake of LLINs in areas of Tanzania identified as malaria hotspots with overall low uptake of LLINs. HASA was implemented in 97 antenatal health facilities; 48 clinics were assigned to the control group (no targeted SMS messages sent to beneficiaries) and 49 in the intervention group (targeted messages sent to beneficiaries). In total, 5396 beneficiaries were randomized through cluster randomization of the health center and had LLIN voucher redemption status recorded. There were 2708 beneficiaries from the intervention clinics, and 2688 beneficiaries from the control clinics. There were 25 urban clinics and 23 rural in each arm. The redemption rate was 70.4% in the intervention sites and 67.4% in the control sites. The absolute difference in the redemption rates was 3.5% (95% CI, -3.8% to 11.0%) p=0.35 according to a Rao-Scott estimate stratifying by urban/rural and clustering by clinic. The odds ratio of redemption in the intervention vs. control sites was 1.13 (95% CI, 0.86 to 1.51,p=0.36) according to the GEE method controlling for urban/rural and the prior redemption rate with a working exchangeable correlation to account for the cluster randomized design. The estimated intraclass correlation coefficient (ICC) is 0.11 meaning that 11% of the total variance in the redemption rates was attributable to the clinic and the remaining 89% was attributable to the beneficiary. The use of behavior change communication via SMS had no significant effect on increasing LLIN uptake among pregnant women in this large cluster randomized trial. This suggests that other factors to uptake of LLINs through a voucher program exist. Evaluation of these factors is essential for future implementation of similar programs.

1842

CONCURRENTLY ESTIMATING THE COMPLEXITY OF INFECTION AND SNP ALLELE FREQUENCY FOR MALARIA PARASITES

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Plasmodium falciparum population genetics can inform malaria epidemiology, but a high prevalence of polygenomic infections (those with more than one genotype) can render estimation of even the most basic parameters, such as allele frequencies, challenging. A method, COIL, has been developed to estimate complexity of infection (COI) and allele frequency from SNP data, but relies entirely on monogenomic infections to estimate allele frequencies. However, allele frequency estimates limited to monogenomic infections are biased, and when the average COI is high they can be difficult or impossible to estimate. Here we develop an iterative approach that simultaneously estimates allele frequency and COI from all samples in a population, irrespective of whether they are monogenomic or polygenomic, and uses Markov chain Monte Carlo method to provide Bayesian inference. The method was tested on a series of simulations and then applied to a real dataset from Uganda. We performed Sequenom typing of 105 SNPs in 868 samples from cross-sectional surveys performed in three regions of varying endemicities in Uganda including Walukuba (low-moderate, EIR 2.8), Kihihi (high, EIR 32), and Nagongera (very high, EIR 310). Allele frequencies were used to calculate F_{ST}, a measure of genetic differentiation. Our results suggest high migration rates and little population substructure between the sites (0.016 Kihihi vs. Nagongera, 0.0 for Walukuba vs. Kihihi or Nagongera). Surprisingly, the mean COI in Walukuba (4.7) was similar to Nagongera (4.4) and significantly higher than Kihihi (2.0) despite much lower transmission in Walukuba; this unexpected finding was not explained by parasite density or age. One possible explanation for this finding is that Walukuba is peri-urban setting with a relatively high proportion of cases coming from surrounding regions with higher transmission intensity. This is also consistent with the absence of population structure observed between the other sites. We conclude that this method allows the interpretation of useful population genetic SNP data from polygenomic infections, which are common in high transmission settings.

1843

USE OF SHARED HAPLOTYPES THAT ARE IDENTICAL-BY-DESCENT TO INFER POPULATION STRUCTURE AND PARASITE MIGRATION WITHIN SOUTHEAST ASIA

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Estimates of parasite gene flow may be important in stratifying malaria risk, but to be useful for this purpose those estimates need to reflect contemporary patterns of parasite migration. Haplotypes identical-by-descent are being increasingly used in human genomics for inference of recent demographic events, and can be used to estimate migration rates. Such methods have not been used to infer migration patterns for malaria parasites, and they are just now beginning to be used to document changes in parasite demography as a result of reduced malaria transmission or the rapid spread of drug resistance mutations. The objective of this study is to examine segments of the parasite genome that are identical-by-descent (IBD) to more finely map patterns of parasite population structure and to infer migration patterns at an increasingly local scale. The extent of shared IBD haplotypes was determined by the program Beagle using SNPs genotyped by a *P. falciparum* DNA microarray from samples collected in Southeast Asia and Bangladesh. The extent of IBD sharing was estimated pair-wise between all samples and aggregated both within each study site and between study sites. Preliminary analyses suggest meaningful sharing of IBD haplotypes within study sites, with median IBD segments upwards of 1MB. There is evidence of increased IBD sharing between sites in close geographic proximity, but also some evidence of IBD sharing between more geographically distant sites, which may represent parasite migration through human movement. Patterns of IBD sharing between sites mirror patterns of spreading artemisinin resistance (based on sites sharing K13 haplotypes). IBD sharing based on SNPs from the DNA microarray will be compared to IBD sharing determined from a subset of samples for which whole genome sequences are available. The study of temporal and geographical dynamics of shared IBD haplotypes is a promising approach for delineating contemporary patterns of parasite migration that can be used to identify sources and sinks of malaria transmission.

1844

WHOLE GENOME SEQUENCING USED TO DISTINGUISH *PLASMODIUM VIVAX* RELAPSE FROM REINFECTION AND PRIMAQUINE RESISTANCE IN PERU

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Plasmodium vivax, the most widespread form of malaria, poses a significant challenge to malaria elimination due to its ability to cause relapsed infections from reactivation of hypnozoites. Distinguishing relapses from reinfections or recrudescence is essential for monitoring malaria transmission patterns and detecting anti-relapse therapy resistance. Current methods for genotyping *P. vivax* rely on microsatellite markers, which reveal a limited region of the parasite's genome, making it difficult to differentiate relapses from reinfections. Whole genome sequencing (WGS) of *P. vivax* is a highly sensitive tool for genotyping recurrent infections that has not been widely deployed in field studies. One main reason is because patients with *P. vivax* infections have low parasitemias, so a small amount of human DNA greatly reduces sequencing efficiency. We used a novel technique called selective whole genome amplification (SWGA) to enrich *P. vivax* DNA from whole blood samples. We performed WGS of 81 isolates of *P. vivax* collected from symptomatic patients in Iquitos, Peru during a study to assess three regimens of primaquine. This included 58 paired samples from a person's initial and recurrent infection after primaquine treatment. We obtained high quality sequences with an average coverage of 22x and up to 80% of the genome covered by >5 reads. We identified thousands of high quality single nucleotide polymorphisms, insertions and deletions, and copy number variants, which we will use for further analysis. We will calculate genetic diversity, linkage disequilibrium, complexity of infection, and genes under balancing or directional selection. We will compare paired samples from recurrent infections using a sliding window principle components analysis approach. In this study, we validate a cost-effective and robust method for genotyping *P. vivax* infections that will significantly improve the ability to track *P. vivax* transmission and monitor the efficacy of anti-relapse medication.

1845

A GENOME-WIDE ANALYSIS OF RECENT SELECTION IN AFRICAN MALARIA VECTOR POPULATIONS

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Land use changes, increasing urbanisation and intensification of malaria control programmes are subjecting malaria vectors to a variety of new and intense selective pressures. Here we describe a genome-wide scan for signatures of recent selection in mosquito populations sampled from 8 African countries, using whole genome sequence data from the *Anopheles gambiae* 1000 genomes (Ag1000G) project. We have integrated results from a number of statistical methods, including tests based on haplotype length (iHS, XP-EHH), haplotype diversity (H12), allele frequency spectra (SweeD, delta Tajima's D) and population differentiation (FST, XP-CLR, PBS). This combination of methods allows us to identify selective events that are specific to a single population or shared across multiple populations, to identify both hard and soft selective sweeps, and to find selection events from recent times and the more distant past. As well as validating several previously observed loci, we identify a number of very strong signals of recent selection at novel loci. Of the top 20 strongest previously unseen signals, 10 coincide with metabolic insecticide resistance genes, 2 coincide with with genes that may be involved in cuticle mediated resistance, 1 coincides with genes associated with gustatory behaviour, and 7 have no candidate phenotype. Although we observe a number of hard sweeps, most loci display soft sweeps involving multiple haplotypes. Several loci have come under selection across a broad geographical range, however the pattern of selection is heterogeneous with a number of hits being restricted to a single population or geographical region and some populations showing almost no evidence for recent selection. All data from these analyses will shortly be made publicly available for download and for interactive exploration via a new release of the Ag1000G web application, providing a community resource for further detailed study of selective forces and adaptive responses in natural vector populations.

1846

GENOME-WIDE ASSOCIATION STUDY OF SUSCEPTIBILITY TO SEVERE MALARIA IN 17,500 INDIVIDUALS FROM AFRICA, ASIA AND OCEANIA

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Genome-wide association studies (GWAS) of susceptibility to malaria have until recently been limited by relatively small sample sizes. In addition, the complex pattern of effects observed for some association signals, and poor representation of relevant genetic variation in available reference panels has hampered progress. Here, we undertake a GWAS in 8931 individuals hospitalised with severe malaria and 8703 population controls from 11 malaria-endemic populations, with replication in a further 15,000 individuals. We sequence the genomes of a further 773 individuals from sub-Saharan African populations, and use this data along with publicly available data from the 1000 Genomes Project to accurately impute genotypes genome-wide. We develop methodology to test for association with severe malaria subphenotypes, and identify and replicate a novel locus on chromosome 6 associated with increased risk of cerebral malaria. Across the genome, at least 10 other loci show substantial evidence for association, and we catalogue these in detail. In specific regions, we survey structural variation and use further refined reference panels to fine-map the signal of association, including at the glycophorin region on chromosome 4 where we identify a strong candidate for the functional protective mutation. Our study represents the largest genome-wide study of an infectious disease to date, and will provide an important resource for future studies into the human genetic basis of malarial disease and, potentially, its interaction with parasite processes.

1847

COMPARATIVE TRANSCRIPTOME ANALYSIS OF THE HOST RESPONSE IN BLOOD AND SPLEEN DURING THE COURSE OF A *PLASMODIUM CHABAUDI CHABAUDI* INFECTION

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During the asexual blood stage of malaria in the mammalian host, *Plasmodium* parasites induce alterations in host haematopoiesis, and in the structure and cellular composition of the spleen. Although this organ plays a critical role in generating anti-parasite immunity, spleen samples are generally not accessible in humans; instead, blood samples are typically used to infer the types of immune responses important for controlling infections. Whether the immune signatures identified in the blood are representative of those within the spleen is still largely not known, however. The objective of our study was to compare parasite-induced whole transcriptome changes in blood and spleen using the rodent malaria model of *P. chabaudi chabaudi* in C57BL/6 mice. Specifically we set out to identify common infection signatures, and those that can only be detected in either the blood or the spleen. Infected blood and spleen samples

were collected every 2 days during the acute phase of infection, until day 12 when the parasitaemia was first controlled. Samples collected from naive uninfected mice at day 0 and day 12 were used as controls. Gene expression was quantified using the Illumina mouse WG6 v2 microarray platform (consisting of 45,281 probes sets, representing 30,854 genes). Data were transformed to log fold change with respect to naive controls, and clustering of these short time-series was performed using a Mixtures of Hierarchical Gaussian Processes (MOHGP), which explicitly model the strong time dependency across successive time points. We identified several pathways that are commonly perturbed in blood and spleen, including anaemia, apoptosis and T-cell activation. We also observed that for some genes, expression peaked in the blood before it was measured in the spleen. Importantly a number of pathways, such as erythrocyte production, were identified that were exclusively activated in the spleen but not the blood. We will discuss the implications of these findings for the interpretation of whole blood transcriptome.

1848

MELDING CHEMOGENOMICS AND CHEMOINFORMATICS TO DEFINE MALARIA'S DRUGGABLE GENOME

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With artemisinin resistance (ART-R) spreading in South East Asia the discovery of new drugs to treat malaria is imperative. Thousands of antimalarial compounds have been identified in high-throughput phenotypic screens, but the mode of action (MOA) for most of these compounds is unknown. Efforts to discover the MOA targets of these compounds is hampered since much of the *Plasmodium falciparum* genome is not functionally annotated and therefore deciphering target information for many, if not most, new lead compounds is severely compromised. Combining chemogenomics and chemoinformatics offers the potential to functionally define the druggable genome of *P. falciparum*. Our approach can functionally link unknown genes to more clearly defined genes and GO pathways through the chemogenomic profiles related to the MOA of characterized antimalarial drugs and unknown lead compounds. Importantly, this type of analysis helps to rationally classify leads most likely to be targeting genes that can counter ART-R. More specifically, we are using chemogenomic profiling of isogenic *P. falciparum* single insertion *piggyBac* mutant clones, including several with differential sensitivity to ART. Specific perturbations in metabolic pathways linked to the genetic mutation caused by the *piggyBac* insertion create unique IC_{50} patterns for each compound and similar IC_{50} profiles identify drugs with MOAs likely to be targeting the same pathway. This information can be combined with chemical similarity measures of around 500 prioritized compounds to increase confidence in target prediction and indicate which molecular features are key to the biological response. We identified distinct mechanisms associated with ART sensitivity and resistance in the current screen by RNA-seq of a dysregulated K13 mutant. Chemogenomic and chemoinformatic characterization of malaria inhibiting compounds will help focus the drug discovery agenda on the most effective targets.

1849

HOW PYRETHROIDS RESISTANCE IN *AEDES AEGYPTI* POPULATIONS FROM BRAZIL AFFECTS *WOLBACHIA* INVASION? EVIDENCES FROM SIMULATIONS AND FIELD RELEASES

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Field trials have started recently in Brazil to evaluate the spread of *Aedes aegypti* with Wolbachia, a bacteria that reduces arboviruses transmission such as dengue and zika viruses. Previous data reported stable and rapid invasion in Austrália and Vietnam using wMel strain. In Rio de Janeiro, we started weekly releases in Sep/2014, but Wolbachia frequency dropped dramatically soon after releases were suspended. Hindsight analysis showed that mosquito colony, which were closed after Australian *Ae. aegypti* females were backcrossed with Brazilian males, lost its alelles to pyrethroid (PI) resistance after only 14 generations in lab, suggesting a strong fitness cost due to insecticide resistance. Therefore, we released mosquito cohorts susceptible to PI in Rio which Ae. agypti populations face high insecticide resistance ratios for PI. Even releasing roughly 10,000 mosquitoes per week for 20 weeks, kdr frequency remained unalterd during releases. We hypothetized the insecticide susceptibility of released mosquitoes hindered Wolbachia invasion in Rio. We performed a new backcrossing with field males to produce wMelRio, a strain with similar insecticide resistance profile and fitness (survival and fecundity) when compared with field population. Thus, Wolbachia presence in a kdr mutated individual may exacerbate the fitness cost and could hinder Wolbachia invasion into a resistant population. In Jan/2016, after 24 weekly releases in field, wMelRio frequency was high as 80%. This frequency remains high (70-80%) even after a five-weeks period in which releases were stopped. Mathematical models were applied to test whether different releasing strategies (changing release number, wild population density, fitness cost of Wolbachia, fitness cost of insecticide resistance) would enhance Wolbachia invasion. Our simulations indicates a successful invasion in two situations: 1) releasing susceptible mosquitos in an environment without insecticide use (may causing a reversal in insecticide resistance levels); and releasing resistant mosquitoes (wMelRio) into a resistant population (such as Rio), even with a high insecticide use.

1850

WOLBACHIA INFECTION DOES NOT AFFECT THE DIVERSITY OF CO-INFECTING NATIVE FLAVIVIRUSES IN ADULT *AEDES AEGYPTI* IN THE FIELD

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Wolbachia (wMel) infections that have been artificially introduced into *Aedes aegypti* limit the ability of the mosquito to become infected with dengue and other flaviviruses in a trait known as pathogen blocking. It is unclear whether these antiviral effects would extend to other native flaviviruses in wild populations of mosquitoes. If so, Wolbachia may be beneficial in cases where these viral infections have negative effects on host fecundity and lifespan or induce energetic investments in immune responses. Here we examine whether the presence of Wolbachia infection in wild caught adults from field release populations in northern Australia reduces the native flavivirus diversity. RNA was extracted from adult mosquitoes collected from two sites within and one site outside of field release zones. Flavivirus specific primers for the NS5 region were then used to amplify from the converted cDNA of individual samples. Six insect flavivirus positive individuals were then selected from each of three sites for deep sequencing. We found that virus diversity was greater in the

Wolbachia infected mosquitoes, returning 10 different viruses versus 6 different viruses in the Wildtype samples. Cell fusion agent virus was found in all 18 samples across the sites and another 5 viruses were present at low levels in both wMel and Wildtype mosquitoes. A total of 6 viruses were unique to the wMel-infected mosquitoes. As the approach required PCR amplification prior to sequencing however it cannot address quantitative differences in the amount of viruses present. Future studies using non-targeted deep sequencing of insect material may address the issue around quantitation and also whether non-flaviviral diversity is impacted. Regardless, our study does not find clear evidence of Wolbachia's potential to benefit wild mosquito populations by limiting native flavivirus infections.

1851

LIGHT MANIPULATION OF MOSQUITO BEHAVIOR: ACUTE AND SUSTAINED PHOTIC SUPPRESSION OF BITING IN THE *ANOPHELES GAMBIAE* MALARIA MOSQUITO

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Host-seeking behaviors in anopheline mosquitoes are time-of-day specific, with a greater propensity of biting occurring during the dark phase of the LD cycle. We investigated how a short exposure to light presented during the night or late day can inhibit biting activity and modulate flight activity behavior. *Anopheles gambiae* s.s., maintained on a 12:12 LD cycle, were exposed to white light at the onset of night and the proportion taking a blood meal in a human biting assay was recorded every 2 hr for 8 hr. The pulse significantly reduced biting propensity in mosquitoes for up to 4 hr following administration, and with no differences detected after 6 hr. Conversely, biting levels were significantly elevated when mosquitoes were exposed to a dark treatment during the late day, suggesting that light suppresses biting behavior even during the late day. These data reveal a potent effect of a discrete light pulse on biting behavior that is both immediate and sustained. We expanded this approach to develop a method to reduce biting propensity throughout the night by exposing mosquitoes to a series of 10 minute pulses presented every 2 hr. We reveal both an immediate suppressive effect of light during the exposure period and 2 hr after the pulse. This response was found to be effective during most times of the night: However, differential responses that were time-of-day specific suggest an underlying circadian property of the mosquito physiology that results in an altered treatment efficacy. Finally we examined the immediate and sustained effects of light on mosquito flight activity behavior following exposure to a 30 minute pulse, and observed activity suppression during early night, and elevated activity during late night. As mosquitoes and malaria parasites are becoming increasingly resistant to insecticidal and drug treatments, there is a necessity for the development of innovative control strategies beyond ITNs. These data revealing the potent inhibitory effects of light exposure and the utility of multiple photic pulses presented at intervals during the night/late day, may prove to be an effective tool that complements established control methods.

1852

ESTIMATION OF ALLELE-SPECIFIC ACE-1 DUPLICATION IN INSECTICIDE-RESISTANT *ANOPHELES* MOSQUITOES FROM WEST AFRICA

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Variation in *Ace-1* copy number and G119S mutation genotype from samples of *Anopheles gambiae* across West Africa are used as appropriate strategies for identifying variation at population and individual levels. The most widespread and economical method, PCR-RFLP, suffers from an inability to discriminate true heterozygotes from heterozygotes with duplication. In addition to PCR-RFLP, in this study we used three different molecular techniques on the same mosquito specimens permitting comparisons. To group heterozygous individuals recorded from the PCR-RFLP analysis into different assumptive genotypes we used K-means clustering on the Z-scores of their correspondent data obtained from both TaqMan and ddPCR methods. Our data suggest that most heterozygotes are duplicated and that G119S mutation must now be regarded as a complex genotype ranging from primarily single-copy susceptible to Glycine and Serine allele balanced and imbalanced heterozygotes, and multiply-amplified resistant Serine allele homozygotes. Whilst qRTPCR-based gene copy analysis suffers from some imprecision, it clearly illustrates differences in copy number among genotype groups identified by TaqMan or ddPCR. Based on TaqMan method properties, and by coupling TaqMan and ddPCR methods simultaneously on the same type of mosquito specimens, we demonstrated that the TaqMan genotype assays associated with the K-means clustering algorithm could provide a useful semi-quantitative estimate method to investigate the level of allele-specific duplication in mosquito populations. *Ace-1* gene duplication is evidently far more complex in *Anopheles gambiae* than *Culex quinquefasciatus*, which consequently can no longer be considered an appropriate model for prediction of phenotypic consequences, which require urgent evaluation. Furthemore, if carbamates and organophosphate will be used as alternative products to pyrethroid for malaria vector control, the monitoring of duplicated alleles in natural populations of *An. gambiae* is essential to guide the rational use of these insecticides.

1853

INSECTICIDE RESISTANCE AND THE FUTURE OF MALARIA CONTROL

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The emerging and rapid spread of resistance to major classes of public health insecticides threatens current malaria vector control efforts namely long lasting insecticidal nets and indoor residual spray, which have contributed substantially to the reduction of malaria since 2000. The decreased ability of current vector control tools to effectively kill mosquitoes may be an early indicator to an increase in malaria cases and attributed deaths. Visualizing the confirmed reports of insecticide resistance in malaria endemic countries provides an indication where resistance may play a role in the persisting burden of malaria. Launched in 2012, IR Mapper is an online geospatial platform for mapping insecticide resistance in malaria vectors, built on a systematic review of peer-reviewed, published literature. The user interface enables filtering by country, year, vector species, insecticide class and type, and resistance mechanisms data including target site mutations and elevated metabolic mechanisms related to the detoxification of insecticides. As of March 2016, IR Mapper

consisted of 13,773 unique field records from 58 countries and 64 *Anopheles* species or species complexes. 78% of countries have reported resistance to at least one of the four classes of insecticides used for adult mosquito control. Examining the top ten countries with the largest burden of malaria today, more reports of confirmed pyrethroid resistance were recorded in the period 2008-2015 than compared to 2000-2007. Kenya and the Democratic Republic of Congo reported no pyrethroid resistance in 2000-2007 but in 2008-2015, 77.5 and 51.0% respectively of the testing conducted on pyrethroids reported resistance. In Burkina Faso, comparing the same time periods, the proportion of reports of confirmed pyrethroid resistance from the total number of tests conducted increased from 19.4 to 94.0%. IR Mapper is a useful tool for visualizing trends in *Anopheles* insecticide resistance and can be used to assist decision making for deployment of the most appropriate tools, which need to be driven by up-to-date data on insecticide resistance in target malaria vector species.

1854

THE EMERGENCE AND SPREAD OF INSECTICIDE RESISTANCE MUTATIONS IN *ANOPHELES GAMBIAE* AND *AN. COLUZZII*: INSIGHTS FROM DEEP WHOLE-GENOME SEQUENCING OF NATURAL POPULATIONS

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Insecticide resistance is a serious challenge to malaria elimination in Africa. We use haplotype data from the *Anopheles gambiae* 1000 genomes project to discover new mutations potentially linked with insecticide resistance, and to analyse the origin, distribution and movement of resistance mutations in populations spanning continental Africa. Within the voltage-gated sodium channel (Vgsc) we find kdr mutations in codon 1014 sweeping to high frequency in almost all Ag1000G phase 1 populations. We infer at least 4 independent origins for the L1014F kdr mutation and a further 4 origins for the L1014S kdr mutation. Some kdr haplotypes are found in a single population and thus have a local origin, whereas others are shared between populations separated by thousands of kilometers. One haplotype carrying L1014F has swept widely throughout West and Central Africa with at least two introgression events between species. Within Vgsc we also find 15 previously unknown non-synonymous mutations at high frequency. Of these, 13 occur exclusively on haplotype backgrounds carrying a mutation in codon 1014, suggesting selection for mutations that enhance or compensate for the resistance phenotype. We also find haplotypes sweeping to high frequency at three loci containing genes linked with metabolic resistance. At the glutathione S-transferase epsilon (gste) gene cluster at least four independent sweeps have occurred. One of the swept haplotypes carries the I114T mutation in gste2 known to enhance DDT metabolism and is found in populations from West, Central and East Africa indicating a continent-wide spread. The other high frequency haplotypes do not carry this mutation but do carry a large number of novel non-synonymous mutations which may be driving a resistance phenotype. We describe similar analyses for the cyp6 gene cluster and a locus on chromosome X containing cyp9k1. For all loci we identify SNPs tagging putatively resistant haplotypes as a basis for future monitoring efforts. These results reveal the threats posed by the capacity for mutations to spread throughout vector populations and to arise multiple times in different locations.

1855

HOUSEHOLD INTERVENTIONS, EXPENDITURES AND BARRIERS TO *AEDES AEGYPTI* CONTROL IN MACHALA, ECUADOR

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The *Aedes aegypti* is an efficient vector for the transmission of Zika, chikungunya and dengue viruses. However, understanding of the household expenditures needed to control this mosquito is relatively sparse. As various countries face the rising epidemic of *Ae. aegypti*-transmitted illnesses such as Zika virus, research on the extent of use and cost of interventions to control the *Ae. aegypti* is urgently needed. Between April to August 2015, we surveyed residents from 40 households in a high risk community in Machala, Ecuador on dengue knowledge and perceptions, vector control interventions, household expenditures, and barriers to employing prevention practices. Additionally, a semi-structured survey was recorded, transcribed and coded to identify the important factors that influence a household's decision to purchase mosquito control products. To determine the various types and cost of products available for sale to households, we surveyed 10 neighborhood stores and three modern supermarkets. The results of this study show that households in this neighborhood spend about 2% of their total family income on *Ae. aegypti* control interventions. On average, households concurrently employed five mosquito control interventions and had access to a variety of products, including aerosols, liquid sprays, repellents, mosquito coils, and unimpregnated bed nets. From our qualitative theme analysis, we found that effectiveness and cost were the most important factors that influence people's decisions to purchase a mosquito control product. These findings show a robust and healthy market for commercial mosquito control products even among the poorest of households in Machala, Ecuador. With the rise in Zika virus transmission, the need for *Ae. aegypti* control has only been exacerbated. Further research will examine how household economics are influenced by the rise of a new disease.

1856

RISK FACTORS FOR PEDIATRIC ENTERIC INFECTION IN A LOW-INCOME URBAN NEIGHBORHOOD: EXAMINING THE CONTRIBUTIONS OF THE HOUSEHOLD ENVIRONMENT, NEIGHBORHOOD GEOGRAPHY AND EXPOSURE BEHAVIORS IN VELLORE, INDIA

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Poor water, sanitation, and hygiene conditions contribute to pediatric enteric infection and longer-term health outcomes. In urban settings, child exposure to fecal contamination may be affected by the population density, physical characteristics of the neighborhood, and frequency of contact with fecal contamination both inside and outside the home. This study examined the contributions of a child's household and neighborhood environments and exposure behaviors to enteric infection risk, by etiologic agent, in an urban slum in India. Diarrheal and routine (monthly) stool were collected from 230 children during the first two years of life and assayed for enteric pathogens as part of the MAL-ED study. Exposures were assessed using spatial data and interviews with

caregivers in 100 of these households and evaluated using mixed effects logistic regression models. Household sanitation coverage (33%) and fecal sludge management (82% of household toilets discharged to open drains) were poor. Significant household risk factors, associated with 44-56% increased risk of any enteric infection, included the presence of, and open defecation by, older siblings and adult caregivers. Reported household-level water treatment (OR: 0.66, 95% CI: 0.50-0.88) and presence of a toilet (OR: 0.73, 95% CI: 0.55-0.97) were associated with reduced enteric infection risk. Residence in the spatial cluster of reported drain flooding, regardless of reported contact with drain or floodwater, was associated with significantly higher risk (OR: 2.39, 95% CI: 1.24-4.63) during the northeast monsoon. The risk factors associated with viral infections differed from those for any enteric infection, and included frequent use (>10 events/month) of public toilets as a unique risk factor for GII norovirus infection (OR: 2.05, 95% CI: 1.09 - 3.86). Overall, while some exposures, like the defecation practices of other household members, may be controllable within the household, conditions in the neighborhood environment may limit a household's ability to control health risks. Thus, interventions to reduce fecal contamination in the public domain are also necessary.

1857

ASSESSING SEROCONVERSION AGAINST ENTERIC PATHOGENS RELATIVE TO REPORTED DIARRHEA AND THE RECEIPT OF A POINT-OF-USE WATER FILTER IN WESTERN PROVINCE, RWANDA

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Diarrhea is a leading contributor to childhood morbidity and mortality in Sub-Saharan Africa. Given the infeasibility of blinding most water, sanitation and hygiene (WASH) interventions, diarrheal disease outcome measures in WASH intervention trials are fraught with potential bias and misclassification. We used the platform of a cluster-randomized controlled trial of a household-based drinking water filter in Western Province, Rwanda to examine the application of enteric seroconversion as an alternative and more objective outcome measure of current and recent infection. All children ≥ 6 and ≤12 months-old among 1582 study households were eligible for enrollment. All enrolled children had their blood drawn through capillary blood draw at baseline and 6 to 9 months after intervention distribution. Multiplex serologic assays for *Giardia lamblia*, *Cryptosporidium* parvum, Entamoeba histolytica, Salmonella spp., norovirus, *Campylobacter* spp., enterotoxigenic *E. coli* and *V. cholerae* were performed to compare seroconversion between the intervention and control groups on an intention-to-treat basis. The water filter was associated with a decrease in *Cryptosporidium* 17 kDa protein (Cp17) seroconversion (RR=0.72, 95%CI: 0.52-1.00) and serologic response against *Cryptosporidium* Cp17 was positively associated with reported diarrhea in the previous seven days (RR=1.78, 95%CI: 1.02-3.12). Children seroconverted against *Cryptosporidium* at relatively early ages (<6 months-old) while Giardia seroconversion typically occurred after 12 months. Serological responses for other antigens increased steadily after 6 months of age, plateauing after 12 months. Seroconversion shows promise as an objective outcome measure for WASH trials among children in this age group in addition to being a potential indicator of recent diarrheal disease.

1858

ASSESSING USE, EXPOSURE AND HEALTH IMPACTS OF AN ADVANCED WATER FILTER AND ADVANCED COOKSTOVE DISTRIBUTION PROGRAM IN RURAL RWANDA

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Unsafe water and air pollution are two major environmental health risks and contribute to child diarrhea and pneumonia. Household water filters and advanced cookstoves could reduce exposure risks, but there is little evidence of medium-term uptake and impact when combined. In 2012, a public-private program provided a free rocket stove and water filter to houses in 15 rural villages. We matched 9 intervention to 9 control villages using propensity score matching. Houses with a child under 5 (n=269) were enrolled and visited in 2 rounds over 12 months starting Nov 2013. At each visit, self-reported use, observed use, and reported health symptoms were recorded; a drinking water sample was also assessed for thermotolerant coliforms (TTC). Personal exposure to fine particulate matter ($PM_{2.5}$) was assessed gravimetrically in cooks (n=211) and children under 5 (n=172) for 48 hours. Use of the advanced filter and stove was assessed using sensors for 8 days each round. Overall, 92.7% of intervention houses had the filter, 90% reported currently using it, and 74.9% had water in it. Sensors indicated a daily average of 1.7L of water filtered/day (SD 2.5L). The control arm had a mean of 0.86 log TTC/100mL, compared to 0.37 log TTC/100mL in the intervention arm (p<.001). 95% of intervention houses had the stove, and 87% reported currently using it. Sensors indicated a daily average of 2.6 uses/day (SD 1.4 uses). Geometric mean (GM) $PM_{2.5}$ for intervention cooks was 156.1 μg/m^3 (95% CI 139.2-175.0) compared to 215.4 μg/m^3 for controls (95% CI 191.1-242.8); GM for intervention children was 171.2 μg/m^3 (95% CI 150.5-194.7) compared to 218.5 μg/m^3 for controls (95% CI 189.2-252.4). Among children in the intervention arm relative to control, mixed effects models showed a significant reduction in the odds of both caretaker-reported diarrhea (OR=.52, p=.04) and cough with difficulty breathing (OR=.11, p<0.001). We found high uptake and sustained use of a home water filter and advanced cookstove 12-24 months after intervention delivery, with evidence of reductions in drinking water contamination, household air pollution and improvements in reported child health symptoms.

1859

ENVIRONMENTAL EXPOSURE OF RURAL BANGLADESHI CHILDREN 3-18 MONTHS OLD FROM HAND- AND OBJECT-MOUTHING

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Children are exposed to environmental contaminants by placing contaminated hands or objects in their mouths. Exposure models based on mouthing data of children from high-income countries may be inappropriate to model exposure of children from different cultural and domestic settings due to differing mouthing frequencies. We sought to quantify hand and object mouthing frequencies of Bangladeshi children and determine if they differ from those of U.S. children, potentially indicating differences in types and levels of exposures. Trained observers used tablet computers to capture hand and object mouthing behaviors of 148 rural Bangladeshi children aged 3-18 months over five hours. For quality control, 11% of children were watched by more than one observer and inter-observer reliability was calculated. We analyzed the effect of

household and child characteristics on mouthing behavior and modeled mouthing frequencies using 2-parameter Weibull distributions to compare the modeled medians with those of U.S. children. Hand- and object-mouthing frequencies decreased with increasing age, and at all ages were higher than those of U.S. children. For hand mouthing, the median hourly frequency for Bangladeshi children 3-6 months old was 37.3 contacts vs. 23.0 contacts for U.S. children; for Bangladeshi children 6-12 months old, 34.4 contacts vs. U.S. 14.0 contacts; and for Bangladeshi children 12-18 months old, 29.7 contacts vs. U.S. 14.0 contacts. For object mouthing, the median hourly frequency for Bangladeshi children 3-6 months old was 23.1 contacts vs. 9.3 contacts for U.S. children; for Bangladeshi children 6-12 months old, 29.6 contacts vs. U.S. 19.0 contacts; and for Bangladeshi children 12-18 months old, 15.2 contacts vs. U.S. 12.3 contacts. Mouthing frequencies were not associated with child location (indoor/outdoor). Using hand- and object-mouthing exposure models from U.S. and other high-income countries might not accurately estimate children's exposure to environmental contaminants via mouthing in low- and middle-income countries.

1860

INCIDENCE OF ADULT DEATHS ASSOCIATED WITH HEPATITIS E VIRUS IN BANGLADESH

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Hepatitis E virus (HEV) causes acute infection of the liver. In addition to sporadic cases, HEV causes epidemic outbreaks in many countries in Asia and Africa, where fecal contamination of drinking water is common. A modelling study estimated 70,000 annual deaths globally from HEV. China has produced and licensed the first effective HEV vaccine but there are limited population-based data on burden of hepatitis E to take a rational decision of introducing HEV vaccine. In this study, we estimated the population-based incidence of adult deaths from HEV infection in Bangladesh. During Jan-Dec 2015, we conducted HEV surveillance in six tertiary hospitals in Bangladesh where we recruited all patients aged ≥15 years admitted with acute jaundice, defined as new onset of either yellow eyes or skin during the past 3 months. We collected blood from all cases to test for anti-HEV IgM using enzyme-linked immunosorbent assay. We conducted a mortality survey in the hospital catchment communites where we asked the caregivers of all deaths aged ≥15 years occurring in the community in the 3 years prior to the survey if the decedent had jaundice, defined as new onset of either yellow eyes or skin during the 3 months prior to death. We administered a verbal autopsy questionnaire for all identified jaundice associated deaths. We used a poisson model to estimate the incidence of jaundice associated mortality in the hospital catchment areas and then applied the proportion of laboratory confirmed HEV cases among patients admitted with jaundice to estimate the population-based incidence of adult deaths from HEV. We identified 519 patients admitted with acute jaundice; 441 of them were tested and 114 (26%) had laboratory confirmed HEV. In the hospital catchment communities with an adult population of 1,219,268, we identified 462 deaths associated with acute jaundice. The incidence of adult deaths associated with HEV was 3.6 (95% CI: 2.9-4.4) per 100,000 adult population per year. The study provides the first population-based estimate of adult mortality associated with HEV in Bangladesh, which can be used in economic evaluations of interventions, including HEV vaccine.

1861

WOMEN'S SANITATION EXPERIENCES ARE ASSOCIATED WITH MENTAL HEALTH IN RURAL, ODISHA INDIA

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Research on the impact of access to water and sanitation on health has focused on infectious agents and diseases, leaving other facets of health—like mental health (MH)—underexplored. No research has quantified how prevalent women's negative sanitation experiences are, how often they occur, and if their frequency and intensity influence MH. Qualitative studies suggest that women suffer assaults to MH due to poor sanitation conditions and negative experiences managing their needs, such as worrying about infection at urination and defecation sites, experiencing fear of potential harm, and limiting food and water to control urges. This study aimed to determine if a woman's negative experiences of sanitation—collectively designated here as sanitation insecurity— and her access to a facility were associated with anxiety, depression, distress, and well-being in rural Odisha India. We used an exploratory, sequential, mixed methods design to create a culturally-grounded measure of sanitation insecurity and test its association with MH. Our measure assessed seven domains of women's sanitation experience: Potential harms; Social expectations and repercussions; Physical exertion or strain; Night concerns; Dependent support; Physical agility; and Defecation location. From a survey with 1347 women, we found access to a latrine to be associated with higher well being scores, but not associated with anxiety, depression or distress. Women's sanitation insecurity domains were associated with all four MH outcomes, with most negatively associated with well being scores and positively associated with anxiety, depression, and distress scores. These relationships existed independent of latrine access. These findings imply that women suffer assaults to MH when urinating and defecating even if they own a facility. They suggest that sanitation interventions should accommodate women's experiences beyond management of excreta to more comprehensively impact health.

1862

THE IMPACT OF SANITATION INTERVENTIONS ON LATRINE COVERAGE AND USE: A SYSTEMATIC REVIEW AND META-ANALYSIS

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It is estimated that 2.4 billion people lack access to improved sanitation and 946 million practice open defecation. A further understanding of how different sanitation interventions impact latrine coverage and latrine use is essential in order to more effectively attain sanitation access for all. We systematically reviewed the literature and used meta-analysis to quantitatively characterize how different sanitation interventions impact latrine coverage and latrine use. We also used both qualitative and quantitative studies to assess how different structural and design characteristics of sanitation impact individual latrine use. A total of 59 studies met our eligibility criteria. We found 36 sanitation trials that, on average, found an increase in latrine coverage of 14% (95% CI: 10%-18%). The interventions with the largest increases in coverage included the Indian government's "Total Sanitation Campaign" (27%; 95% CI: 14%-39%), education interventions (17%; 95% CI 5%-30%), sewerage interventions (14%; 95% CI: 1%-28%), Community-Led Total Sanitation interventions (10%; 95% CI: 0%-20%), and other latrine subsidy/provision interventions that incorporated education components (17%; 95% CI: -5%-38%). Only 11 of these trials also assessed latrine use and in these the interventions had an average increase in latrine use of 13% (95% CI: 5%-21%). Individual study success was often context specific. Although many studies showed improvements in coverage and use compared to controls, these studies usually did not reach sufficient

coverage and use thresholds to translate into health impacts. We found 17 studies that examined how structural and design characteristics of sanitation were associated with latrine use. Better latrine maintenance, accessibility, privacy, facility type, cleanliness, newer latrine age, and better hygiene access were all frequently associated with higher latrine use, whereas poorer sanitation conditions were associated with lower use. A deeper understanding of how to effectively increase sanitation coverage and use could accelerate progress in eliminating open defecation and ultimately improve health.

1863

DISCOVERING AND OPTIMIZING BROAD-BASED ANTHELMINTICS USING PAN-PHYLUM ANALYSIS OF METABOLIC CHOKEPOINTS

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Parasitic nematodes infect more than two billion people, resulting in significant morbidity and mortality. The development of new therapeutics is indispensable due to the limited number of currently available drugs, their limited efficacy against some species (or life-cycle stages) and increasing anti-drug resistance. We undertook a systems biology approach to reconstruct metabolic pathways and to identify, characterize and prioritize chokepoint reactions and enzymes (that produce a unique product or consume a unique substrate) that we could target with test compounds to evaluate their potential for developing selective inhibitors against them. This is facilitated by recent extensive sequencing and annotation of genomes and transcriptomes of parasites spanning the phylum Nematoda, along with the availability of binding, structure, pharmacology and toxicology data for a large set of small molecules in publicly available databases. Preliminary work on a broadly conserved chokepoint enzyme CPT (Carnitine palmitoyl transferase) resulted in compiling of a small library of 12 CPT inhibitors (from DrugBank and ChEMBL databases or synthesized by us) that we screened against five parasitic nematode species spanning the phylum Nematoda. A worm motility assay identified 8 effective compounds, including 3 with potential for broad applicability across clades. An extension of this work to identify more such chokepoints is currently underway using enzyme annotation and metabolic reconstruction of 17 species spanning the phylum. A comparison of these resulted in identification of 202 chokepoints conserved across all taxonomic clades, including 87 that are conserved across all 17 species. These are currently being analyzed in order to prioritize a small number using multiple factors including level of conservation in nematodes and host, orthology in drug target databases, RNAi phenotype, expression profile across tissue and developmental stages, function in multiple pathways of interest, gene copy number etc. The chokepoints will be linked to inhibitors, and the predictions will be validated in multiple intestinal and filarial species.

1864

TRANSGENESIS IN *STRONGYLOIDES*: FREE-LIVING MALE WORMS AS TARGETS FOR GENE TRANSFER AND TRANSGENE PROPAGATION

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The capacity of parasitic nematodes in the genus *Strongyloides* to under go a generation of free-living development has enabled transgenesis in these worms by DNA transfer into oocyte nuclei. Anatomical similarity of free-living *Strongyloides* females to hermaphrodites of the free-living nematode *Caenorhabditis elegans* has made it easy to adapt DNA transformation techniques developed for this model organism to *S. stercoralis* and *S. ratti*. The technique involves microinjecting DNA constructs into the ovarian syncytium of free-living female *Strongyloides* and mating these with wild type male worms. This process yields only small numbers of F1 transgenic progeny (<100), a large proportion of which are somatic transformants only. In light of this, we examined free-living male *Strongyloides* as targets of gene transfer, reasoning that transgenic males could propagate transgenes to multiple progeny by mating and that the majority of transformed progeny would carry transgenes in their germlines. To this end we microinjected testes of 10 free-living male *S. stercoralis* with a solution containing 100 ng/µl of a proven reporter construct linking the coding sequence of green fluorescent protein (*gfp*) to the promoter of the ubiquitously expressed gene *Ss-eft-3* and the *Ss-era-1* 3'UTR. Microinjected males were plated along with 20 wild type free-living females on NGM agar with a lawn of *E. coli* OP50 and incubated at 22° C for mating. F1 larvae were screened for GFP expression at 24 and 48 hours in culture. Of 150 progeny screened, 3 (2%) were transgenic as indicated by GFP fluorescence in the anatomical patterns typical of the *Ss-eft-3* promoter. This indicates that the male germline of *S. stercoralis* may be transformed by microinjection of plasmid DNA and that transgenes may then be propagated to F1 progeny by mating with one or more wild type free-living females.

1865

MODULATION OF HUMAN DENDRITIC CELL ACTIVITY BY THE HELMINTH PARASITE *ASCARIS SUUM*

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Ascariasis currently affects more than 1 billion people worldwide. Like many other helminths, *Ascaris* is thought to actively modulate and/or down-regulate host immune responses and inflammation. The mechanisms behind this immune-regulation have not been fully elucidated, although modulation of dendritic cell (DC) and macrophage function is likely to be involved. Here, we investigated *in vitro* modulatory effects of *Ascaris suum* (a swine parasite closely related to the human *A. lumbricoides*) on human DC function. Monocyte-derived DCs were matured with the TLR-agonist lipopolysaccharide (LPS) in either the presence or absence of *A. suum* body fluid (ABF). DC function was assessed by analysis of cytokine secretion combined with transcriptomic and gene-set enrichment analysis (GSEA). ABF profoundly impacted on the response of DC to LPS. Secretion of the inflammatory cytokines IL-6, IL-12p70, IL-23 and TNF-α was strongly suppressed in ABF-treated DCs. Microarray analysis of ABF-treated DCs indicated a down-regulation of numerous genes encoding cytokines and chemokines, as well as molecules involved in intracellular inflammatory pathways and DC adhesion and migration. Selected genes were verified by qPCR and/or Western blotting. GSEA indicated significant disruption by ABF of numerous pathways involved in inflammation and DC maturation. Thus, we have demonstrated that *Ascaris* parasites strongly supress human DC function, suggesting that the parasite likely exerts a strong modulatory effect on the development of host immunity. These results increase our

understanding the host-parasite relationship in *Ascaris* infections and may contribute to the design of effective vaccines and other interventions for control of Ascariasis.

1866

GUT MICROBIOME CHANGES INDUCED BY EXPERIMENTAL *TRICHURIS MURIS* INFECTION ARE ASSOCIATED WITH DECREASED COGNITIVE FUNCTION IN MICE

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The effect of helminth infections on cognitive function of children has recently been under increased scrutiny. The gastrointestinal microbiome can modulate the functional development of the central nervous system. Recently we have demonstrated that helminth infections are able to induce important changes in the gastrointestinal microbiome of children. However, to date no studies have sought to evaluate the effect that microbial shifts associated with helminth infections may have on the host's cognition. This study aimed to investigate whether gastrointestinal helminthic infections are associated with decreased cognitive function as a result of changes to the gut microbiome. A chronic infection model was set-up using three groups of mice: two groups of 12 animals infected with *Trichuris muris* (a low and a high infection group) and 12 non-infected animals (control group). Mice were followed for 9 months and faecal samples were collected and stored in dichromate. Total DNA was extracted from the collected samples and changes in the structure and diversity of the gastrointestinal microbiome of mice in each group were done by evaluation of next-generation 16S rDNA sequencing. Cognitive function of mice was tested using the forced swim test (to identify depression-like endophenotype), working memory test (to measure general activity), a social interaction test and the reference Y-maze test (working memory). Our results indicate significant differences in diversity and abundance in the gut microbiome of mice in the control group compared to the low and high infection groups. Our results also indicated that mice in the high infection group show a deficit in reference memory compared to control and low infection groups associated with those alterations. This study demonstrates an alternative mechanism through which helminth infections can result in deficits in cognitive function. The functional profile of the groups of bacteria found altered and the clinical repercussions on cognitive delays of these alterations deserve further empirical studies in populations where both helminth infections and cognitive delays are highly prevalent.

1867

ASSESSING THE IMPACT OF MASS DEWORMING ON CO-INFECTIONS WITH OTHER PARASITES AND COMMENSALS USING MOLECULAR TECHNIQUES

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Studies in mice suggest that the presence of soil-transmitted helminths (STH) is associated with alterations in microbial community diversity. Some of these effects extend to anatomic locations within the gastrointestinal tract remote from where these helminths reside, and may persist after helminth clearance. Studies in humans have mostly failed to corroborate these findings. To demonstrate how molecular approaches to the human gut pathobiome and bacterial microbiome can provide insights into the complex interplay among disparate organisms, DNA was extracted from cryopreserved stools from subjects in 5 rural Kenyan villages and examined by qPCR for 9 intestinal parasites and by MiSeq 16S rRNA sequencing for bacterial communities before and 3 months following albendazole (ALB) therapy. Among 796 people surveyed by qPCR, 23% (186) had 2 or more gastrointestinal parasites concurrently. There were no strong inter-species relationships between the presence of one infection and the presence of any other parasite, except for an association between *Ascaris lumbricoides* and *Giardia lamblia* (Pearson chi-square, $p < 0.001$). Based on 16S rRNA sequence from 192 pre-ALB samples, there was no significant association between STH infection and microbial community composition. However, when a measure of microbial species diversity (Shannon index) was applied to 39 pairs of samples from individuals pre- and post-ALB, there was a significantly higher microbial diversity post ALB ($p=0.04$) in individuals who had *Necator americanus* infection pre-ALB, whereas there were no significant differences in microbial diversity pre and post-ALB in those with *A. lumbricoides* or those without any STH infection. We are currently sequencing additional samples, so that our final dataset will include pre- and post-ALB pathobiome and microbiome data from a much larger sample size (at least 60 pairs of samples for each of the important STHs and appropriate controls). This increased sample size will sharpen our understanding of the broader impact of mass deworming programs on microbial communities and ultimately on human health.

1868

CONTROLLED HUMAN HOOKWORM INFECTION MODEL FOR TESTING THE EFFICACY OF EXPERIMENTAL HOOKWORM VACCINES

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A controlled human hookworm infection model is being developed to provide early proof-of-concept that experimental hookworm vaccine candidates are feasible and efficacious. The proposed model consists of vaccinating healthy, hookworm-naïve adults with a candidate hookworm vaccine, followed by challenging them with infectious *Necator americanus* larvae (L3). We are conducting a feasibility study in Washington, DC, in which different doses of L3 are administered to healthy adult hookworm-naïve volunteers to determine the optimal dose that is safe, well-tolerated and results in consistent levels of infection. 3 cohorts of 10 healthy, hookworm-naive adult volunteers are receiving 25, 50, or 75 L3 in a dose-escalating design. L3 are obtained from the feces of an infected donor who is regularly screened for blood borne pathogens. Batches of L3 are tested for identity, motility/viability, and bacterial/fungal growth prior to release for use. Doses are prepared by counting motile L3 by microscopy; these are then applied to a gauze pad that is placed on the subject's forearm for 1 hour. Subjects are seen weekly until 12-18 weeks post-infection, when they are treated with albendazole. Results from the 25 and 50 L3 cohorts indicate that these doses are well tolerated by volunteers. Early manifestations of infection included mild-to-severe pruritus, erythema, pain, and papulovesicular rash (duration: 4-76 days) at the skin application site. Gastrointestinal complaints (abdominal bloating, flatulence, nausea and abdominal pain) were frequent starting between weeks 4-5 post-infection although these were mostly mild or moderate in intensity. Eosinophilia developed in 9/10 and 10/10 in the first and second cohorts, respectively (range: 0.5-4.9 x 10^3/mm^3). Hookworm eggs were detectable by microscopy in 3/10 (range by McMaster method: 0-33.3

eggs per gram [epg] feces) and 9/10 (range: 0-166.66 epg) in the first and second cohorts, respectively. Controlled hookworm infection with at least 50 L3 appears necessary to induce consistent infection in controls for future vaccination-challenge clinical trials.

1869

PHASE 1 TESTING OF THE NA-APR-1/ALHYDROGEL HOOKWORM VACCINE IN HEALTHY, HOOKWORM-NAIVE ADULTS

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Necator americanus Aspartic Protease-1 (*Na*-APR-1) is a 42.2-kDa protein produced by adult hookworms that is the first enzyme in the ordered cascade of hemoglobinases that the worms use to digest host hemoglobin. Vaccination of laboratory dogs and hamsters with recombinant APR-1 resulted in reduced hookworm fecal egg counts and reduced adult worm burden following challenge with infective larvae. Recombinant *Na*-APR-1 was derived from *Agrobacterium tumefaciens* genetically engineered to express the antigen in *Nicotiana benthamiana* tobacco plants and formulated on Alhydrogel. A Phase 1 trial was conducted in Washington, DC. 40 healthy, hookworm-naïve adults were vaccinated with 1 of 2 different dose concentrations of *Na*-APR-1 (30 or 100 µg) either with or without the point-of-injection addition of 2.5 or 5 µg of an aqueous formulation of glucopyranosyl lipid A [GLA-AF], a synthetic Toll-like receptor-4 agonist. Subjects received 3 intramuscular injections at 2-month intervals. In this study, the vaccine was well tolerated: common adverse events included mild to moderate injection site pain and tenderness, headache, and nausea. No differences were observed in adverse events between dose groups or GLA formulations. Mean anti-*Na*-APR-1 IgG antibody levels as measured by qualified indirect ELISA were modest after the 2nd vaccination, but increased significantly from baseline after the 3rd vaccination in those who received 100 µg *Na*-APR-1 (with or without GLA-AF). The highest peak IgG levels were observed in the cohort that received 100 µg *Na*-APR-1 in combination with 5 µg GLA-AF. In the 30 µg *Na*-APR-1 groups, mean IgG levels did not increase above baseline in those who received the Alhydrogel-only formulation whereas significant increases were observed after the 2nd and 3rd vaccinations in those who received *Na*-ARP-1/Alhydrogel plus GLA-AF. IgG responses were sustained until the end of the trial, 6 months post-final vaccination. This first-in-humans trial of the *Na*-APR-1 hookworm vaccine demonstrates that it is well-tolerated and immunogenic in unexposed healthy adults and justifies further clinical testing of this vaccine in endemic areas.

1870

IMPAIRED NEUTROPHIL RECRUITMENT TO INVADING *LITOMOSOIDES SIGMODONTIS* L3 LARVAE LEADS TO AN INCREASED WORM BURDEN IN NOD2 RECEPTOR AND IL-6 DEFICIENT MICE

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The NOD2 receptor is a widely spread intracellular pattern recognition receptor that is activated by muramyl-dipeptide (MDP), a bacterial cell wall component, triggering NFκB-induced pro-inflammatory responses. Since most human pathogenic filariae as well as the rodent filariae *Litomosoides sigmodontis* (L.s.) harbor endosymbiotic Wolbachia bacteria that synthesize the cell wall precursor lipid II, which contains MDP, we investigated the role of the NOD2 receptor during L.s. infection. Crude L.s. adult worm extract induced both NOD1 and NOD2 activation in NFkB reporter cell lines in a Wolbachia dependent manner. Upon infection with L.s., NOD2-/- mice harbored significantly more worms compared to wild-type (WT) controls. Lack of the NOD2 receptor did not change the cellular composition and analyzed cytokine/chemokine levels within the thoracic cavity, the site of worm residency. However, the skin stage of infection was essentially modulated in NOD2-/- mice, and bypassing the skin barrier by subcutaneous L3 injection resulted in a comparable worm burden in NOD2-/- and WT animals. Flow cytometric analyses and PCR arrays showed a significantly reduced neutrophil recruitment in the skin of NOD2-/- mice following intradermal injection of crude worm extract or L3 larvae, respectively. Further support that an impaired neutrophil recruitment mediates the increased worm burden in NOD2-/- mice was obtained by neutrophil depletion before natural L.s. infection, which significantly increased the worm recovery in WT, but did not alter the already elevated worm counts in NOD2-/- mice. That neutrophils are in general an essential part of the initial protective immune response against invading L3 larvae was further shown in IL-6-/- mice, which also had a delayed neutrophil recruitment within the skin resulting in an increased worm burden, which was not observed after subcutaneous infections. This study demonstrates that the NOD2 receptor is involved in protective immune responses against filarial nematodes by triggering the neutrophil-driven initial protective immune response against invading L3 larvae within the skin.

1871

MICROFILARIAE OF *BRUGIA MALAYI* INDUCES AUTOPHAGY THROUGH THE INDUCTION OF INDOLEAMINE 2,3-DIOXYGENASE (IDO) AND INTERFERON-γ (IFN-γ)

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Monocyte dysfunction in filarial infection is one of the mechanisms proposed to explain the diminished parasite antigen-specific T cell responses seen with patent filarial infection. In fact, monocytes from filariae-infected individuals demonstrate internalized filarial antigens and, as a consequence, express inhibitory surface molecules and have diminished cytokine production. To investigate the mechanisms underlying these phenotypic and functional changes induced by filarial antigens in monocytes we exposed purified human monocytes to live microfilariae (mf) of *Brugia malayi* and compared the mRNA and protein expression of important inhibitory immune checkpoint molecules to mf-unexposed monocytes. Our results indicate that mf significantly induced the mRNA expression of indolamine 2,3-dioxygenase (IDO) - a tryptophan catabolic enzyme with immune-inhibitory properties- in human monocytes and also significantly enhanced tryptophan degradation (an indicator of IDO activity; $p<0.005$) in these cells. As IDO induces autophagy through the upregulation and activation of GCN2 (a serine/threonine protein kinase), we next examined the expression of this kinase and autophagy related genes BCN1, LC3B, ATG5, and ATG7. Interestingly, mf significantly induced the mRNA expression of GCN2 and each of these autophagy related genes ($p<0.05$) in human monocytes. This upregulation was shown to be dependent on interferon-γ (IFN-γ) as mf significantly induced the production of this cytokine in monocytes ($p=0.03$) and a neutralizing anti-IFN-γ antibody reversed the expression of autophagy-related genes almost to the basal levels. Our data suggest that mf of *B. malayi* alter the function of monocytes by inducing IDO and IFN-γ, molecules that lead to monocyte autophagy that may in turn alter the host immune response.

1872

IMPACT OF MATERNAL PRAZIQUANTEL TREATMENT DURING PREGNANCY ON OFFSPRING IMMUNE RESPONSES TO SCHISTOSOME ANTIGENS AT SIX YEARS OF AGE IN LEYTE, PHILIPPINES: RESULTS FROM A RANDOMIZED CONTROLLED TRIAL

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Previous studies have suggested that schistosome antigens cross the placenta during pregnancy and influence newborn immune responses. We conducted a placebo-controlled trial of Praziquantel (PZQ) to schistosomiasis infected pregnant women in Leyte, Philippines. Here, we assess the impact of treatment on *in utero* sensitization to schistosome specific immune responses for N=107 six year old offspring of these mothers (55 PZQ, 52 placebo). We found no difference in schistosomiasis prevalence at age 6 (8.9% vs 13.5% in PZQ versus placebo, P=0.45). We purified PBMC from these children and stimulated them with schistosome worm (SWAP) and egg (SEA) antigens, and paramyosin. Cytokines (Interleukin-1, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, Interferon γ, TNF α) were measured in tissue culture supernatants using a multiplexed platform (Luminex). We evaluated differences in immune responses based on maternal treatment allocation using multivariate models adjusting for sex, schistosomiasis infection intensity and constitutive cytokine expression. Strikingly, children born to PZQ-treated mothers consistently demonstrated decreased levels (17% - 69%) of both Th1 and Th2 cytokines to SWAP (IL-12 1.33 vs 1.10 pg/ml (placebo vs PZQ), P=0.02; IL-13 53.7 vs 16.6 pg/ml, P=0.04, marginal for IL-4, IL-5, IL-13, IFNγ & TNF-α). We also detected a trend toward decreased levels of Th2 cytokines in response to SEA among children born to PZQ-treated mothers (IL-5 63.2 vs 23.9 pg/ml & IL-13 75.9 vs 29.4 pg/ml, both P=0.1). Interestingly, children born to PZQ-treated mothers had increased Th2 cytokine responses to paramyosin (IL-4 2.0 vs 2.3 pg/ml P=0.02; IL-5 3.2 vs 4.4 pg/ml, P=0.05), an immune response we have previously reported is associated with significant protection from infection. *In utero* sensitization was not associated with differences in IL-10 levels. Our data indicate that treatment modifies *in utero* immune sensitization to schistosome antigens, having potentially profound effects on parasite-specific cytokine profiles even at age six. We will present data from an additional 87 children by the time of the meeting.

1873

EFFECT OF PRENATAL EXPOSURE TO SCHISTOSOMIASIS AND CO-INFECTIONS WITH SCHISTOSOMIASIS ON FETAL IMMUNE RESPONSES

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Prenatal maternal infections are known to influence fetal immune responses. This fetal priming can enhance or suppress responses to parasitic antigens, which may influence the child's response to infection. Helminth infections are known to induce T-cell hyporesponsiveness and immunoregulatory mechanism. However, little is known on how helminth co-infections during pregnancy may impact on fetal immunity. We investigated immune responses from infants born to mothers with single or multiple infections. 79 Kenyan women were tested for the presence of *Schistosoma haematobium* (SH) and schistosome co-infection with filariasis and malaria (SH co-infected). Neonates' cord blood lymphocyte responses to SH worm (SWAP) and egg antigens (SEA) were analyzed for proliferation and production of IL-2, IL-5, IL-6 IL-10, IL-12, IL-13, IFN-γ, TNF-α and GM-CSF. Proliferation stimulation index (SI) and cytokine levels were compared between mothers with single (n=36), multiple (n=16) and no infection (n=27). Mothers with single infection at delivery were further classified based on treatment during pregnancy. Analysis was done using unpaired t test with welch's correction. Results showed a higher SI to SWAP, SEA and PHA (p=0.047) in the SH co-infected group (3.22, 2.77, 14.85) compared to the non-infected (1.26, 1.27, 4.96). Reduced IL-2, IL-5, IL-10, IL-12 and IFN- γ responses were recorded in SH and SH co-infected groups but levels did not differ significantly. In contrast, significantly lower levels of SEA stimulated GM-CSF (p=0.021) was recorded in the SH compared to the SH co-infected group. Mothers in the SH group who received anti-malarial drugs had higher levels of immune responses compared to the group not exposed to malaria. We noted spontaneous production of IL-6 in all groups. Conversely, significantly higher levels of spontaneous production of GM-CSF (p=0.041) and TNF-α was recorded in the SH co-infected group compared to the SH group. These preliminary results indicate that while schistosomiasis infection results in immunosuppression, fetal immune priming is enhanced with multiple infections and is sustained post treatment.

1874

LYMPHATIC FILARIASIS: HOST AND PARASITE FACTORS AND THE PATHOGENESIS OF SYSTEMIC ADVERSE EVENTS FOLLOWING TREATMENT

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Lymphatic filariasis (LF, aka "elephantiasis") is a neglected tropical disease (NTD) that is caused by the nematode parasite *Wuchereria bancrofti*. Some 800 million people in 73 countries are at risk for infection and disability caused by these parasites. Mild to moderate systemic adverse events (AEs) such as fever, myalgia, and headache are common after treatment of LF, and these AEs pose a major challenge for the global LF elimination program that is using mass drug administration (MDA) to interrupt transmission of the disease. We are studying the pathogenesis of AEs with blood samples collected before and after treatment in infected volunteers in clinical trials in Côte d'Ivoire and Papua New Guinea. We have used a Bio-Plex cytokine panel to measure 27 cytokines in 24 LF-infected individuals at seven time-points, from pre-treatment up until 72 hours post-treatment. Results show that 19 out of the 27 cytokines were significantly increased in post-treatment plasma in individuals with moderate AEs compared to individuals with no/or mild AEs. This included the three main pro-inflammatory cytokines (IL-6, TNF-α and IL-1β) that were all increased in people with moderate AEs between 8-36 hours post-treatment. Another interesting, and unexpected result was observed for Eotaxin-1. This eosinophil-specific chemokine was significantly up-regulated at baseline in individuals that would go on to develop moderate AEs after treatment. Eotaxin-1 could therefore be a potential biomarker for AEs risk. Preliminary results from global gene expression studies (RNAseq) suggest that several immune pathways are up-regulated in host leukocytes following treatment, and we hope to identify specific transcriptional signatures that are associated with AEs. Additionally, we have developed a qPCR assay for the detection of Wolbachia DNA in human plasma, and we have found that post-treatment plasma samples are more likely to test positive for Wolbachia. Improved understanding of the causes of post-treatment AEs may lead to improved methods for their prevention or management and increase compliance in mass drug administration programs that aim to eliminate LF.

1875

ONCHOCERCA VOLVULUS ANTIGEN PEPTIDE IMMUNOREACTIVITY DISTINGUISHES PARASITE POPULATIONS IN THE AMERICAS, WEST AFRICA, CENTRAL AFRICA AND EAST AFRICA

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Studies of *Onchocerca volvulus* (Ov) population biology may help elucidate its transmission, spread, emergence of drug resistance, and persistence despite control measures. Such studies are currently limited because they rely on the extraction of parasite material from their human hosts, material that is often limited in availability. Thus, we have developed a novel, serologically-based immunotyping approach to the study of *Ov* population diversity and spatial distribution. Using genomic sequence data and PCR-based genotyping, we identified non-synonymous single nucleotide polymorphisms (SNPs) in the coding sequences of many of the major known immunogenic *Ov* proteins: Ov 7, Ov 16, Ov ASP1, Ov CHI1, M3, Ov ALT1, Ov TMY1, Ov B8, Ov FAR1, Ov SOD1, Ov CPI1, Ov B20, Ov RAL1 and Ov RAL2. Using immunoassays to assess the antibody reactivity against synthetic SNP-containing peptides derived from these immunogenic proteins and well-characterized sera from a large cohort of patients (n=114) from multiple regions across Africa and the Americas, we have found statistically different geolocation-specific immunophenotypes (by Chi-Square analyses) against variant peptides derived from M3, Ov RAL1, Ov RAL 2, Ov SOD1, Ov CPI1, Ov B20, Ov RAL2, Ov TMY1, Ov16 and OvCAL1. Hierarchical clustering analysis also identified immunotype differences by region. Specific patterns of immunoreactivity against variant peptides from Ov B20, Ov TMY1, Ov16 and OvCAL1 clearly distinguished East African samples from those originating from Central Africa, West Africa and the Americas. Our data show that differences in immunoreactivity to variant antigenic peptides may be used to characterize populations of *Ov*, thereby shedding light on features of *Ov* population biology that may have been inaccessible because of the reliance on archived parasite material of limited availability.

1876

HAPLOTYPES WITHIN NFKBIA PROMOTER ARE ASSOCIATED WITH SEVERE MALARIAL ANEMIA AND CIRCULATING IL-10 AND IP-10 LEVELS IN CHILDREN WITH *PLASMODIUM FALCIPARUM* MALARIA

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Pathogenesis of severe malarial anemia (SMA, Hb<5.0g/dL and any density parasitemia) in children has been described as a multifactorial process. Genetic susceptibility factors have been proposed as elements of this pathogenesis. Transcription factors are important in regulating cellular processes including immunity. The inhibitor of nuclear factor of kappa light enhancer in B-cells (IκBs) plays important roles in infectious and autoimmune diseases through their ability to regulate the production of soluble immune modulators such as cytokines whose imbalance have been shown to characterize SMA. Due to the important roles of NFκB in immunity, we hypothesized that genetic variations within the promoter region of its inhibitor, IκBs (NFKBIA) gene affect its production thereby the downstream modulators of immunity and hence influence *Plasmodium falciparum* malaria outcome. The association between NFKBIA (-826 G/A, rs2233406 and -310 G/A; rs2233409 and SMA in children (n=1,026, aged 6-36mos.) with *P. falciparum* malaria from Siaya County, western Kenya, a *P. falciparum* holoendemic transmission area was determined. NFKBIA genotypes were determined using Taqman® genotyping assay. Bivariate regression analysis controlling for confounders revealed that existence of AA haplotype (NFKBIA-826A/-310A) was associated with risk of SMA (OR 1.60, 95%CI 1.01-2.55, P=0.047) while the AG haplotype (NFKBIA-826A/-310G) was associated with protection from SMA (OR; 0.58, 95% CI; 0.34-0.98). To identify the downstream target mediators modulated by NFKBIA, we used 25 mediators from Hu Cytokine 25-plex Ab Bead Kit. Additional analysis revealed that the AG haplotype (NFKBIA-826A/-310G) was associated with elevated levels of IL-10 and IP-10 (P=0.0050 and P=0.008, respectively). Moreover, SMA was associated with low levels of IL-10 and IP-10 (P=0.048 and P=0.025). These results demonstrate that genetic variation in the regulatory region of NFKBIA are associated with susceptibility to SMA and influence changes in the levels of circulating IL-10 and IP-10 during *P. falciparum* infection.

1877

PLASMODIUM MTRAP IS ESSENTIAL FOR GAMETE EGRESS AND PARASITE TRANSMISSION TO MOSQUITOES

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Apicomplexan parasites use an actin-myosin motor to glide on substrates and actively invade host cells. The TRAP (thrombospondin-related anonymous protein) family of transmembrane proteins, which is conserved in the apicomplexan phylum, is involved in parasite motility and cell invasion by integrating actin in the parasite and ligands in the matrix/cell surfaces. In *Plasmodium*, the family member expressed in the merozoite stage, called merozoite-TRAP (MTRAP), is thought to act during merozoite invasion into erythrocytes in the mammalian host. We show here that MTRAP is dispensable for this step but essential in the mosquito vector for gamete egress from erythrocytes, an actin and myosin-dependent process, by allowing the disruption of the membrane of the gamete-containing vacuole. This indicates that the apicomplexan TRAP protein family mediates more than parasite motility and cell invasion, and that vacuolar membrane disruption may result, at least in part, of a motor- and TRAP-dependent process.

1878

PROFILING GENE EXPRESSION IN A PHASE II *PLASMODIUM VIVAX* IRRADIATED SPOROZOITE VACCINE TRIAL

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Malaria remains an important public health problem worldwide, with 13.8 million cases caused by *Plasmodium vivax*, a parasite species that predominates in South-East Asia and the American continent. Despite the epidemiological importance of this species, studies of the immune response and their potential for vaccine development are limited. The vaccine that is in the most advanced stage of development is the anti-*P. falciparum* RTS,S vaccine, which has been reported to provide partial immunity when tested on a population of newborns and young children in West Africa. Development of more protective vaccines requires a better understanding of the human immune response. Here we report

initial results of gene expression profiling of peripheral blood before and after seven rounds of immunization with radiation attenuated *P. vivax* sporozoites (RAS) in 20 volunteers, as well as after controlled challenge with live *P. vivax*. RNASeq was used to generate whole transcriptome profiles for three non-immunized controls, five protected Duffy Fy-, five protected volunteers immunized with RAS, and seven not protected volunteers. The most remarkable changes in gene expression were observed between baseline and post- challenge, with distinct signatures differentiating protected and susceptible individuals. Analysis of transcriptional modules shows that B-cell signaling is reduced while cell cycle regulation and interferon response are highly elevated in individuals not protected by RAS, whereas T-cell signaling and an inflammatory response are elevated in protected individuals. Furthermore, some differences in the profiles associated with protection as a result of Duffy negative status and RAS immunization were observed, while vaccination itself also modified aspects of B and T cell gene expression. Combined with immune cell profiling we expect the systems biology approach may suggest novel approaches to improving the efficacy of malaria vaccines.

1879

HIGH-THROUGHPUT GENOMIC SURVEILLANCE OF *PLASMODIUM* INFECTIONS IN INDIA

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High-throughput genomic sequencing technologies provide the resolution, quality and rapid turnover required for routine large-scale surveillance of *Plasmodium* - for example for the spread of drug resistance genes or determining the complexity of infection. Here, we describe the development of a high-throughput amplicon sequencing protocol for multiplexed sequencing of multiple *Plasmodium* genes for routine surveillance purposes. We are using this in our Genomics Core Facility at the National Institute Malaria Research in New Delhi, India as part of an NIH-funded International Center of Excellence for Malaria Research, for surveillance of *Plasmodium* samples collected during our epidemiology studies. The amplicon sequencing protocol has been designed for the bench-top Ion Torrent PGM platform and can be operated with minimal bioinformatics infrastructure making it ideal for use in endemic country settings. In one assay, deep sequencing of a panel of six *P. falciparum* genes including *k13, crt, dhfr, dhps, mdr1* and *mrp1*, in ~150 clinical isolates from three epidemiologically diverse sites in India revealed a number of known and novel single nucleotide polymorphisms (e.g., in *Pfk13*), which could be associated with antimalarial drug resistance. In a second assay, we have shortlisted a panel of five *Plasmodium vivax* genes, including *msp1, msp3, sera5, msp7* and *clag* identified as being highly polymorphic across 200 *P. vivax* genomes, to estimate the number of clones in ~150 *P. vivax* infections. Our studies are revealing the within-host diversity of these isolates by using SeekDeep haplotype frequency estimation to infer the number of parasite clones and their change in frequencies after drug treatment. Our next-generation amplicon sequencing method facilitates surveillance of antimalarial drug resistance and helps elucidate the role of complexity of infection in disease outcome.

1880

REVERSIBLE HOST CELL REMODELING UNDERPINS DEFORMABILITY CHANGES IN MALARIA PARASITE SEXUAL BLOOD STAGES

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Survival of the human malaria parasite *Plasmodium falciparum* in the circulation of the host relies on its ability to drastically alter its red blood cell (RBC) host cell. The sexual blood stage (gametocyte) of the human malaria parasite *P. falciparum* undergoes remarkable biophysical changes as it prepares for transmission to mosquitoes. Developing mid-stage gametocytes show low deformability and sequester in the bone marrow, avoiding clearance during passage through splenic sinuses. Mature gametocytes exhibit increased deformability and reappear in the peripheral circulation, allowing uptake by mosquitoes. Here we define the reversible changes in RBC membrane organization that underpin this biomechanical transformation. Using a combination of biophysical techniques such as ektocytometry, spleen mimic filtration assays along with super resolution microscopy and atomic force microscopy techniques we functionally assess the role that RBC membrane skeleton remodelling plays in this reversible shift in deformability. We show that the length of the spectrin cross-members and the membrane skeleton mesh size increases in the non-deformable early gametocyte. These changes are accompanied by relocation of actin from the RBC membrane to the Maurer's clefts. These changes are reversed in the late stage gametocyte allowing parasite survival within the host and disease transmission.

1881

SPATIAL HETEROGENEITY CAN UNDERMINE THE EFFECTIVENESS OF COUNTRY-LEVEL TEST AND TREAT POLICY FOR MALARIA: A CASE STUDY FROM BURKINA FASO USING RDT AND HEMOGLOBIN

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Considerable debate has arisen regarding the appropriateness of the test and treat malaria policy recommended by the World Health Organization (WHO). While presumptive treatment has important drawbacks, the usefulness of test and treat can vary considerably across space, depending on several factors such as baseline malaria prevalence and RDT performance characteristics. Using 2010 Demographic and Health Survey (DHS) data, we fitted generalized linear mixed effects models to hemoglobin measurements, rapid diagnostic test (RDT), and microscopy results on 6,510 children under 5 years of age from Burkina Faso. Our statistical models revealed substantial variation in RDT performance, baseline prevalence, and hemoglobin measurements, both in space and as a function of covariates. As a result, an individual with a positive RDT result in one region can surprisingly have the same malaria infection probability as another individual with a negative RDT result in another region. These findings reveal that a test and treat policy might be reasonable in some settings but might be unacceptable in others given the high proportion of false negatives. Our results also suggest that in some regions RDT negative children that are severely anemic should be treated anyway for malaria. To aid the formulation of region-specific guidelines for malaria diagnosis

and treatment, we created proof-of-concept web-based tools for decision makers that enables them to interact with our modeling results. Our methods and results are likely to help improve current malaria policies in Burkina Faso and other malaria endemic countries.

1882

IMPROVING THE QUALITY OF MALARIA CASE MANAGEMENT IN PUBLIC HEALTH FACILITIES - THE MALARIACARE EXPERIENCE IN WESTERN KENYA

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Quality malaria case management remains a challenge in Kenya. To date, most health workers only receive occasional updates and irregular supervision visits which focus on infrastructural needs, not on skills assessment and improvement. In 2015, to address the deficits, MalariaCare is implementing a case management quality assurance (QA) program in eight counties around Lake Victoria. The QA strategy focuses on training QA teams of clinicians and laboratory technicians in the principles of quality malaria case management and mentoring and then supporting them in quarterly outreach training and support supervision (OTSS) visits using a structured checklist mentoring tool. After each OTSS round, county supervisors and health management teams will review data collected and design short-term approaches to address gaps at the county-level. To complete the QA cycle, following the first few rounds of OTSS, supervisors from multiple intervention counties will meet, along with national-level representatives, in lessons learned workshops, to exchange lessons learned across counties and develop action plans to address identified weaknesses. In addition, the project is working with hospitals to establish Medicines and Therapeutics Committees (MTCs) to assure that each case is managed according to quality assured protocols. The majority of public health facilities within the eight counties - including 71 hospitals, 185 health centers and 584 dispensaries - are being enrolled in three phases over 18 months. Using specific selection criteria, 75 laboratory technicians are receiving microscopy refresher training, 75 clinicians are receiving case management refresher training, and all are receiving OTSS supervision training. This report will describe the outcomes of these trainings and three rounds of on-site OTSS. The key indicators for microscopy, RDT, and clinical care performance will be discussed. The lessons learned from large-scale roll-out implementation of an electronic tablet-based and DHIS2-linked data collection system will be discussed, and the initial findings for implementation of the MTC system will be shared.

1883

FINDINGS FROM THE FIRST MALARIA MOLECULAR EQA SCHEME LAUNCHED BY UK NEQAS (UNITED KINGDOM NATIONAL EXTERNAL QUALITY ASSESSMENT SERVICE) PARASITOLOGY

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The WHO policy brief on malaria diagnostics in low-transmission settings recommends use of Nucleic Acid Amplification (NAA), and an international External Quality Assurance (EQA) system to ensure that data obtained are reliable and comparable. UKNEQAS Parasitology has developed such an EQA and the outcome of the pre-pilot and pilot surveys plus development of the EQA will be presented in this talk. A pre-pilot followed by a pilot survey for malaria molecular diagnosis using freeze dried blood samples was run. For the surveys, two distributions each containing lyophilised blood specimens were dispatched to an overall of 60 participants in 24 countries. The pre-pilot blood specimens contained parasite densities ranging from 20parasites/μL to 1parasite/μL. The pilot blood specimens contained parasite densities ranging from 40parasites/μL to 1parasite/μL. Both the surveys contained samples from single infections of *Plasmodium falciparum*, *P. vivax*, *P. malariae* and *P. ovale*. Negatives were also sent. Participants were requested to report on the presence or absence of malarial nucleic acid in reconstituted lyophilised blood using either qualitative or quantitative methods. The results showed reporting of higher false negatives at lower parasite densities whilst using both real time and nested PCR. Although a range of CT values and parasitaemia were reported by participants for each specimen, in general within a given assay the CT values increased with a decrease in the parasitaemia of the intended results, suggesting their ability to produce semi-quantitative results. In conclusion, the pre-pilot and pilot surveys demonstrate that NAA EQA schemes can successfully be run using lyophilised blood. Secondly, the majority of participants' results were in good agreement with the intended results. Thirdly, it fulfilled the EQA criteria in that the specimens might help participants take individual action to investigate and remedy any discrepant results. Fourthly, lyophilised blood usage obviates the need for a cold chain distribution, significantly reducing associated costs and opening up the distribution of such samples to a global audience.

1884

BUILDING A SYSTEM OF QUALITY ASSURED MALARIA CASE MANAGEMENT IN THE DEMOCRATIC REPUBLIC OF THE CONGO

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In the Democratic Republic of the Congo (DRC), assuring that microscopy and malaria rapid diagnostic tests (RDTs) are done properly and that test results are used appropriately remains a difficult challenge. To address this, the MalariaCare partnership has supported the National Malaria Control Program (NMCP) to implement a system of quality assured (QA) malaria case management. This system is designed on continuous improvement principles and includes updated training, certification of trainers, and on-site supportive supervision linked with regular review and feedback by supervisors. Over the past four years, 97 diagnostics experts have been trained in microscopy skills and RDT performance, and 17 clinical experts have been trained in malaria treatment. Best performers are then trained as onsite laboratory and clinical outreach training and support supervision (OTSS) supervisors and perform joint supervision and mentoring for local laboratory and clinical staff in 13 of the DRC's 26 provinces, including Kinshasa. The OTSS visits focus on skills observation and on-the-spot problem-solving, with primary goals of improving preparation and accuracy of malaria slide reading, assuring appropriate RDT results, and improving adherence by clinicians to test results. Between OTSS rounds 6 (June 2015) and 7 (February 2016), microscopy performance improved by 18 points—from 67% to 85% overall—and RDT performance improved by 50 percentage points—from 42% to 92% compliance with the performance checklist. Supervisors also observed an increase in clinicians correctly ordering a malaria test, from 64% to 90%, and correct prescription per diagnosis, from 8% to 52%. To assure steady quality improvement in the program, the supervisors meet to review outcomes and share best practices during annual lessons learned workshops. Based on these program improvements, the NMCP recently adopted this QA system as one of the key components in its new national strategic plan and will expand use of the system outside PMI supported health zones.

1885

SHIFTING THE PARADIGM: WHAT CAN BE DONE TO PROTECT COMMUNITIES AGAINST THE THREAT OF SUBSTANDARD AND FALSIFIED MALARIA MEDICINES?

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Substandard, spurious, falsified, falsely-labeled and counterfeit (SSFFC) malaria medicines pose an incredible threat to malaria endemic countries, as they not only put the individual at risk of treatment failure and death, but also increase artemisinin resistance, waste national healthcare system's limited financial resources and create distrust in the healthcare system. Unfortunately, these harmful medicines are quite common in low-income, malaria endemic countries, with recent quality assurance research finding that approximately one in ten doses of artemisinin-combination therapy (ACT) in sub-Sarahan Africa is poor quality. Most interventions designed to combat SSFFC malaria medicines work to improve quality assurance, address regulation policy reform, or operate within the criminal justice system, but few provide strategies to influence the demand for and purchasing practices regarding quality medicines. To this end, the Health Communication Capacity Collaborative (HC3) has developed a global initiative to unite stakeholders from regulatory agencies, criminal investigation units, clinical and pharmaceutical industries, national policymakers and program managers around promoting positive behaviors around malaria medicine purchasing, use, and reporting. HC3 will present findings from their pilot project in Akwa Ibom Nigeria, as well as introduce a step-by-step toolkit (Implementation Kit, or I-Kit) that can be used by any national or local entity to design and launch an effective program to combat substandard and falsified malaria medicines in their country or community.

1886

ANALYSIS OF ULTRA LOW COST NEAR-INFRARED SPECTROMETERS FOR DRUG AND BED NET QUALITY MONITORING

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The prevalence of falsified and substandard drugs is a barrier to effective management of malaria and other disease in resource constrained settings. Beyond drugs, other important healthcare interventions, such as long-lasting insecticide-treated bed nets. A simple low-cost tool to identify falsified and degraded commodities would secure supply chains, inform planning for product replacement, greatly reducing avoidable mortality. Handheld near-infrared spectroscopy (NIRS) systems have recently been developed for consumer use capable of distinguishing chemical composition of various materials, with hardware costs of $250 - 500. Spectroscopy is an attractive solution to drug and net quality testing due to low cost-per-test and the non-destructive nature of testing. The portability of these systems offer utility throughout supply chains. We investigated the capabilities of two portable NIRS devices, the Consumer Physics SCiO and the Texas Instrument NIRscan Nano, to perform quality assurance testing on a variety of drugs and nets. Spectral libraries were built for several classes of drugs including contraceptives, artemesinin combination therapies (ACTs), antibiotics, and others. The performance of these libraries was then tested in the laboratory and in field conditions with target users. Results were compared to reference testing with established reference standards, and with a laboratory-grade NIRS. Results include analysis of performance in falsified drug identification, active ingredient quantification (for finding substandard drugs), and active ingredient determination (for identifying unmarked pills). A bed net spectral library was compiled to quantify the presence of insecticide on the net. Both NIRS systems performed with high accuracy in identifying falsified drugs. In certain applications, active ingredients could be quantified sufficiently to assess degradation. Hand-held NIRS systems offer potential to revolutionize quality assurance of pharmaceuticals and other commodities in resource constrained settings.

1887

MALARIA INTERVENTION ASSESSMENT IN FOUR STATES OF NIGERIA: AN INNOVATIVE, COMPREHENSIVE, MIXED-METHODS EVALUATION

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The significant expansion of malaria control interventions in recent years has reduced the malaria disease burden in many countries, leading the global malaria community to set goals for elimination. In this context, there is need for appropriate tools and methods to document efforts and measure achievements. The Malaria Intervention Assessment (MIA), an innovative comprehensive evaluation methodology, funded by PMI, led by MEASURE Evaluation in partnership with the NMEP, is being implemented in four states of Nigeria supported by PMI: Cross River, Ebonyi, Nasarawa and Sokoto. The objective of MIA is to document progress in malaria control interventions from 2008-2016 in the four states. Specifically, MIA will describe the state-level malaria interventions; document trends in key malaria prevention and case management indicators and assess quality of care among PMI-supported and non-PMI-supported primary healthcare facilities (PHCs); document trends in malaria morbidity and mortality at the hospital level; assess the quality of monthly malaria data at PHCs; and document changes in contextual factors likely to affect malaria interventions and outcomes. MIA uses a quasi-experimental design and a comprehensive mixed-methods approach consisting of: (1) secondary data collation of malaria indicators from the routine health information system (RHIS) at 560 PHCs and their referral hospitals; (2) primary data collection, including: 2800 exit client interviews (5 at each PHC visited), 38 qualitative key informant interviews, and observations of the availability of malaria commodities at the PHCs; (3) secondary data analysis of state-level representative household surveys, and (4) document review. Using a stratified random sample with probability proportional to size, 140 facilities were selected in each state, 70 PMI-supported and 70 non-PMI-supported. Fieldwork will be completed in May 2016 so MIA results will be available to present at the ASTMH conference. The presentation will include key findings, strengths and challenges of MIA, and lessons learned for improving malaria control interventions and the RHIS.

1888

RAPID ACTIVE SEROPREVALENCE SURVEYS AS A TOOL TO MEASURE NOROVIRUS DISEASE BURDEN IN RESOURCE-LIMITED SETTINGS

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Cost-effective surveillance systems capable of accurately detecting acute gastroenteritis (AGE) are necessary to estimate the burden of pathogens such as norovirus (NoV) and the potential effectiveness of vaccines. Cross-sectional seroprevalence surveys are commonly used for outbreak investigations, but they have not been validated as a timely, community-based alternative to active surveillance. We used a 2-stage cluster design (30 clusters of 7 households) to enroll children age 0-17 years in a rural, resource-limited region of Guatemala into two parallel surveillance systems to estimate the burden of NoV-associated AGE. In the prospective Participatory Syndromic Surveillance (PSS) arm, 207 households with 483 children (enrolled Apr-Sep 2015) were provided an internet-connected smartphone with a symptom diary application and asked to submit weekly reports of AGE symptoms. Subjects meeting case criteria of 3+ days of vomiting/diarrhea or 1+ day of both were visited and offered NoV PCR testing via rectal or fresh stool swabs. In the Rapid Active cross-sectional Surveys (RAS), 377 children from 209 households (cycle 1), and 369 children from 210 households (cycle 2) from the same community were surveyed for AGE within the past 7 days and tested for NoV via PCR, regardless of symptoms. In the PSS arm, 50 children met AGE criteria during 362 person-years of observation (13.8 cases/100 person-years), and 9 of 34 (26%) tested were NoV+. In RAS cycles 1 (Oct-Nov 2015) and 2 (Jan-Feb 2016), 53 (14%) and 29 (8%) children had AGE in the preceding week and 6/39 (15%) and 5/24 (21%) tested were NoV-positive, respectively; the asymptomatic:symptomatic NoV ratio was 3.2:1; 79 (89%) of NoV isolates were genogroup I (GI) and 10 (11%) were GII. In logistic regression models adjusted for sex, younger age was a significant predictor of AGE but not NoV+ AGE. Our data demonstrate a large burden of NoV+ AGE and asymptomatic NoV shedding in this Guatemalan community. The more cost-effective RAS cross-sectional surveys provided comparable AGE incidence and NoV infection rates to the smartphone-based PSS active surveillance cohort, and further surveillance is planned.

1889

HIGH HEPATITIS E SEROPREVALENCE AMONG DISPLACED PERSONS IN SOUTH SUDAN: EVIDENCE OF UNDETECTED TRANSMISSION AND IMPLICATIONS FOR VACCINATION

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Hepatitis E (HEV) is responsible for significant morbidity and mortality worldwide, especially among pregnant women. Large protracted outbreaks have been documented in East African IDP and refugee camps over the past decade though little data on burden and transmission exist outside these exceptional settings. Characterizing the population-level exposure to the pathogen through age-stratified serological studies has the potential to improve our understanding of this disease and provide new insights for improving surveillance and control. We conducted an age-stratified serological survey among 206 residents in a camp for internally displaced persons in Juba, South Sudan where no clinical cases of HEV had been reported. We tested serum for HEV IgM and IgG using standard ELISAs and estimated the population-level prevalence of seroprevalence to each. Using data on individuals' sero-status, date of arrival in the camp and state of origin, we were able to construct a series of statistical models to estimate the rate of infection in the camp and that in the participants' previous residence. The age-adjusted seroprevalence was 61% (95% CI 54-69%) and we found evidence of recent exposure in 3 participants (1.5%). We found increasing IgG seroprevalence with age and higher seroprevalence in women compared to men. We estimate that the rate of HEV exposure was nearly 5-fold (95% CrI 1.2-10.2) higher in the PoC camp than our estimates in the participants' home states. The high seroprevalence estimated within this population suggests that HEV transmission may be much more common than previously thought, even in the absence of a detected outbreaks. The results suggest that the population is immunologically primed, which may have implications for control strategies, including vaccination, where a reduced-dose schedule may provide high levels of protection in immunologically primed individuals. Improved HEV surveillance is needed to understand the true burden of disease and to minimize the impact of epidemics.

1890

POSSIBLE HIGH EXPOSURE TO EBOLA AMONG NON-FORMAL HEALTH CARE PROVIDERS IN A PREVIOUS OUTBREAK SITE, BOENDE, DEMOCRATIC REPUBLIC OF CONGO

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During an Ebola virus disease (EVD) outbreak, health care workers (HCWs) are on the frontline of the response, and their occupational health and safety is critical to control and elimination of the outbreak. Thus they may be at increased risk of disease acquisition given the presence of virus in bodily fluids and lack of compliance with precautions to prevent exposure. Therefore, we conducted a serosurvey among formal and informal HCWs in the Boende health zone in Tshuapa District, DRC, the site of the 2014 Ebola outbreak. Field collection occurred in November 2015. Interviews and blood specimens were collected from all consenting individuals. Serum samples from 441 formal (doctors, nurses, midwives, and room attendants) and informal (religious leaders/pastors and traditional healers) HCW's were screened for ZEBOV GP Ig detection using Human Anti-Zaire Ebola Virus Glycoprotein (GP) IgG ELISA Assay kits (Alpha diagnostic International, Inc.) in Kinshasa, DRC. Among the HCWs, 21% (93) were seropositive for ZEBOV GP IgG, of those, 25% of pastors (n=27) were seropositive, 37% of traditional healers (n=27) were seropositive compared to 19.6% of formal HCWs. 27.6% of pastors reported that they had come in contact with suspected cases of Ebola, and of those, 87.5% reported that they did not use PPE compared to the 28.6% of formal HCWs reporting contact with suspected Ebola cases. Both formal and informal HCWs in Boende appear to be highly exposed to Ebola virus. While there appears to be no significant difference between formal and informal HCWs, the number of informal HCWs participating in the last outbreak was high. It is important that informal HCWs, especially in areas that have experienced EVD outbreaks, be included in surveillance and biosafety training in order to help prevent disease circulation in future outbreaks.

1891

SIERRA LEONE TRIAL TO INTRODUCE A VACCINE AGAINST EBOLA (STRIVE): IMPLEMENTATION CHALLENGES, SUCCESSES AND LESSONS LEARNED

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STRIVE, a phase 2/3 trial of investigational rVSV-ZEBOV vaccine was conducted during an unprecedented Ebola epidemic. Eligible health care workers and front line Ebola response workers were individually randomized to immediate (within 7 days) or deferred (within 18-24 weeks) vaccination and followed for 6 months for serious adverse events and Ebola. We describe lessons learned during trial implementation. Substantial infrastructure investments, including renovation of government cold chain facilities and importation of equipment to store and transport vaccine at -80oC, were needed. Generators and solar battery systems were used for backup. Staffing challenges centered on lack of experience with Investigational New Drug (IND) trials and their regulatory requirements. STRIVE built capacity by training >350 staff on IND research, including medical, pharmacy and nursing students whose classes had been cancelled during the outbreak. Didactic and practical training was reinforced with daily review and feedback meetings. CDC staff were paired long-term with local counterparts for role-specific skills transfer. The operational challenges of safety follow-up were addressed by issuing mobile phones to participants, establishing a nurse triage hotline, and providing access to free medical care. The effectiveness of these solutions was limited by frequent loss, breakage, or selling of study phones and frequent medical visits for minor ailments. Lessons learned include the need for back-up electrical and cold chain equipment, the importance of daily ongoing training supported by train-the-trainer approaches, the value of multiple participant locator information sources—including home visits—for participant follow-up, and the need for adequate staffing, systems, and guidance for free medical care. STRIVE enrolled ~8650 participants and vaccinated ~8,000 with excellent follow-up. Before the Ebola outbreak, Sierra Leone had limited infrastructure and staff to conduct clinical trials. Without interfering with the outbreak response, STRIVE responded to an urgent need and helped build this capacity.

1892

ASSESSING THE HETEROGENEITIES IN VIRAL HEMORRHAGIC FEVER OUTBREAK POTENTIAL ACROSS AFRICA

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As the Ebola virus disease outbreak in West Africa comes to an end, considering where to prioritise reinforcing response capacities to viral hemorrhagic fevers (VHFs) such as Ebola, Marburg, Lassa fever and Crimean-Congo hemorrhagic fever will be a key focus. By characterizing three key transition points in a potential VHF outbreak, this output provides the first quantitative assessment for districts in Africa which identifies those that are more likely to see index cases of VHFs, and should insufficient timely intervention measures be put in place, districts more likely to see localized outbreaks as well as those which are more likely to seed infection in other districts leading to a widespread outbreak. Information derived from zoonotic niche maps defining the geographic extent of the virus, coupled with measures of in-district healthcare infrastructural capacity and population vulnerabilities (inspired by a pre-existing index for risk management INFORM) as well as travel time surfaces are incorporated to provide this assessment across the African continent. The methodological framework is shown to be flexible to allow for improved, more disease specific covariates to be added as-and-when they become available. By understanding the inherent differences that exist across Africa, this method provides an alternative approach for identifying which districts to be targeted for broad scale healthcare improvement (focusing on VHF measures), as well as those to be prioritized for surveillance prior to outbreaks or the focus of rapid intervention should undiagnosed hemorrhagic fevers be reported.

1893

SPATIAL DETERMINANTS OF EBOLA VIRUS DISEASE RISK FOR THE WEST AFRICAN EPIDEMIC

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Although many studies have investigated the probability of Ebola virus disease (EVD) outbreaks while other studies have simulated the size and speed of EVD outbreaks, few have investigated the environmental and population-level predictors of Ebola transmission once an outbreak is underway. Identifying strong predictors of transmission could help guide and target limited public health resources during an EVD outbreak. A

Bayesian hierarchical Poisson model was used to estimate EVD risk and to evaluate the spatial variability explained by the selected predictors. We categorized our predictors into terciles, and found that districts had greater risk of EVD with increasing proportion of households not possessing a radio (RRRadio2 2.79, 0.90-8.78; RRRadio3 4.23, 1.16-15.93), increasing rainfall (RRRainfall2 2.18; 95% credible interval 0.66-7.20; RRRainfall3 5.34, 1.20-23.90), urban land cover (RRUrban2 4.87, 1.56-15.40; RRUrban3 5.74, 1.68-19.67), and years of education (RREducation3 1.58, 0.40-6.25). We found that districts with higher proportion of radio ownership had reduced EVD transmission risk, suggesting that that the use of radio messaging for control and prevention purposes may have been crucial in reducing the EVD transmission risk in certain districts, a potential modifiable risk factor for future outbreaks. Additionally, in areas with low proportion of radio ownership, public health authorities may need to develop and introduce different communication strategies. Future research should examine the etiologic relationships between the identified risk factors and human-to-human transmission of EVD with a focus on factors related to population mobility and healthcare accessibility, which are critical features of epidemic propagation and control.

1894

RE-CURRENT EPIZOOTICS OF HIGHLY PATHOGENIC AVIAN INFLUENZA IN NIGERIA AND STATUS OF VACCINATION AS ALTERNATE CONTROL

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Episodes of HPAI H5N1 in Nigeria are evidence of the risk of re-introduction of the virus through annual migration of infected waterfowls from Asia and Europe. Concerns are rife on the possibility of the virus becoming endemic in domestic poultry. This is moreso that the current wave of outbreak was not sufficiently contained leading to more cases of infection within six months than was ever experienced between 2006-2008. Current Federal Government status remains depopulation and decontamination without the use of vaccines. We however investigate evidence of vaccination by some poultry farmers desperate to prevention infection. In a limited prospective study in seven commercial poultry farms in South West Nigeria, 161 sera were randomly collected and tested by Agar Gel Immuno-diffusion (AGID) test to detect group specific nucleoprotein antigen of influenza A. Thereafter, Haemagglutinin Inhibition (HI) test was carried out using antigen directed against monospecific H5 subtype in a V-bottom microtitre plate with 1% solution of pooled chicken red blood cell as indicator. Eight (5%) sera had evidence of influenza A antibody shown by distinct precipitation line between antigen and antiserum in agar gel. Further analysis by HI showed three (2%) sera were positive for influenza antibody at low titer HI of 3log 2. This study for the first time, showed evidence of antibody to avian influenza in domestic flock in Nigeria that is most likely due to vaccination. Previous serological tests in farms infected with HPAI H5N1 were negative. There are unconfirmed reports of vaccination in southwestern region, the hub of poultry production in Nigeria. Unregulated and inappropriate application of vaccine may results in poor antibody responses as demonstrated in this study. In view of these possibilities, it is in the best interest of avian influenza disease control to monitor the use of vaccines in commercial poultry and immune responses thereof.

1895

USE OF A QUANTIFIABLE STOOL RT-PCR ASSAY INCREASES DIAGNOSTIC YIELD IN CHILDHOOD TB

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Quantification of Mycobacterium tuberculosis (Mtb) has potential to improve TB diagnostics and treatment monitoring, with particular relevance in childhood TB as its paucibacillary nature presents a diagnostic challenge. We evaluated the diagnostic yield of quantitative real-time PCR for the detection of Mtb in stool. Stool was collected from a cohort of adults and children with confirmed or clinically diagnosed TB (n=67). DNA was isolated from 50mg of stool using the MP Fast DNA soil kit. Tuberculosis-specific primers were designed from the IS6110 insertion element found exclusively within the Mtb complex. To determine a limit of detection (LOD), 10 to 106 CFU of H37Rv Mtb was spiked into 50mg of healthy stool and the DNA was isolated. All samples were analyzed using quantitative real-time PCR. The LOD of Mtb was < 10 CFU of Mtb per 50mg of stool. The CFU of Mtb spiked into stool and DNA quantified by PCR was well correlated (Figure 1A: Spearman r = 0.998, p< 0.0001). The quantity of Mtb DNA detected inversely correlated with time on ATT (Figure 1B; Spearman r = -0.4219; p = 0.04). The quantified Mtb DNA in stool at 2 months was lower than baseline levels (Figure 1C, Wilcox signed rank test; p< 0.0001). Stool qPCR had similar diagnostic accuracy as sputum GeneXpert Mtb/RIF (Xpert) amongst individuals who had stool collected within 72 hours of ATT initiation (Fisher´s exact p-value 0.32). Stool PCR identified 15% (4 of 26) children who were clinically diagnosed with TB despite having negative Xpert and culture results. In conclusion, detection of Mtb DNA from stool provides a quantifiable measure of an individual's Mtb burden. PCR detection of Mtb in stool of children with clinically diagnosed TB (Xpert and culture negative) highlights the potential for this assay to increase bacteriologic confirmation of childhood TB.

1896

IS IT TINDZHAKA OR TUBERCULOSIS? A STUDY OF TRADITIONAL DIAGNOSIS AND TREATMENT AMONG HEALERS IN BUSHBUCKRIDGE, SOUTH AFRICA

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The prevalence of Tuberculosis (TB) in South Africa (SA) has increased significantly over the last 30 years, with an annual incidence rate of 1% (roughly 450,000 new cases per annum). In Bushbuckridge, SA, Tindzhaka is a common condition for which people seek the services of a traditional healer. Tindzhaka is an ailment that affects the lungs and can eventually lead to death. There is concern among clinicians that Tindzhaka and TB are the same illness; increased understanding about the causes, symptoms, treatment, and expected outcomes associated with Tindzhaka can be used to engage healers to support testing of suspected TB patients. In 2015, we completed 27 in-depth interviews and 133 surveys with a simple random sample of traditional healers in rural Bushbuckridge, SA. Healers were mostly female (77%), older (median = 58 [IQR: 50-67 years]), with low levels of education (median = 3.7 [IQR: 3.2-4.2]) years). Seventy-three percent of healers claimed to treat Tindzhaka (while less than 10% claimed to treat TB). Our research has revealed the overlapping symptoms of Tindzhaka and TB, including coughing, difficulty breathing, loss of body weight, fevers and, ultimately - if there is no treatment - death. Healers argue these to be two distinct illnesses. Color of the sputum (white indicates Tindzhaka, while yellow indicates TB) was identified as one means of distinguishing the two illnesses. Several social transgressions are

believed to cause Tindzhaka infection: (1) having sex with one's partner before the family member's death ceremonies are completed; (2) having sex with one's partner too quickly after a funeral; or (3) bringing home any items (including food from the funeral) from the deceased member's house. On average, healers charged patients with Tindzhaka 1376 SA Rand (IQR: 600-1500; 82 United States Dollars (USD)) and patients with TB 700 SA Rand (IQR: 400-1000; 47 USD) for treatment. With 11% mortality among those who contract TB in SA, widespread acceptability of traditional treatments for an illness with similar presentation may contribute to poor patient outcomes. Further engagement with traditional healers is required.

1897

FOLLOW-UP EVALUATION IN THE UNITED STATES OF NEWLY ARRIVED IMMIGRANTS AND REFUGEES AT HIGH RISK FOR TUBERCULOSIS, 2009-2015

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Required culture-based overseas tuberculosis (TB) screening in U.S.-bound immigrants and refugees reduces importation of TB to the United States. It also identifies persons at high risk for TB, for whom post-arrival follow-up evaluation is recommended. We analyzed data collected by state and local health departments to determine TB rates among at-risk newly arrived immigrants and refugees. During 2009-2015, overseas screening identified 92,248 U.S.-bound immigrants and refugees with Class B1 Pulmonary TB (chest radiograph, medical history, or examination suggestive of TB but smear- and culture-negative) and 57,475 with Class B2 Latent TB Infection (LTBI). Of 62,131 persons with Class B1 Pulmonary TB who completed follow-up evaluation, 1,028 were diagnosed with active TB within 1 year after arrival; of 403 culture-confirmed cases, 3.0% (12) had multi-drug resistant TB, 0.7% (3) were resistant to isoniazid, 6.2% (25) resistant to rifampin, and 3.7% (15) resistant to other first-line drugs. Of 36,068 persons with Class B2 LTBI who completed follow-up evaluation, 121 were diagnosed with active TB; of 13 culture-confirmed cases, 7.7% (1) were resistant to first-line drugs other than isoniazid or rifampin. TB rates were 1,655 and 336 cases per 100,000 persons for those with Class B1 Pulmonary TB and Class B2 LTBI within 1 year after their arrival, respectively. For persons with Class B2 LTBI, TB rates were 3,268, 308, and 531 cases per 100,000 persons for those aged <2, 2-14, and ≥15 years, respectively. Of 98,199 persons with a Class B1 Pulmonary TB or Class B2 LTBI, 40,718 persons were diagnosed with LTBI by follow-up evaluation, 25,054 (61.5%) initiated preventive therapy but only 11,118 (27.3%) completed their treatment. Newly arrived immigrants and refugees have high rates of active TB, despite overseas screening. High TB rates among persons with Class B2 LTBI in all ages suggest that expanding overseas LTBI screening beyond the currently required 2-14 years should be considered. To further prevent TB in the United States, strategies are needed to improve the completion of follow-up evaluations for all persons, and preventive therapy for LTBI.

1898

USING POINT-OF-CARE C-REACTIVE PROTEIN TEST RESULTS TO TARGET ANTIBIOTIC PRESCRIPTION FOR RESPIRATORY ILLNESSES IN UNDER-FIVES: EXPERIENCE FROM A CLINICAL TRIAL IN DAR ES SALAAM, TANZANIA

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We sought to determine the usefulness and safety of using C-reactive protein (CRP) test results in deciding on antibiotic prescription for respiratory illnesses among febrile children presenting to outpatient care. A sub-cohort of all patients with cough and no signs of severe illness from a larger trial that investigates a novel electronic algorithm for management of fever among under-fives in Dar es Salaam, Tanzania, was included. A two-step diagnostic approach was used to decide on antibiotic prescription: amoxicillin was given if a patient presented with i) respiratory rate (RR) between the 75th and <97th %ile for age and temperature based on a European derivation study as well as ii) CRP ≥80mg/L using a point-of-care assay (Bionexia™, Biomerieux). All children were followed until clinical cure or death. Out of the 922 patients with cough, 428 patients met the 75th %ile cutoff for RR, of which 277 (64.7%), 115 (26.9%), 26 (6.1%), and 10 (1.1%) patients had CRP levels of 0-9, 10-39, 40-79, and ≥80mg/L, respectively. Antibiotics were thus prescribed in 10 (1.1%) of patients. Out of the 428 patients, 9 patients met clinical failure criteria per the main study at day (D)3 or D7 (7 had CRP values of 0-9, 2 of 10-39mg/L) : 2 developed severe respiratory symptoms, 3 had persistent fever at D7, 4 still had clinical pneumonia with low CRP values at D3 but recovered before D7 without antibiotic treatment, and 1 patient had clinical pneumonia at D7. There were no deaths. Using current IMCI cut-offs, 226 (53%) out of these 428 patients would have been prescribed an antibiotic at presentation. A two-step diagnostic approach using respiratory rate and a CRP ≥80mg/L is safe for deciding on antibiotic prescription among febrile children with respiratory symptoms and has the potential to significantly reduce antibiotic prescriptions. Further research should be conducted in children at higher risk for bacterial pneumonia, i.e. in areas with high rates of malnutrition and low immunization coverage. In addition, newer host biomarkers with better performance should be evaluated in clinical studies.

1899

WHAT DROVE THE DECLINE IN PNEUMONIA-SPECIFIC UNDER-FIVE DEATH IN MALAWI FROM 2000-2014?

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Globally, pneumonia is the leading cause of mortality in post-neonatal children under 5 years of age. More than 95% of the estimated 0.9 million under-five children who died from pneumonia in 2013 lived in low and middle income countries. Overall mortality in children under five years of age has declined dramatically in the past ten years in Malawi, partly due to decreases in childhood pneumonia. We explore how scale-up of specific interventions contributed to this decline. Our objective is to determine

which interventions contributed the most to reducing pneumonia-specific mortality in Malawi from 2000 to 2014. We used the Lives Saved Tool (LiST; Spectrum v5.41 Beta 6) to conduct a retrospective analysis to estimate change in pneumonia-specific under-five mortality over the study period. Estimates of intervention coverage were drawn from Malawi Demographic Health Surveys (MDHSs) from 2000, 2004, and 2010, and the Multiple Indicator Cluster Survey (MICS) from 2006 and 2014. Data were interpolated from existing data for years without coverage estimates. Key outcomes included reduction in under-five mortality due to pneumonia and lives saved by pneumonia-specific interventions. Preliminary results show that among children under five, pneumonia-specific mortality declined by 59% among children aged 1-59 months from 2000 to 2014. Although the number of neonatal deaths due to pneumonia has been decreasing since 2006, neonatal pneumonia deaths was slightly higher (8%) in 2014 relative to 2000. Nearly all (98%) of the lives saved were attributable to vaccination (37%), including H. influenzae b and pneumococcal conjugate vaccine, antibiotics for treatment of pneumonia (34%), and various interventions to reduce stunting and wasting (27%). Overall pneumonia-specific mortality in children under-five has declined sharply in Malawi since 2000. Treatment and prevention both played key roles in saving lives. Ongoing implementation of interventions is essential to maintain this trend.

1900

EPIDEMIOLOGY OF HUMAN METAPNEUMOVIRUS IN CHILDREN UNDER AGE FIVE — DAMANHOUR DISTRICT, EGYPT, 2009-2015

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In 2013,Acute Respiratory Infection (ARI)was the 5thleading cause of under-five mortality in Egypt.Human metapneumovirus (hMPV) was the second most common cause of ARI. However, data is only available through sentinel surveillance. We sought to estimate the incidence and describe the characteristics of hMPV among ARI cases from population-based surveillance in Damanhour, Egypt. During June 2009-December 2015, hospitalized ARI patients were enrolled from three government referral and two private hospitals. An ARI patient was defined as having a temperature ≥38°C or <35.5°C, abnormal white blood cell count or differential, at least one respiratory symptom, and age<5 years. Nasopharyngeal and oropharyngeal specimens were tested by real-time reverse transcriptase polymerase chain reaction (rt-PCR) for hMPV. Data from a 2012 healthcare utilization survey were used to determine the proportion of individuals who sought care for ARI. Frequencies and chi-square test were used for data analysis. Among 4,400ARI cases, 4181(95.0 %) had rt-PCR testing for hMPV; of these, 322 (7.7%) were positive. hMPV was the only pathogen in 276 (85.7%) cases. Overall, 255 (79.2%) were rural residents and 185 (57.5%) were male. The highest proportion of hMPV infections among ARI cases occurred during December-February (214/1503, 14.2 %) compared to other months (108/2897, 3.7%), (p<0.01).Overall incidence of hMPV infection was 2.3 per 1,000 child-years. hMPV infection increased over time (p<0.01) with the highest proportion occurring in 2010(12.5%).hMPV patients presented with sudden onset of fever (99.4%), cough (99.4%), abnormal breathing (82.9%),and tachypnea (55%).Among 197 patients with chest radiography, 43(21.8%) had consolidation. Mean duration of symptoms was 4.9+3.1days and hospitalization was 4.0+2.6 days. Thirteen (4.0%) patients were admitted to intensive care for a mean duration of 4.8 (+2.5) days. Two patients died, both under age two years. hMPV infection peaked during winter and is a significant cause of ARI in children under age five years in Damanhour District, Egypt.

1901

ETIOLOGY OF ACUTE LOWER RESPIRATORY INFECTIONS IN INPATIENT CHILDREN IN GHANA - A CASE-CONTROL STUDY

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Background: An estimated 14.5 million pediatric hospital admissions are caused by acute lower respiratory tract infections (ALRI) each year. Molecular diagnostic tools have led to the detection of a large number of organisms claimed to be causal in ALRI. However, current data are insufficient to determine whether these viruses are truly pathogenic. The aim of this study was to assess the prevalence, pathogenicity and clinical relevance of organisms in respiratory infections of Ghanaian children using a case-control design. Methods: From September 2014 to August 2015 all children admitted to a Hospital in Ghana with symptoms of ALRI were recruited. Healthy controls were recruited from the community. Pharyngeal swabs were analysed by PCR for Pneumococci, Mycoplasma, Influenza (A, B), Parainfluenza (1-4), hMPV, RSV, Entero-. Rhino-, Adeno-, Parechovirus and Human Corona Viruses (NL63, 229E, OC43, HKU-1). The frequency of organisms in both groups was determined and age-adjusted odds ratios (OR) for association with ALRI calculated using logistic regression models. The attributable risk fraction of ALRI for each organism was estimated. Results: 337 children were recruited as cases and 573 as healthy controls. Of these, 235 (69.7%) and 271 (47.3%) tested positive for at least one organism in the case and control group, respectively. The most common organisms in cases were pneumococci (163, 48.4%), Adenoviruses (60, 17.8%) and Rhinoviruses (59, 17.5%). In the control group, pneumococci (176, 30.7%), Rhinoviruses (145, 25.3%) and Enteroviruses (113, 19.7%) were the most frequent organisms. The strongest association with ALRI symptoms was seen for influenza viruses (OR=32.3; 95% CI 7.7-136.0; p<0.001) and RSV (OR=12.3; 95% CI 3.6-42.0; p<0.001). The highest attributable fractions were 28.4% for pneumococcal infection and 9.6% for influenza virus infection. Discussion: Strong associations of influenza, RSV and hMPV with disease, indicate that these are most likely causative if detected in a child with ALRI. Despite the introduction of a vaccine 2 years prior to the study, pneumococcal infection was still the most important cause of ALRI.

1902

COMPARISON OF THE HUMORAL RESPONSE INDUCED BY DIFFERENT LINEAGES OF *TRYPANOSOMA CRUZI* IN A MURINE MODEL

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Trypanosoma cruzi is classified based on genetic variability into six discrete typing units (DTU TcI to TcVI). This genetic diversity may be related with the clinical features and humoral immunity observed in Chagas´s disease. The aim of this work was to evaluate the humoral responses induced by two different strains of *T. cruzi* (DTUs TcI and TcV), from an endemic area of Argentina. We used female BALB/c mice 45 days old infected intraperitoneally with 10.000 metacyclic trypomastigote forms. For the

measurement of antibody titers induced by the parasites we used an in-house ELISA. Serum samples were taken at different time points, measured in days post-infection (dpi), since 8 to 120 dpi. As capture antigens we used protein homogenates (HP) of two different strains of *T. cruzi* (TcI, TcV). The homogenates were characterized by SDS-PAGE. We carried out two tests: ELISA-HPTcI and ELISA-HPTcV for each experimental group. Each serum set reacted with the antigens, demonstrating the presence of antibody anti-*T. cruzi* in the experimental groups. We observed a high sensitivity and specificity of the reaction between serum and antigen of the same DTU. The values of optical density (OD) in serums of mice infected with TcI were significantly higher ($p < 0.05$) than serum of animals infected with TcV, when HPTcI was used. However, when HPTcV was used, we observed the highest OD in mice infected with TcV. On the other hand, we observed difference in the kinetics of antibodies. Serum of TcI-mice presented an exponential increase in the antibody titers along time of the infection. While serum of TcV-mice showed the highest antibody titers at 90 dpi and then decreases. By SDS-PAGE technique we observed differences in the protein profile of each homogenate. In conclusion the results suggest that strains TcI and TcV induce different serological responses according to the antigen used. On the other hand the different proteins of each antigen could participate in the specificity and sensitivity of the technique used. These findings are potentially useful in the search for new antigens to be applied in serological tests or as molecular markers.

1903

PHENOTYPIC AND FUNCTIONAL CHARACTERISTICS OF HLA-DR+ NEUTROPHILS IDENTIFIED IN CIRCULATION OF BRAZILIAN CUTANEOUS LEISHMANIASIS PATIENTS

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The vector-borne protozoan *Leishmania braziliensis* causes the chronic ulcerative skin disease cutaneous leishmaniasis (CL) in individuals living in endemic regions. In murine models, neutrophils (PMNs) are recruited to the site of infection minutes after parasite inoculation, but their role during chronic infection, and the role of PMNs in human disease, remain undefined. We hypothesized that PMNs from patients with active CL would exhibit different functional properties compared to healthy subjects. Despite the fact that CL is a localized disease, a subset of CL patients had circulating neutrophils expressing HLA-DR, a molecule thought to be restricted to professional antigen presenting cells. We also examined lesion-recruited PMNs for these same surface markers. Circulating HLA-DR+ PMNs also expressed the co-stimulatory molecules CD80, CD86 and CD40. Recently described low-density PMNs contain a high percentage of HLA-DR+ PMNs. Sorted HLA-DR+ PMNs morphologically resembled conventional PMNs, and they were capable of phagocytosis and reactive oxidant generation. Nonetheless, PMNs from subjects with high proportions of HLA-DR+ PMNs promoted significant *in vitro* proliferation of T cells. Compared to conventional HLA-DR- PMNs, HLA-DR+ PMNs showed increased activation, degranulation, oxidant generation and phagocytosis of parasites and zymosan particles. Incubation of whole blood with inflammatory cytokines resulted in increased HLA-DR+ PMNs, suggesting a connection between neutrophil "priming" and upregulation of HLA-DR. These data suggest that CL causes expansion of a subset of HLA-DR+ PMNs that are primed for activation.

1904

HLA DR EXPRESSING LOW DENSITY NEUTROPHIL SUBSETS EXPAND DURING HUMAN VISCERAL LEISHMANIASIS AND CAN CONTRIBUTE TO T CELL PROLIFERATION

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Visceral leishmaniasis (VL) is a chronic infectious parasitic disease, which if left untreated is almost always fatal.The role of neutrophils and how they affect or are affected during active VL is still unknown. Abebe et al reported that depletion of arginine was associated with suppressed T cell activity. We know that T cells from our subjects with visceral leishmaniasis respond differently to antigen when studied in isolated PBMCs or in whole blood, so we examined the hypothesis that there might be distinct subsets of circulating neutrophils in subjects with this infection. Indeed our studies revealed an enhanced population of low density neutrophils, similar to Abebe et al (2013 reference above).We fractionated whole blood over Ficoll to obtain both low density and normal density neutrophil populations. The neutrophils were identified and gated on Forward Side scatter and stained cells were CD66b+ CD14-, CD3-. The neutrophils were stained with HLA DR, CD80, CD86, CD63, CD11b, CD62L in our studies. For Neutrophil T cell cocultures we MACS purified CD66b cells and CD3 T cells from PBMCs and CD15 cells from NDG.In whole blood and fractions of whole blood i.e NDG and LDG we found that LDG were much more abundant during active disease and stained strongly for HLA DR. These cells were of different density and also expressed co-stimulatory molecules like CD80 and CD86. We performed Neutrophil-T cell co-culture experiments that lead us to this interesting finding that neutrophils can contribute to antigen presentation and proliferation in T cells. The CD66b+ neutrophils in VL subjects were CD62L low, CD11b high and are CD63 high which indicates that they are activated and de-granulating. This study was performed on 83 Active VL subjects (29 female and 54 male). Our data indicate there are indeed unusual neutrophil subsets that expand during visceral leishmaniasis, and underscore the need to further our understanding of neutrophil populations in infectious and inflammatory diseases.

1905

ACTIVATION OF HUMAN KERATINOCYTES BY *LEISHMANIA* SPP.: DIVERGENT EFFECTS OF *LEISHMANIA INFANTUM* VERSUS *LEISHMANIA MAJOR*

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All *Leishmania* infections are initiated in skin, but clinical manifestations vary greatly with infecting species. *Leishmania major* (*Lm*) causes localized cutaneous lesions, whereas *L. infantum* (*Li*) causes potentially fatal disseminated disease without skin pathology. Early responses at the skin infection site influence adaptive responses to *Leishmania*, yet little is known of the role of keratinocytes. We hypothesized that *Leishmania* induce keratinocytes to produce factors influencing the immune response. Incubation of *Li* with primary or immortalized human keratinocytes caused a significant increase in pro-inflammatory cytokine transcripts *il6*, *il8*, *tnfa,* and *il1b* measured by RT-qPCR. However, keratinocytes exposed to five distinct *Lm* isolates did not induce these transcripts, highlighting a species-specific difference in inflammatory response. Similar to live parasites, *Li*-derived exosomes induced more *il8* mRNA compared to control ($p<0.01$) or *Lm*-derived exosomes ($p<0.05$). Western blotting confirmed NFkB-p65 phosphorylation in keratinocytes exposed to *Li* but not *Lm*. To examine whether soluble keratinocyte factors influence nearby immune cells, *Li*-

infected human monocytes were co-cultured with keratinocytes across a trans well membrane. Soluble products of *Li*-exposed keratinocytes improved monocyte control of parasite replication compared with unexposed controls ($p<0.01$). However, culture with *Lm*-exposed keratinocytes across the trans well did not affect monocyte *Lm* infection. These data suggest that (1) activated keratinocytes may increase monocyte leishmanicidal activity and (2) keratinocytes support an early inflammatory environment, uniquely tailored to each *Leishmania* species, at the infection site.

1906

HEMOPHAGOCYTOSIS IN EXPERIMENTAL VISCERAL LEISHMANIASIS BY *LEISHMANIA DONOVANI*

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Visceral leishmaniasis (VL), also known as kala-azar, is caused by parasitic protozoa of the genus *Leishmania*. VL is characterized by clinical manifestations such as fever, weight loss, hepatosplenomegaly and anemia. Hemophagocytosis is a phenomenon of macrophages or histiocytes phagocytosing blood cells. There are reports on up-regulated hemophagocytosis in patients with infectious diseases including typhoid fever, tuberculosis, influenza and VL. However, mechanisms of infection-associated hemophagocytosis remained elusive due to lack of appropriate animal models. Here, we have established a mouse model of VL representing hemophagocytosis in order to elucidate mechanisms behind this phenomenon in VL. At 24 weeks after infection with 1 x 10^7 promastigotes of *Leishmania donovani*, BALB/cA mice exhibited splenomegaly with an average tissue weight of 10 times as those of naïve mice and anemia with lower hematocrit, hemoglobin and red blood cell counts than the naïve mice. In the spleen, 28.6% of macrophages contained erythrocytes. All of hemophagocytosing macrophages were parasitized by *L. donovani*. When hemophagocytes were categorized based on the number of parasites per macrophage, higher levels of hemophagocytosis were observed in heavily infected cells (more than 20 amastigotes). Besides, more than half of these hemophagocytes had two or more nuclei per cell whereas only 15.0% of splenic macrophages were multi-nucleated. Such multi-nucleated cells were not observed in spleens of uninfected mice. From these histological observations, hemophagocytes were presumed to be macrophages which acquired abnormal character by *L. donovani* infection. Through *in vitro* experiment with RAW264.7 cells, enhanced hemophagocytosis by macrophages was reproduced by infection with *L. donovani* in the presence of IFN-γ. These results suggested that *L. donovani* causes hyper-activation of macrophages to hemophagocytose. To our knowledge, this is the first report on hemophagocytosis in experimental *Leishmania* infections and may be useful to further understanding of the pathogenesis.

1907

INVOLVEMENT OF NUCLEOTIDE-BINDING DOMAIN LEUCINE-RICH REPEAT PROTEIN 12 (NLRP12) IN VISCERAL LEISHMANIASIS (VL)

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Leishmania infantum chagasi (Lic) causes VL, with suppression of type 1 immune responses. The NLR proteins include >20 cytosolic proteins that regulate inflammation and immunity. Activation of three NLRs or AIM2 can cause assembly of an inflammasome leading to IL-1β and IL-18 release. Functions of non-inflammasome forming NLRs are not as well understood. We hypothesized that NLR proteins influence the course of VL by modifying the localized inflammatory response to Lic. We screened for NLR effects by infecting NLR pathway gene knockout or wild type (WT) mice with Lic coexpressing luciferase and mCherry. Progressive parasite expansion was monitored by *in vivo* imaging (IVIS), qPCR and, Luciferase assay. The screens suggested involvement of the non-inflammasome forming Nlrp12 in progression of VL. Lic parasite loads expanded early (day 28) but were controlled in WT mice, whereas Lic continued to expand and were 2-fold higher than WT on day 56 of Nlrp12-/- infection. Consistently, liver-derived infiltrating cells from Nlrp12-/- mice released less antigen-induced IFNgamma than WT cells on infection day 56 (24 vs. 41 pg/mL). Flow Cytometry showed inflammatory monocytes expanded on day 28 in WT but not Nlrp12-/- mice, preceding parasite clearance from WT. Instead, resident macrophages expanded in Nlrp12-/- mice in parallel with the late expanding parasite load (day 56). The kinetics of monocyte derived dendritic cell (MNDC) recruitment paralleled parasite load, with recruitment at 28 days in WT but recruitment at 56 days in Nlrp12-/- mice. These data suggest that Nlrp12 plays a protective role in VL, associated with recruitment of both inflammatory monocytes and MNDCs at the time of peak parasite growth, followed by parasite clearance. Infiltration of inflammatory monocytes is impaired in the absence of Nlrp12, leading to delayed MNDC influx, impaired IFN-gamma, and expansion of resident macrophages, which permit parasite growth.

1908

EVALUATION OF THE USE OF *LEISHMANIA DONOVANI* DOUBLE KNOCK-OUT PARASITES (LDCEN-/-MIF-/-) AS PROTECTIVE VACCINE AGAINST VISCERAL LEISHMANIASIS

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Visceral leishmaniasis (VL) is a neglected tropical disease, and fatal if untreated. There is no vaccine available against VL. Parasite persistence is thought to be important for an effective protective response. Such protection may also be achieved by immunization with gene-deleted live attenuated parasites that do not cause disease. We have previously reported on a genetically modified live attenuated parasite, with a cell division specific centrin1 gene deletion, producing strong immune protection in mice, hamsters and dogs. *Leishmania* parasites are shown to secrete an inflammatory cytokine, macrophage migration inhibitory factor (MIF) that causes poor T cell responses in the infected host due to excessive inflammation. In this study we have tested the *L. donovani* double gene deletion mutant strains deficient for centrin and MIF genes (LdCen-/-MIF-/-) for their safety and efficacy as a candidate vaccine. Our hypothesis is the double-attenuated strain induces more effective immune response through production of long-term memory T cells, being able to promptly respond to infection inducing protective response. Balb/c mice were immunized with LdCen-/-, LdMIF-/- or LdCen-/-MIF-/- parasites, and the immune responses were compared to a control group (PBS). After 4 weeks of immunization (4wpi), some mutant parasites were detected in spleen and liver by serial dilution. Our preliminary results showed that, at 4wpi, LdCen-/-MIF-/- immunized group presented higher percentage of CD4 and CD8 central memory T cells, higher percentage of CD8 late effector memory T cells, and increased CD8 T cells proliferation after specific stimulation compared to PBS and LdMIF-/-. Protective immunity induced by LdCen-/-MIF-/- parasites is currently being evaluated in mice by parasitological and immunological assays following 4 wpi, and 4, 8 and 12 weeks of challenge with wild type strain of L. infantum. These results demonstrate the role of parasite products involved in manipulating the host immunity and manipulating these mechanisms might enhance the vaccine induced protective immunity and help further development of vaccines against VL.

1909

TEST AND NOT TREAT (TNT): A SAFE STRATEGY TO PROVIDE COMMUNITY-BASED TREATMENT WITH IVERMECTIN IN *LOA LOA* ENDEMIC AREAS

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Implementation of ivermectin (IVM)-based community treatment for onchocerciasis or lymphatic filariasis (LF) control/elimination has been delayed in Central Africa because IVM can induce serious adverse effects (SAE) in people with *Loa loa* microfilaremia exceeding 30,000 microfilariae (mf)/mL blood. The recent development of CellScope-Loa, a rapid field-friendly diagnostic tool to quantify *L. loa* mf in peripheral blood, permits point-of-care (POC) identification of the few "at risk" individuals for exclusion from IVM treatment (to prevent SAEs) while the rest of the population can be safely treated. This "Test and not Treat" (TNT) strategy was evaluated in Okola district (Central Cameroon) where onchocerciasis and loiasis are co-endemic and where IVM distribution was halted in 1999, after the occurrence of SAEs including fatalities. Between August and October 2015, 16,205 individuals from a target population (>5 years) of 22,800 (participation: 71.1%) were tested at the point of care (POC) using the CellScope-Loa; those with fewer than a pre-determined threshold (20,000 mf/mL) were given IVM (n=15,469), whereas those above this threshold (n=343, 2.1%) were excluded from IVM treatment, in addition to 167 pregnant women and 226 people in a poor state of health). Adverse events were closely monitored by local volunteers and mobile medical teams visiting each village 1, 2, 3 and 6 days after treatment. No SAE was observed. A total of 970 individuals (6.3% of the IVM-treated population) experienced mild adverse effects (itching, rash, headache, arthralgia, myalgia, fever) that resolved within one week. About half of adverse events occurred in individuals who had no Loa mf before treatment. The TNT strategy based on the CellScope-Loa is an extremely promising and practical approach to the safe implementation of large-scale IVM-based treatment for LF and onchocerciasis elimination in Loa endemic areas.

1910

THE MACROFILARICIDAL ACTIVITY OF A SINGLE DOSE OF IVERMECTIN, ALBENDAZOLE AND DIETHYLCARBAMAZINE AGAINST *WUCHERERIA BANCROFTI* IN CÔTE D'IVOIRE

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Current single dose treatments for lymphatic Filariasis (LF) have limited ability to kill adult worms. In a recent pilot study in Papua New Guinea we showed a single dose of co-administered ivermectin (IVM, 200ug/kg), diethylcarbamazine (DEC, 6mg/kg), and albendazole (ALB, 400mg, IDA) completely cleared microfilaria (mg) 1 year after treatment compared to 8% clearance with DEC/ALB. However, the effect of IDA on macrofilaria is not known. We used ultrasounds of the spermatic cord and inguinal lymphatic vessels immediately prior to treatment and 6 months later to compare the effects of two drug regimens on adult filarial worms in infected men in Côte d'Ivoire. The first group included 46 men treated with a single dose of IVM+ALB (IA, mean number of worms nests=3.2±1.4 [range 1-13]) and the other group included 28 men who received IDA. Number of worm nests was the same at baseline (IA=3.2±1.4 range [1-13], IDA=3.0±1.4 [1-8]). Thirty-six men treated with IA and 21 men treated with IDA underwent repeat ultrasound after 6 months. Worm nests were cleared more often after IDA (15 of 21, 71%) than after IA (9 of 36, 25%, P=0.0009). IDA also showed a reduction in nest size of 83%, compared to 9% in the IA group, as well as 95.3% clearance of mf compared to 28.6% clearance of mf in IA (P<0.0001). These results suggest that a single dose of IDA killed most adult W. bancrofti and that IDA is more effective against adult filarial worms than IA.

1911

NEXT GENERATION IMMUNOASSAYS PROVIDE ONE-STEP SPECIES-SPECIFICITY FOR THE DIAGNOSIS OF FILARIAL INFECTIONS AND *STRONGYLOIDES STERCORALIS IN* TRAVELERS AND IMMIGRANTS

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Antifilarial antibody testing in the evaluation of returned travelers and immigrants to North America has relied on IgG- and IgG4-specific responses to crude filarial extracts (BmA). The anti-BmA IgG response is highly sensitive (~100%), but suffers from relatively poor (50%) specificity. It also suffers from significant cross-reactivity with *Strongyloides stercoralis* (Ss) and cannot distinguish among the infecting filarial species. Conversely, the IgG4 anti-BmA antibody test is close to 100% specific but has sensitivities that range between 50%-70%. Of the 10173 CLIA-certified antifilarial antibody tests performed, 1809 (18%) filarial infections were diagnosed based on a positive IgG4 anti-BmA antibody response, and 4908 (48%) were excluded using an IgG anti-BmA test below the defined cutoff. Over the same period, filarial- (Ov16, Wb123, LL-SXP1) and Ss (SsIR, Ss-NIE)-species-specific recombinants have been identified and characterized. Each of these, when configured in a variety of single antigen IgG4-based immunoassay formats have demonstrated close to 100% specificity for the species of interest but with variable sensitivities depending on the antigen. Thus, to create an all-in-one assay for screening of returned travelers and immigrants where infections with filariae or Ss

is being considered, we configured a multiplex suspension array assay to measure the IgG or IgG4 responses to BmA, LlSXP1, Ov16, Wb123, SsNIE and SsIR. When these multiplexed assays were assessed using serum samples from parasite-uninfected (n=70) subjects compare to definitively diagnosed (parasite-positive) infected patients with *Loa loa* (N=37), *Onchocerca volvulus* (n=185), *Wuchereria bancrofti* (N=24), and Ss (N=41) we were able to get IgG4 based assays that achieved 100% specificity for all infections and sensitivities that ranged from 67% for LL-SXP1 to 92% for Wb123. Using this novel multiplexed immunoassay, we have been able to de-convolute the anti-BMA reactivity and identify the species of infecting parasite responsible for the antibody positivity for better accuracy in the diagnosis of individual filarial and Ss infections.

1912

HIGH EFFICACY OF SINGLE DOSE OF CO-ADMINISTERED IVERMECTIN, DIETHYLCARBAMAZINE AND ALBENDAZOLE IN TREATMENT OF LYMPHATIC FILARIASIS IN CÔTE D'IVOIRE

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Available treatments for lymphatic filariasis (LF) are limited in their long-term clearance of microfilaria (mf) from the blood. Recently we reported that a single dose of co-administered Ivermectin (IVM, 200ug/kg), Diethylcarbamazine (DEC, 6mg/kg), and Albendazole (ALB, 400mg) completely cleared mf 1 year following treatment, compared to 8% clearance with DEC/ALB alone in a small pilot study in Papua New Guinea. In order to confirm and expand these results in a different population we performed an open-labeled, single-blinded clinical trial where microfilaremic infected individuals were randomized into those treated with IVM/DEC/ALB (IDA, N=42) or IVM/ALB (IA, N=55) and mf levels measured immediately prior to and 6 months following treatment. Of those enrolled 83% are men, with median age of 37 years and overall geometric mean mf of 191.4 mf/ml (range 51-2,250). In the IDA group 22 of 25 (88%) completely cleared their microfilaria. The remaining 3 each had a single mf per 2 ml of blood. By contrast only 32.4% of individuals treated with IA completely cleared their mf, with the remaining participants averaged 92.8% (range 58.2-98.6%) reduction in mf levels. Adverse events (AEs) particularly fevers, myalgias, and pruritus were common, occurring in 54.8% vs 40.4% of those receiving triple-drug compared to 2-drug treatment respectively (P=0.18); all symptoms resolved within 7 days after treatment. IDA had more level 2 (scale 1-3) reactions [9 (21%) vs 1 (2%) in IA], however no serious AEs were observed in either group. This confirms that triple-drug therapy is safe and more effective than IVM/ALB for Bancroftian filariasis and has the potential to accelerate elimination of lymphatic filariasis.

1913

EFFECTIVENESS AND SAFETY OF ALBENDAZOLE FOR THE TREATMENT OF HYPERMICROFILAREMIC LOIASIS IN GABON

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Loiasis is endemic in Gabon with prevalences ranging from 10 and 35 %. Ivermectin, the drug known to be associated with serious adverse reactions in case of *Loa loa* hypermicrofilaremia, is used for the prevention of lymphatic filariasis and onchocerchiasis in areas where they coexist with loiasis. Albendazole is an alternative to ivermectin. Its efficacy and safety for the treatment of loiasis microfilaraemia was assessed in 128 individuals with more than 8000mf/mL. Parasitological data and clinical symptoms were monitored at days 7, 14, 21 and 63 after administration of increasing doses of albendazole (50 or 100mg to 400mg and 800mg/day) during six to eight weeks. Asthenia was the predominent drug-related adverse event recorded. The percentage of participants with ≥ 50% decrease of microfilaraemia from pre-treatment to 1 month was 69%. At 3 months post-treatment, 82% of patients had no microfilaraemia detected after leuconcentration of 4mL blood. Objective symptoms were not noticed after 3 months and pruritus was the most frequently reported post-treatment clinical symptom. Data analysis is still ongoing. In conclusion, treatment of hypermicrofilaraemic loiasis with albendazole was safe and efficacious in continuously exposed patients.

1914

DEVELOPMENT OF MURINE MODELS OF LOIASIS TO ASSESS MICROFILARICIDAL ACTIVITY OF PRE-CLINICAL CANDIDATE ANTI-FILARIAL DRUGS

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Development of new macrofilaricides to eliminate onchocerciasis in Africa requires assessments of safety for potential indications in loiasis co-endemic regions. This is because rapid killing of *Loa loa* microfilariae (mf) following ivermectin (IVM) treatment in patients with high parasitaemias has been linked to the development of severe neurological adverse reactions. Via human pharmacokinetic profiling of IVM and the related macrocyclic lactone moxidectin (MOX) we define a mis-match between *in vitro* and *in vivo* drug sensitivities to the bloodborne human filariae *Brugia malayi* and *L. loa*. This indicates that safety evaluation of potential macrofilaricides requires screening *in vivo* against blood-stage *L. loa* mf. Here we describe the development of mouse models of loiasis with the goal of evaluating them as *in vivo* microfilaricide drug screens. BALB/c WT or SCID (+/- splenectomy) were perfused with Loa or Brugia mf. CB.17 SCID, NOD.SCID or NOD.SCID IL-2gc-/- (NSG) strains were infected with Loa L3 and evaluated at 3-5 months post-inoculation. Recovered worms were then surgically re-implanted in NSG mice and evaluated 1 month post-implantation. To evaluate drug responsiveness, microfilariaemic mice were treated with bio-equivalent IVM. The vast majority of perfused mf (~10% of initial inoculates) were sequestered in the cardiopulmonary circulation. Splenectomy increased both the incidence of peripheral Loa mf in WT mice and the overall yield of cardiopulmonary mf six days post infusion. IVM induced a rapid decline (>80%) in circulating mf in WT and SCID mice. For patent *Loa* infections, NSG mice yielded an average recovery of adult worms of 33% of the initial inoculate at +5 months. No circulating mf were observed although embryograms of female worms identified occurrence of embryogenesis and inter-uterine mf. For the adult

Loa implanted NSG mice, circulating mf were observed both centrally and peripherally. IVM treatment reduced microfilaraemia in these mice. In conclusion, preliminary validation demonstrates both models could be implemented as pre-clinical macrofilaricide counter-screens and are thus in further development.

1915

PET/CT LYMPHOSCINTIGRAPHY DEMONSTRATES EARLY CHANGES IN LYMPHATIC FUNCTION IN THE *BRUGIA MALAYI*/FERRET MODEL OF LYMPHATIC FILARIASIS

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The goal of this study was to evaluate changes in lymphatic function in the *Brugia malayi*/ferret model of lymphatic filariasis. Injection of L3 larvae into the ferret footpad results in intralymphatic infection of the femoral and inguinal lymphatics. Development of microfilaremia and eosinophilia begin at 12-14 weeks post-infection. Ferrets were imaged at baseline, and again at 2 and 16 weeks post-infection. Imaging was performed using a Simeons Inveon Multimodality PET/CT scanner. Anesthetized animals were subcutaneously injected (between the toes of their right hind limb) with 18F-FDG (100-150 uCi), and monitored for tracer uptake for 90 minutes. A CT scan for anatomical localization followed PET analysis. This approach enabled assessment of lymphatic function by quantification of tracer uptake into the inguinal lymph nodes over time. While single infectious challenges with L3s did not result in clinical disease as monitored by measurements of ankle and limb circumferences, they clearly caused marked changes in lymphatic anatomy and function as early as 2 weeks post infection. Compared to baseline imaging, expanded networks of tortuous and dilated lymphatic vessels were observed at all infected timepoints, along with the generation of collateral lymphatic vasculature. Whereas peak tracer uptake into the inguinal lymph nodes was observed by 20 minutes post-tracer injection in uninfected animals, peak uptake did not occur until 25-30 minutes after two weeks of infection and until 30-35 minutes after 16 weeks of infection. Additionally, maximal intensities of tracer signal in the inguinal lymph nodes was reduced by 50% in infected animals at all timepoints evaluated. These results demonstrate that *Brugia malayi* cause marked alterations in lymphatic vessel anatomy and function early in the course of LF infection; occuring prior to microfilaria production and in the absence of frank clinical disease. In current studies we are evaluating whether this imaging protocol can be used to assess alterations in lymphatic function induced by treatment with antifilarial agents.

1916

MICROBIOLOGY AND OUTCOMES IN HOSPITALIZED NEONATES WITH SEPSIS: A ZAMBIAN COHORT STUDY

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Sepsis is a major cause of mortality in neonates in sub-Saharan Africa, yet is not well-studied. We are conducting an ongoing cohort study of infants hospitalized in a large neonatal intensive care unit in a public hospital in Zambia. Clinical and demographic data were captured by maternal interview and chart review. Blood cultures were obtained on all neonates with suspected sepsis. We examined the microbiology and outcomes of neonates with all-cause bacteremia and those with *Klebsiella* infection. From September 1, 2015 to March 31, 2016, we enrolled 711 neonates, of whom 376 (53%) were male, and 543 (76%) were born at the study hospital. The median birth weight was 2690g (IQR 1600-3135g) and 539 (77%) were born vaginally without instrumentation. Most enrolled infants had suspected sepsis (n=520; 73%), of whom half (n=265; 51%) had a culture-confirmed bacteremia. The most common isolates were *K. pneumoniae* (n=187; 70%), common skin commensal organisms (n=55; 21%), and *Enterococcus* spp. (n=22; 8%). There was one isolate each of *Staphylococcus aureus* and *streptococcus agalactiae*. Overall mortality was 31% and was greater among bacteremic infants than septic, non-bacteremic infants (36% vs. 27%, p= 0.04). Among bacteremic neonates, low birth weight (aOR=1.02) and *Kleb.* infection (aOR=1.80) were independent risk factors for death. Most *Kleb.* isolates were resistant to fluoroquinolones (n=177, 95%) and half were resistant to β-lactam antibiotics, (n=93, 50%), with 29 (15%) ESBL. Mortality among neonates with *Kleb.* infections was 42%. The risk of death was similar among infants with β-lactam-resistant as compared to susceptible strains of *Kleb.* (44% vs. 41%, p= 0.77). Age at sepsis onset, maternal HIV status, and birth location were not associated with death in either the all-cause bacteremia or the *Kleb.*-infected cohorts. Neonatal sepsis was common, often caused by multi-drug resistant organisms, and associated with a high case-fatality rate in this large NICU in Zambia. *Kleb.* infection was associated with an increased risk of death; however, infection with β-lactam-resistant *Kleb.* was not associated with an increased risk of death.

1917

HIGH SERUM ZINC LEVELS PROTECT AGAINST ROTAVIRUS INFECTION BUT NOT OTHER DIARRHEA-ASSOCIATED PATHOGENS IN A BIRTH COHORT IN BANGLADESH

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Diarrhea remains the world's second leading cause of death in children. In combatting this challenge, zinc supplementation has been shown to reduce diarrheal morbidity and mortality, though the mechanisms are unclear. In most zinc efficacy trials, diarrhea has been treated as pathogen-agnostic; however rotavirus causes an estimated 40% of acute diarrhea in developing countries where rotavirus vaccines perform poorly and alternative methods for diarrheal management are critical. In the PROVIDE study, a randomized controlled trial of oral rotavirus vaccine in a birth cohort in Dhaka, Bangladesh, we found a significant protective effect of serum zinc concentration at age 18 weeks on the risk of rotavirus diarrhea through one year based on rotavirus antigen detection by ELISA in diarrheal stools. Using advanced molecular techniques, here we interrogate whether the protective effect of zinc is specific to rotavirus or extends to other diarrheal pathogens. We performed multiplex PCR on 1,448 diarrheal specimens collected between weeks 18-52 with the following targets: rotavirus, norovirus, sapovirus, astrovirus, Giardia, Cryptosporidium, and Entamoeba histolytica. Among these pathogens, only rotavirus correlated with zinc status (P=0.016, Kruskal-Wallis test). This relationship was further tested by logistic regression to include variables that may modify the effect of zinc: courses of zinc supplementation, sex, urinary mannitol at week 40, household food deficit, exclusive breast feeding and stunting. In the final model of risk of rotavirus diarrhea, children with zinc levels in the highest quartile compared to children at risk of zinc deficiency were nearly four times more likely to have diarrhea without any rotavirus detected versus rotavirus

infections (OR 3.93, 95% CI 1.28 - 12.05, P=0.017). Other factors in the model were not associated with rotavirus infection and did not significantly modify the effect of zinc. These results suggest a particular utility of zinc interventions in reducing the burden of rotavirus diarrhea compared to other etiologies. Future research will examine mechanisms of zinc protection in rotavirus.

1918

DECLINING CHILD MORTALITY DUE TO INFECTIOUS DISEASES IN AN URBAN SLUM IN NAIROBI KENYA

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Examining trends in causes of child mortality over time can help measure progress towards achieving Millennium Development Goal #4. Little is known about trends in child mortality in urban slums, where living conditions are precarious. We assessed child mortality rates and cause of death (COD) from 2009-2014 in a population of ~25,000 residents of Kibera slum in Nairobi, Kenya, under demographic surveillance. Participants were visited biweekly in their homes and queried about any deaths. Trained verbal autopsy (VA) interviewers used standardized World Health Organization VA questionnaires to gather data from a credible respondent on the circumstances leading up to death. InterVA-4 was used to code the most likely COD. We calculated rates of death per 1000 person-years-observation (pyo); for cause-specific rates, we extrapolated the observed proportion of COD by year to those with missing VA data. From 2009-2014, 336 deaths were reported among children aged <5 years. The child mortality rate declined from a high of 18.3/1000 pyo in 2009 to a low of 9.8/1000 pyo in 2013, then increased slightly to 11.4/1000 pyo in 2014. VA was completed on 255 (76%); 18 (7%) were classified as 'indeterminate' and 5 (2%) as 'other and unspecified neonatal'. Among the remaining 232 with a likely COD identified, the leading cause was acute respiratory infection/pneumonia, including 13/35 (37%) neonatal deaths and 102/197 (52%) in children aged 29 days to <5 years. Other common causes included malaria (n=29, 12%), diarrhea (n=12, 5%), and HIV/AIDS-related (n=12, 5%). Rates of pneumonia deaths peaked in 2010 at 9.8/1000 pyo, then fell to a low of 4.5/1000 pyo in 2014. Rates of all other infectious causes combined (excluding pneumonia) decreased from 9.8/1000 pyo in 2009 to 2.9/1000 pyo in 2013 and 2014. We observed a reduction in child mortality of more than 35% in recent years in Kibera. The decline was driven by falling mortality due to pneumonia and other infectious diseases. Possible factors contributing to improved survival include introduction of the pneumococcal conjugate vaccine in 2011 and scaled up malaria control and HIV prevention efforts.

1919

A SYSTEMATIC REVIEW ON THE EFFECTIVENESS OF STRATEGIES TO IMPROVE HEALTH WORKER PERFORMANCE IN LOW- AND MIDDLE-INCOME COUNTRIES: PRELIMINARY RESULTS ON UTILIZATION OF HEALTH SERVICES

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Improving health worker (HW) performance is a global health priority. Strategies that improve performance might also increase utilization of health services. To characterize the effectiveness of such strategies in low- and middle-income countries (LMICs), we conducted a systematic review of 15 electronic databases, 30 document inventories of international organizations, and bibliographies of 510 articles. We included studies meeting accepted criteria for methodological adequacy (e.g., trials with comparison groups) of any strategy to improve HW performance on any health topic in any language, published or not. This analysis focuses on studies that measured continuous outcomes on the utilization of health services (e.g., number of patients seen per month). Effect sizes were calculated as percent change over time in the intervention group minus percent change over time among controls. We screened >105,000 citations, and 822 reports met inclusion criteria. Fifty-seven studies measured continuous utilization outcomes and were included in the analysis. Many strategies have been tested, usually with multiple intervention components. However, most strategies were tested by only one study. The median effect size (MES) across all studies was an improvement of 15 percentage-points (%-points) (interquartile range [IQR]: -14, 57). Among studies of facility-based HWs, three strategies tended to increase utilization: strategies that included financial incentives for HWs or health facilities (MES = 67 %-points, IQR: 2, 119), insurance schemes (MES = 16 %-points, IQR: -44, 47), and reducing or removing user fees (MES = 15 %-points, IQR: -12, 42). Introducing or increasing user fees tended to decrease utilization (MES = -53 %-points, IQR: -82, -17). Among studies of lay HWs, no clear patterns were identified. For example, strategies that included the combination of HW training + supervision + patient or community education had effect sizes of -29, 5, 79, and 306 %-points. Contextual and methodological heterogeneity made comparisons difficult. These results should inform decision-making on increasing utilization of health services in LMICs.

1920

THE IMPACT OF ANEMIA DURING PREGNANCY AND ITS RISK FACTORS ON THE COGNITIVE DEVELOPMENT OF ONE-YEAR-OLD CHILDREN

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The aim was to investigate the impact of anemia during pregnancy and its risk factors on the cognitive development of one-year-old children. Our prospective cohort study included 636 mother-singleton child pairs from 828 eligible pregnant women who were enrolled during their first antenatal care visit (ANV) in Allada, Benin, into the MiPPAD clinical trial. Venous blood samples of women were assessed for ferritin and hemoglobin (Hb) concentrations at the first and second ANV of at least one-month interval and at delivery. Stool samples of pregnant women were also collected during these follow-up periods to test for helminths using the Kato-Katz technique. All pregnant women were administered a total of 600 mg of mebendazole (100 mg two times daily for 3 days) to be taken after the first ANV. Women were also given daily iron and folic acid supplements throughout pregnancy. The intake was not directly observed. At age one year, cognitive and motor functions of children were assessed using the Mullen Scales of Early Learning. The prevalence of iron deficiency (ID) among pregnant women at first and second ANC visits, and at delivery was 30.5%, 34.0% and 28.4%, respectively. Prevalence of helminth infection was 11.5%, 7.5% and 3.0% at first, second ANV and at delivery, respectively. Prevalence of anemia decreased from 67.1% at first ANV [mean gestational age (Standard deviation), 22.1(4.0) weeks] to 40.1% at delivery. Children of mothers who were infected with hookworms at first ANV had 4.9 (95% confidence interval, CI: 1.3 - 8.6) lower mean gross motor scores compared to those whose mothers were not infected with hookworms at the first ANV. We observed a significant negative quadratic relationship between infant gross motor function and Hb concentration at first and second ANVs. Prenatal helminth infection is associated with poor with infant cognitive and motor development. However, in the presence of iron supplementation, ID is not associated with infant neurocognitive development. Further, there appears to be an Hb concentration range (90-110 g/L) that may be optimal for better gross motor function of one-year-old children.

1921

MATERNAL AND INFANT FACTORS MEDIATING COGNITIVE DEVELOPMENT AT 12 MONTHS AMONG FILIPINO INFANTS

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The objective of this study was to identify pre- and post-natal predictors that directly or indirectly affect infant cognitive, language, and motor development at 12 months of age among Filipino infants and the pathways through which they act. The Bayley Scales of Infant Development, 3rd edition, was used to assess the development of 314 infants who were enrolled in a trial to examine the effects of Praziquantel for the treatment of schistosomiasis given at 12-16 weeks gestation on pregnancy outcomes. Covariates evaluated from the trial included maternal iron, socio-economic, and nutritional status, as well as birthweight and newborn iron status. Infant nutritional status, iron and hemoglobin were captured at 1, 6, and 12 months of age and the Philippines Nonverbal Intelligence Test (PNIT) was administered to mothers. Multivariable linear regression and structural equation modeling were used to identify significant factors associated with infant development. In multivariable regression models, maternal treatment with Praziquantel, education, PNIT score, and iron status as well as infant WAZ, WLZ, and WAZ gains were significantly associated with specific domains of infant development at 12 months of age. Structural equation models demonstrated that maternal PNIT scores [standardized β (sβ) for cognitive=0.073, sβ for language=0.061, sβ for motor=0.20, all P <0.05] directly influenced most subscales of infant development and indirectly impacted development through birthweight and/or infant weight gain. Maternal iron status during gestation was a stronger predictor of development than infant iron status. Infant change in nutritional status was related to language and motor development (eg, sβs of WAZ gain/mo for language=0.15 and motor=0.079, all P <0.05), suggesting catch up growth may ameliorate some cognitive deficits among LBW infants. Finally, exclusive breast feeding had a direct effect on infant expressive language, rather than through improved iron or nutritional status. This study identifies modifiable risk factors for impaired infant development beginning *in utero* and the key pathways through which they act.

1922

MOTHERS SCREENING FOR MALNUTRITION BY MUAC IS NON-INFERIOR TO COMMUNITY HEALTH WORKERS: RESULTS FROM A LARGE-SCALE PRAGMATIC TRIAL IN RURAL NIGER

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Community health workers (CHWs) commonly screen for acute malnutrition in the community by assessing mid-upper arm circumference (MUAC) on children aged 6-59 months. MUAC is a simple screening tool that has been shown to be a better predictor of mortality in acutely malnourished children than other practicable anthropometric indicators. This study compared, under program conditions, mothers and CHWs in screening for severe acute malnutrition (SAM) with color-banded MUAC tapes and checking for edema. It took place from May 2013 to April 2014 in two health zones of Niger's Mirriah District. Mothers in Dogo (Mothers zone) were trained to screen children for malnutrition in their household and CHWs in Takieta (CHWs zone) were trained to screen children in the community. Exhaustive coverage surveys were conducted quarterly, and relevant data collected routinely in the health and nutrition program. An efficacy and cost analysis of each screening strategy was performed. 12,893 mothers were trained in the mothers zone and 36 CHWs in the CHWs zone, and point coverage was similar in both zones at the end of the study (35% [26/74] Mothers Zone vs 32% [11/34] CHWs zone; p=0.7772).The rate of MUAC agreement (compared with health center agent) was higher in the Mothers zone (75.4% [721/956] vs 40.1% [221/551]; p<0.0001) and cases were detected earlier, with median MUAC at admission for those enrolled by MUAC <115 mm estimated to be 1.56 mm (95%CI 0.65-1.87) higher using a smoothed bootstrap procedure. Children in the mothers zone were less likely to need inpatient care, both at admission and during treatment, with the most pronounced difference at admission for those enrolled by MUAC <115 mm (0.7% [4/569] vs 7.8% [32/413]; risk ratio 0.09 [95%CI 0.03-0.25]; p<0.0001). Training mothers required higher up-front costs, but overall costs were much lower ($8,600 USD vs $21,980 USD). Mothers were not inferior to CHWs in screening for malnutrition at a substantially lower cost, and children in the Mothers zone were admitted at an earlier stage of SAM with fewer hospitalizations. Empowering mothers to screen for malnutrition should be a part of treatment programs globally.

1923

INTERMITTENT PREVENTIVE TREATMENT OF MALARIA IN HIV-INFECTED PREGNANT WOMEN WITH DIHYDROARTEMISININ-PIPERAQUINE: A DOUBLE BLINDED RANDOMIZED CONTROLLED TRIAL

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Intermittent preventive therapy with sulfadoxine-pyrimethamine (IPT-SP) is recommended for the prevention of malaria among HIV-uninfected pregnant women in sub-Saharan Africa. The WHO recommends that HIV-infected pregnant women receiving daily trimethoprim-sulfamethoxazole (TS) prophylaxis should not be given SP due to drug toxicity concerns. Although daily TS has been shown to be more effective than IPT-SP, resistance to this class of antimalarials is widespread, especially in East Africa. We recently showed that IPT with dihydroartemisinin-piperaquine (DP) was more effective than SP for the prevention of malaria in pregnancy in HIV-uninfected women. To extend this approach to HIV-infected women, we are conducting a double blind randomized placebo controlled trial comparing daily TS alone with daily TS plus monthly DP in Tororo District, Uganda. 200 HIV-infected pregnant women between 12-28 weeks gestational age were enrolled between December 2014 and October 2015. At enrollment, all women received a long lasting insecticide treated bed net and ensured to be taking combination antiretroviral therapy. Participants are being followed in a dedicated study clinic for all their medical care and encouraged to deliver at an adjacent hospital.The primary outcome is the risk of placental malaria defined by histopathology. Secondary outcomes include placental malaria defined by placental blood smear, birth outcomes, and the incidence of adverse events. As of 29th February 2016, 188 women had delivered and 12 were still being followed during pregnancy. The risk of placental malaria was 6.4% by histopathology and 0.6% by placental blood smear. Adverse birth outcomes include 3 spontaneous abortions (1.6%),1 stillbirth (0.5%), 5 congenital anomalies (2.7%),16preterm deliveries (8.7%), and 23 with low birth weight (12.4%). It is anticipated that all women will have delivered by April 2016 and that the final un-blinded results of the trial will be presented at the meeting.

1924

IMPACT OF EFAVIRENZ AND PREGNANCY ON PIPERAQUINE EXPOSURE IN UGANDAN PREGNANT WOMEN

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In malaria endemic areas HIV+ pregnant women receiving EFV-based combination antiretroviral therapy (EFV-cART) may receive artemisinin-based combination therapies (ACTs) for the treatment or prevention of malaria. One ACT, dihydroartemisinin-piperaquine (DHA-PQ), has shown excellent efficacy for the treatment of falciparum malaria and for intermittent preventive therapy (IPT) in pregnancy. We evaluated PQ pharmacokinetics in the setting of DHA-PQ and EFV-cART in pregnant (28 wks gestation) and postpartum Ugandan women using an intensive design. These studies were included as part of our trials (PROMOTE) in Tororo, Uganda to inform IPT dosing guidelines. PQ levels after standard dosing of DHA-PQ (qD x 3d) were compared between a) HIV- (no cART, n=30) and HIV+ (EFV-cART, n=26) pregnant women to determine the impact of EFV and b) HIV - antepartum (n=30) and postpartum (n=23) women to determine the impact of pregnancy. The area under the concentration-time curve (AUC) was measured over 21 d. PQ levels were measured by LC tandem MS. We found highly significant decreases in PQ exposure for HIV+ women on EFV compared to HIV- women, as measured by AUC (6.60 vs 10.6 hr.ug/mL; GMR:0.62, p<0.005) and Day 7,14, and 21 PQ levels (5.46, 1.62, 0.668 ng/mL vs 15.5, 5.37, 3.78 ng/mL; GMR:0.35, 0.30, and 0.18, respectively, all p values <0.0001). Pregnancy was also associated with decreased PQ AUC when comparing ante-partum and post-partum women (10.6 vs 17.2 hr.ug/mL; GMR:0.63, p<0.0001), and day 7, 14, and 21 PQ concentrations (15.5, 5.37, 3.78 ng/mL vs 32.8, 17.4, 11.4 ng/mL; GMR: 0.47, 0.31, 0.33, respectively, all p values ≤0.0001). EFV and pregnancy resulted in significant reductions in PQ exposure. For both HIV+ and HIV- pregnant women, mean PQ terminal concentrations were consistently <10 ng/mL, lower than the concentration previously estimated to be required for effective chemoprevention (30 ng/mL). Clinical correlates of these findings are underway. DHA-PQ dose escalation for pregnant women and those receiving EFV-cART may merit study.

1925

MALARIA IN HIV-INFECTED CHILDREN RECEIVING HIV PROTEASE-INHIBITOR-COMPARED WITH NON-NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITOR-BASED ANTIRETROVIRAL THERAPY

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HIV and malaria geographically overlap. HIV protease inhibitors kill malaria parasites *in vitro* and *in vivo*, but further evaluation in clinical studies is needed. Children from Malawi, Uganda, and Zambia were enrolled in P1068s, a substudy within a larger randomized HIV treatment study. Children aged 4-36 months were followed every 3 months and at intercurrent illness visits for up to 47 months between September 2009 and December 2011. We compared malaria parasite carriage by blood smear microscopy (BS) and confirmed clinical malaria incidence (CCM, or positive BS with malaria symptoms) in children initiated on HIV antiretroviral therapy (ART) with zidovudine, lamivudine, and either nevirapine (NVP), a non-nucleoside reverse transcriptase inhibitor, or lopinavir-ritonavir (LPV-rtv), a protease inhibitor. Because of low rates of BS positivity at Ugandan and Zambian sites, we analyzed results from Malawi only, an area of low to moderate malaria transmission intensity. We found an association between increased time to recurrent positive BS, but not CCM, when anti-malarial treatment and LPV-rtv based ART were used concurrently and when accounting for a LPV-rtv and antimalarial treatment interaction (adjusted HR 0·39; 95% CI (0·17,0·89); p=0·03). In our study, LPV-rtv in combination with malaria treatment is associated with lower risk of recurrent positive BS, but not CCM, in HIV-infected children. Larger, randomized studies are needed to confirm these findings which may permit ART optimization for malaria-endemic settings.

1926

EFFECT OF DAILY TRIMETHOPRIM SULFAMETHOXAZOLE PROPHYLAXIS ON THE LONG-TERM CLINICAL IMPACT OF MALARIA INFECTION AMONG HIV INFECTED ADULTS ON SUCCESSFUL ART IN BLANTYRE, MALAWI

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Sub-Saharan Africa has 90% and 70% of all new cases of malaria and HIV respectively. The risk of malaria infection is higher in HIV infected adults. Malaria infection in HIV positive individuals is associated with increased HIV plasma viral load (VL) and decreased CD4+ T cells. Daily trimethoprim sulfamethoxazole (TS) reduces the risk of malaria infection in HIV positive individuals but its long term benefit after successful ART has not been well documented. To determine the impact of TS on malaria infection and disease, we analyzed data from clinically stable, non-pregnant HIV infected adults on non protease inhibitor ART enrolled in an ongoing randomized controlled trial in Blantyre, an area with low to moderate malaria transmission. Participants with CD4 count >250 cells/mm3 and HIV VL of <400 copies/ml were enrolled and randomized to continue daily TS, discontinue TS, or discontinue TS and begin chloroquine. During the rainy season, we measured asymptomatic infection by quantitative PCR of dried blood spots. Clinical malaria was diagnosed in participants with symptoms suggestive of malaria and positive malaria smear by microscopy. We included only a subset of participants who continued on TS prophylaxis (n=34) or stopped prophylaxis (n=27). The two groups were similar in age, gender distribution, CD4 count, hemoglobin level and bed net use. Four participants in the TS discontinuation group developed clinical malaria compared to only one from the daily TS group. No episodes of asymptomatic malaria infection were detected by qPCR. Even in this lower transmission setting, TS prophylaxis was associated with protection against clinical malaria disease. The absence of asymptomatic malaria infection is in contrast with the common finding of high rates of low-level asymptomatic parasitemia in Malawi. HIV infected adults may be more likely to develop symptomatic disease; another possible interpretation is that ART or malaria prophylaxis confer some protection. We are currently undertaking immunological evaluation to determine mechanism of this observed phenomenon.

1927

PERSISTENCE OF LOWER ANTIBODY LEVELS TO VAR2CSA IN HIV-POSITIVE KENYAN PREGNANT WOMEN DESPITE HAART

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Malaria and HIV epidemics intersect in sub-Saharan Africa, disproportionally affecting young women, including those of childbearing age. Pregnancy provides *Plasmodium falciparum* parasite an additional niche for evading the immune system. Parasite-infected erythrocytes sequester in the placenta using the VAR2CSA adhesion molecule, causing placental malaria. Antibodies (Ab) against VAR2CSA improve pregnancy outcomes and a vaccine based on VAR2CSA is under clinical evaluation. Ab levels to VAR2CSA are lower in HIV-positive women compared to healthy controls. It is not clear whether widespread HAART implementation and immune reconstitution in HIV-positive pregnant women will improve their Ab responses to VAR2CSA. In a longitudinal case-control study we compared Ab levels to the full—length VAR2CSA (FV2) and its individual DBL domains (DBL1-DBL6), antibody avidity and cytokine levels between HIV-positive pregnant women receiving HAART and HIV-negative Kenyan pregnant women. At delivery no significant differences were observed in peripheral plasma levels of IL1β, IL2, IL4, IL6, IL7, IL8, IL10, IL12, IL21, IFNγ, TNFα, MIP1α, MIP1β between HIV-positive and HIV-negative women (all p>0.05) after adjusting for malaria status (PCR) and gravidity. In a multiple regression model adjusted for malaria status and gravidity, HIV was associated with significantly lower Ab levels at delivery to FV2, DBL1+2, DBL3 and DBL5 (all FCR3 strain); no significant differences were observed for DBL2, DBL4 and DBL6. In addition, HIV was associated with 5% decrease of Ab avidity to FV2 (p=0.03). Ab data from earlier visits during pregnancy are currently being analyzed. Lower Ab levels at delivery in HIV-positive women could contribute to less protection from placental malaria during the next pregnancy. Data on Ab responses to VAR2CSA in HIV-positive women on HAART are important in order to guide VAR2CSA-based vaccine regiments for HIV-positive women in sub-Saharan Africa.

1928

ASSESSING IMPACT OF COMMUNITY-BASED ANTIRETROVIRAL THERAPY AND ITS SCALE UP: PERSPECTIVES FROM FOUR PRIORITY LOCAL GOVERNMENT AREAS IN LAGOS, NIGERIA

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Community-based antiretroviral therapy (cART) delivery is effective in improving the early identification of HIV-positive clients, access to treatment and quality of health outcomes of people living with HIV. The cART project scale-up (cART Plus) aimed to further improve on those gains in achieving more individual testing in the community, improve ART coverage, strengthen linkage and retention in care and subsequently achieve viral suppression over the long-term; in line with the UNAIDS 90-90-90 targets. The cART project is being supported by USAID through SIDHAS. 4 LGAs in Lagos Nigeria were selected based on epidemiological and mapping indices. Community volunteers were recruited and trained in areas subsequently working with the CBOs to carry out community mobilization, household testing and counselling, identify positives and enrol to care, track and follow up on defaulters, documentation and reporting of service output data using approved tools. Data analysis spanning October to December 2015 in these 4 LGAs showed that the indices assessed significantly improved upon cART scale-up (cART Plus). Number of individuals counselled, tested and received results for HIV in the community {cART (Oct: 24 307); cART Plus (Nov: 61 532; Dec: 50 049)}. Number of individuals tested HIV-positive {cART (Oct: 65); cART Plus (Nov: 364; Dec: 268)}. The positivity rate in the general population being {cART (Oct: 0.27%); cART Plus (Nov: 0.59%; Dec: 0.54%)}. Number of persons newly enrolled into the ART programme for PreART care in the community {cART (Oct: 34); cART Plus (Nov: 283; Dec: 214)}. Percentage enrolment {cART (Oct: 52.3%; cART Plus (Nov: 77.7%; Dec: 79.9%)}. Number of persons newly started on ART in the community {cART (Oct: 13; cART Plus (Nov: 106; Dec: 123)}. In conclusion, the cART delivery scale-up has shown to improve uptake and accessibility of treatment. This concept could be adopted in more resource-limited settings to improve ART coverage. However, efforts need to be channeled into advocacy for community ownership as community programs need to be driven, owned by and embedded in the communities.

1929

DEVELOPMENT OF A MUCOSAL VACCINE AGAINST HIV BASED ON GENETICALLY-ENGINEERED *SACCHAROMYCES CEREVISIAE* PROBIOTIC STRAINS

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Human immunodeficiency virus (HIV) is a major public health problem. It is estimated that 37 million people worldwide are infected with HIV and 2 million new infections are reported each year. A vaccine against HIV is urgently required to stop this epidemic. An efficient prophylactic vaccine strategy must induce a mucosal immune response, since most infections occur during sexual intercourse through vaginal and rectal mucosae. Probiotic Saccharomyces cerevisiae strains are known to provide health benefits in the gut when administered in correct doses, including stimulation of secretion in the colon. We engineered several probiotic *S. cerevisiae* strains to express the HIV GAG antigen on their surface, and are assessing the GAG expression levels and the genetically-engineered strains ability to resist gastrointestinal stresses. We are also quantifying the *in vitro* phagocytosis rates of GAG-expressing yeasts by macrophages and quantifying the levels of tumor necrosis factor-alpha (TNF-α), interferon-gamma, (IFN-γ), interleukin (IL)-1β, IL-5, IL-6, IL-8, IL-10, and IL-12 secreted by these antigen presenting cells following contact with the fungal engineered vectors. We are currently developing a humanized mouse model to evaluate the efficacy of genetically-modified S. cerevisiae probiotic strains as a potential prophylactic vaccine against HIV.

1930

DIALOGUE BETWEEN NEUTROPHILS AND HOOKWORMS DETERMINES PARASITE DEVELOPMENT

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Hookworms are skin-penetrating parasites infecting about 700 million people, principally within improvised communities. The skin has recently been shown to be an important bulwark against parasite establishment in immune hosts. However, the initial interaction between the host and parasite within the skin following the first encounter with the parasite is still poorly characterized. Here, we investigate the fate of the larvae from their skin penetration to their migration to the lungs using intravital microscopy. We observe that neutrophils are rapidly recruited to the site of infection and adhere to the larvae. Surprisingly however, neutrophils are not sufficient to cause parasite killing. We further show that the parasite adjusts its development to the presence of the neutrophils by an evasion demarche: on one hand, the parasite delays its exsheathment to benefit from an additional layer of cuticle protection; on the other, in response to bleach induced by the neutrophils, the parasite secretes specific Excretory-Secretory (ES) products with anti-neutrophil activity. Building on these observations, we show that vaccination with parasitic ES products renders the parasite susceptible to killing by neutrophils, presumably by allowing the host to neutralization parasitic products capable of interfering with neutrophil activity. Altogether, this study highlights that targeting both the nematode's sensing mechanism and its secretory products with neutrophil inhibitory potential could enable parasite killing early during its migration and thus block its transmission.

1931

GENOMIC ABLATION OF CYST-WALL-PROTEIN-1 PREVENTS STAGE-SPECIFIC FORMATION OF GOLGI-LIKE ORGANELLES AND REGULATED SECRETION OF A CYST WALL IN *G. LAMBLIA*

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The genome of the ubiquitous protozoan parasite G. lamblia is organized in two diploid nuclei, which has so far precluded complete analysis of gene function. Here we used a previously developed Cre-loxPbased knock-out and selection marker salvage strategy in the human-derived isolate WB-C6 to eliminate all four copies of the Cyst Wall Protein 1 locus (cwp1). Because the cwp1 loci are silenced in proliferating trophozoites and expressed only in encysting cells, complete CWP1 ablation allowed functional characterization of a conditional phenotype in differentiating cells. Induced -cwp1 cells show morphological hallmarks of cyst development as well as karyokinesis, but are unable to establish the stage-regulated trafficking machinery with Golgi-like encystation-specific vesicles required for cyst wall formation. The wall-less "pseudocyst" phenotype could be rescued by transfection with an episomallymaintained CWP1 expression vector. This is the first example of genome editing and functional analysis or a locus essential for transmission between hosts in a diplomonad parasitic species.

1932

ADIPOSE TISSUE IS A MAJOR RESERVOIR OF FUNCTIONALLY DISTINCT *TRYPANOSOMA BRUCEI* PARASITES

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In the mammalian host, *Trypanosoma brucei* parasites are thought to reside mainly in the blood. Although these parasites are also present in the interstitial spaces of organs, such as brain, the extent of these extravascular sites has never been assessed. Using a mouse model, in this study, we identified the adipose tissue as a previously unknown major reservoir of *T. brucei* parasites. Histology and quantitative studies revealed that, in chronic stages of disease, there are 100-fold more parasites in adipose tissue than blood and 800-fold more than brain. Morphometric analysis of a GFP::$PAD1_{UTR}$ reporter cell-line showed that adipose tissue parasites (ATFs) can be found as slender, intermediate and stumpy forms. We also showed that ATFs are capable of infecting naïve mice, suggesting they are viable and can reestablish a blood infection. To test if parasites from adipose tissue and blood are functionally different, we performed RNA-seq of these parasites. ATFs are remarkably distinct from their blood counterparts in several key regulatory processes, including putative fatty acid –oxidation enzymes. Pulse-chase biochemical assays confirmed that ATFs are indeed able to catabolize exogenous myristate and form –oxidation intermediates, suggesting that ATF parasites can use fatty acids as an external carbon source, a behavior never previously reported for any life cycle stage of this parasite. All together, these findings identify the adipose tissue as a niche for *T. brucei* during its mammalian life cycle. In the future, it will be interesting to test if this is the cause of the weight loss associated with sleeping sickness and to investigate how such a large parasite reservoir impacts population dynamics and transmission to other hosts.

1933

ANALYZING THE CRYPTIC STATOR OF THE ATP SYNTHASE COMPLEX IN *TOXOPLASMA GONDII*

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The mitochondrial F_o-F_1 ATP synthase is a macromolecular complex present in almost every organism that couples the proton-motive force generated by respiration to synthesis of ATP. The complex can be divided in two main portions: F_1, which has the catalytic sites for ATP synthesis; and F_o, which forms a channel allowing protons to move down their electrochemical gradient. The F_o portion of the ATP synthase also contains a stator, which is needed to resist the rotational torque of F_1. In apicomplexans, little is known about the organization and function of the ATP synthase. While all the F_1 constituents have been identified, the information about the F_o subunits is fragmentary, and sequence-based searches have failed to identify any stator subunits. By performing a genome-wide CRISPR-based screen in *Toxoplasma gondii*, we identified several mitochondrially-localized proteins, unique to apicomplexans, and essential for survival in human fibroblasts. One such subunit had structural similarity to the ATP synthase b subunit, a central stator component. Tagging the protein endogenously showed that it is localized to the parasite mitochondria and that it co-immunoprecipitates with all the ATP synthase F_1 subunits, consistent with its putative role as the b subunit. Visualized by negative stain electron microscopy, the complex assumes the typical organization, as well as unusual higher-order arrangements. We are currently studying the function of the putative stator through a series of genetic and biochemical approaches. The study of the cryptic apicomplexan stator will yield new knowledge about the function of the ATP synthase in these parasites, and uncover potential therapeutic susceptibilities.

1934

TOXOPLASMA GONDII INTERACTIONS WITH THE HOST LIPID DROPLETS: RECRUITMENT, NEUTRAL LIPID SCAVENGING AND CONSEQUENCES

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Toxoplasma gondii has evolved to recruit host mammalian organelles to its parasitophorous vacuole (PV) in part to divert essential nutrients present in host organelles. We explored the role of host lipid droplets (LD) as sources of neutral lipids for the parasite. We demonstrate that host LD cluster around the PV and LD numbers increase and then decrease with infection, suggesting that *Toxoplasma* manipulates these structures. Indeed, *Toxoplasma* scavenges lipids from host LD, in part through the interception of Rab7-associated LD, and through the translocation of intact host LD into its PV. In mammalian cells, the exogenous addition of oleic acid (OA) up to 1mM is non-toxic and stimulates LD biogenesis. When exposed to 0.2mM OA, intravacuolar *Toxoplasma* profusely scavenges OA, channels this fatty acid to newly formed LD, with a concurrent increase in transcriptional activities of neutral lipid-generating enzymes However, this condition slows down both parasite replication and egress. By comparison, 0.2mM palmitic acid added in the medium does not affect parasite development whereas 1mM jasmonic acid boosts parasite growth. Our ultrastructural analyses of OA-loaded *Toxoplasma* reveal, for the first time, the presence of coated pits at the parasite's plasma membrane and additional structures potentially involved in endocytosis. More dramatically, exogenous addition of 0.4 mM OA results in the massive accumulation of lipid deposits in the PV and within parasite organelles, leading to replication defects and death. This highlights the high sensitivity of *Toxoplasma* towards deleterious effects of accumulating OA. Deciphering the lipotoxic response of the parasite may reveal new vulnerabilities amenable to controlling *Toxoplasma* infections.<!--EndFragment-->

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IDENTIFICATION OF BROADLY CONSERVED CROSS-SPECIES PROTECTIVE *LEISHMANIA* ANTIGEN AND ITS RESPONDING CD4$^+$ T CELLS

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Despite a plethora of publication on immunology of leishmaniasis, there is still no vaccine against the disease. Recovery from natural or experimental infection with *Leishmania major* induces long-term protection to reinfection collectively known as infection-induced resistance. However, it is not known what antigens induce and maintain this resistance and whether these antigens preferentially favor the development of memory T cells. To identify protective *Leishmania* antigens, we eluted and identified naturally processed *L. major* peptides from I-A^b MHC II molecules on infected BMDCs by immunoproteomics approach. One of the peptides activated *Leishmania*-reactive T cells from mice that have healed their primary *L. major* infection *in vitro*. Interestingly, the source protein of this peptide, glycosomal phosphoenolpyruvate carboxykinase (PEPCK), was expressed in both the promastigote and amastigote stages of the parasite. Also, cellular immune responses against PEPCK were detected in *L. major*-infected patients, while antibody responses were detected in infected mice, dogs and human. I-A^b-PEPCK$_{335\text{-}351}$ tetramer identified for the first time protective Leishmania-specific CD4$^+$ T cells at clonal level, which comprised ~ 20% of all Leishmania-reactive CD4$^+$ T cells at the peak of infection. PEPCK$_{335\text{-}351}$-specific CD4$^+$ T cells are oligoclonal in their TCR usage, produce polyfunctional cytokines (IL-2, IFN-- and TNF) and undergo expansion, effector activities, contraction and stable maintenance following lesion resolution. Vaccination with PEPCK peptide, DNA expressing full length PEPCK or rPEPCK induced strong durable cross-species protection in both resistant and susceptible mice. Given the remarkable effectiveness and durability of protection in vaccinated mice, our study suggests a real possibility for development of a broadly cross-species protective vaccine against different forms leishmaniasis by targeting PEPCK.

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A NOVEL POPULATION OF NATURAL KILLER CELLS PLAYS A CRITICAL ROLE IN THE DEPLETION OF SPLENIC B2 B CELLS DURING EXPERIMENTAL AFRICAN TRYPANOSOMIASIS

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Mice infected with Trypanosoma brucei, the causative agent of human sleeping sickness and a contributor to nagana in cattle, rapidly lose the capacity to mount VSG-specific antibody responses, and die with uncontrolled parasitemia. We have shown (Bockstal et al., 2011, PLOS Pathogens) that the loss of humoral immune competence in the infected mice results from depletion of developing and mature splenic B cells. We now report that T. brucei-induced splenic B cell depletion is dependent upon the presence of the pore forming molecule perforin which is present in the cytotoxic granules of cytotoxic T lymphocytes, natural killer T cells and natural killer cells, occurs in the absence of T cells (and natural killer T cells), i.e., in T cell receptor ($\alpha\beta\gamma\delta$)-/- mice, but does not occur in intact mice that are depleted of natural killer (NK) cells by treatment with monoclonal antibody specific for the NK1.1 differentiation antigen. In the intact mice, B cells are deleted after remission of the first T. brucei parasitemic wave. At this time natural killer cells are expressing the cytotoxic granule marker CD107a, indicating that they have degranulated, executing their effector function. Moreover, in vitro assays show that

B cells from T. *brucei* infected mice are killed by natural killer cells from uninfected C57BL/6 mice but not efficiently killed by CD107a positive natural killer cells isolated from infected mice, which may be functionally exhausted.

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ENDOGENOUS PHOSPHOLIPASE A2 GROUP 1B (PLA2G1B) HAS DIRECT ANTI-HELMINTH PROPERTIES AND IS ESSENTIAL FOR IMMUNITY TO *HELIGMOSOMOIDES POLYGYRUS*

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With emerging evidence of drug-resistant helminths, it is important to identify mechanisms of anti-helminth immunity to provide new avenues of therapeutic intervention. To identify novel mechanisms of immunity we compared the small intestinal transcriptome of mice that were susceptible (primary infected, *H. p.* 1°) or resistant (secondary challenge infected, *H. p.* 2°) to the evolutionally adapted murine intestinal helminth *Heligmosomoides polygyrus*. We identified distinct clusters of genes in resistant mice, some of which have previously been described, and many that have not. In particular, we identified elevated expression of lipid metabolism pathways and the lipid catabolising enzyme, Phospholipase A_2 Group 1B (*Pla2g1b*), in resistant, but not susceptible mice. Elevated expression of *Pla2g1b* was dependent upon drug-mediated killing of *H. polygyrus* and was restricted to epithelial cells of the small intestine. Importantly, elevated expression of *Pla2g1b* was critical for immunity to *H. polygyrus*, as *Pla2g1b*$^{-/-}$ mice failed to expel a challenge infection with *H. polygyrus*. Proficient immunity to *Nippostrongylus brasiliensis*, but not *Trichuris muris,* also required *Pla2g1b* suggesting preferential requirement for *Pla2g1b* in the small intestine, but not large intestine. The failure to expel *H. polygyrus* in *Pla2g1b*$^{-/-}$ mice was not due to an ineffectual or aberrant immune response. Instead we show that Phospholipase A_2 Group 1B had a direct effect on *H. polygyrus* larvae, with *in vitro* treatment of L3 larvae compromising their ability to establish *in vivo* and treatment of *H. polygyrus* larvae restoring immunity in *Pla2g1b*$^{-/-}$ mice. Together, these data indicate that endogenous epithelial cell-associated *Pla2g1b* is required for direct killing of invading larvae; revealing a previously unrecognised *Pla2g1b*-dependent mechanism of anti-helminth immunity.

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A SERUM FACTOR REGULATES SEXUAL COMMITMENT IN P. FALCIPARUM

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Sexual commitment initiates production of the transmission-competent gametocyte stage in malaria parasites. Chromatin remodeling events at the *ap2-g* locus and activation of the encoded transcription factor are the earliest steps known in this differentiation process. Interestingly, the rate of gametocyte formation is not fixed and variation is thought to depend on environmental cues.

Using an assay to probe gametocyte formation *in vitro*, we found that parasites induce sexual commitment in response to depletion of human serum components. Fractionation experiments identified lysophosphatidylcholine (LysoPC) - a major phospholipid component of serum - as the active host factor. Low micromolar concentrations of LysoPC are sufficient to prevent sexual differentiation in *P. falciparum* cultured under otherwise commitment-inducing conditions. Metabolic labeling experiments revealed that parasites readily use LysoPC as a substrate for the synthesis of other lipids, including phosphatidylcholine (PC). In the absence of LysoPC, parasites induce a switch in lipid metabolism that is accompanied by the transcriptional up-regulation of enzymes used for *de novo* synthesis of PC (Kennedy pathway). RNAseq data revealed that LysoPC-depletion further induces the expression of both known (i.e. *ap2-g*) and new markers that define the transcriptional signature of commitment. A subset of those markers, including several kinases and cell cycle regulators, are currently under investigation. Altogether, our results provide unprecedented insights on how malaria parasites integrate external stimuli into the decision-making process of sexual differentiation.

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MOLECULAR DISSECTION OF *CRYPTOSPORIDIUM* LIFECYCLE

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Cryptosporidium is a leading cause of diarrhea and an important contributor to infant mortality. Neither efficacious drugs nor vaccines are available and our knowledge of the Cryptosporidium biology to drive their development is scant. Cryptosporidium has a single-host lifecycle, and completes its asexual and sexual phases in the same host. While one model suggests that Cryptosporidium sustains continued infection of a single host through an asexual cycle, we believe progression from asexual to sexual stages to be obligatory, and favor a developmental model of continued autoinfection with sporozoites. Sex is thus a requirement of chronicity and an important target of therapy. To unravel this process at the molecular level we have developed a series of transgenic C. parvum strains that mark different stages of the lifecycle with fluorescent reporters. Using these tools we demonstrate and define asexual and sexual stages in tissue culture and infected animals and we observe mating. These strains also allow us to enrich specific stages by flow cytometry to discover sets of genes uniquely expressed at different points of the parasite's lifecycle. ApiAP2 transcription factors are key regulators of apicomplexan development making them ideal targets to disrupt lifecycle progression. We have identified and tagged ApiAP2 factors that are expressed exclusively in early trophozoites, schizonts and female gamonts of Cryptosporidium parvum, respectively. Our current work uses conditional ablation of key AP2 genes to dissect the cellular mechanisms and transcriptional regulation of sex in Cryptosporidium.

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