1

THE IMPACT OF AN INTRODUCED HOMING ENDONUCLEASE GENE ON A REGIONAL MOSQUITO POPULATION

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Homing endonuclease genes (HEGs) exist naturally in many single-celled organisms and can show extremely strong genetic drive allowing them to spread through populations into which they are introduced. They are being investigated as tools to manipulate the populations of important vectors of human disease, in particular the mosquitoes that transmit malaria. Before HEGs can be deployed, it is important to study their spatial spread in order to design efficient release strategies. We recently analysed an individual-based simulation model which demonstrated that a HEG which acts to either male-bias the mosquito sex-ratio or reduce female fecundity could potentially eliminate local mosquito populations, as reported previously. Questions remain, however, about how readily this gene-drive mechanism will spread across larger landscapes comprised of numerous interacting local mosquito populations. In this talk I present a metapopulation model which explores how gene-drive, which facilitates the spread of a HEG, interacts with the local elimination of populations, which curtails spread. The model predicts that a HEG may persistently reduce the number of mosquito populations in a region although without causing global extinction. The load imposed by the HEG increases in a correlated landscape where mosquito population sites are clumped in space. Since the HEG effectively acts as a contagious and virulent pathogen on the mosquito population, the model can be summarised as an 'SIER' (Susceptible-Infected-Eliminated-Recolonised) metapopulation model. Implications of the model to other infectious diseases are discussed.

2

OPTIMIZING FIELD RELEASES OF ENGINEERED MALE MOSQUITOES

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Control of *Aedes* spp, the mosquito vectors of the dengue virus, remains the primary approach in the prevention of dengue. However, there is a lack of effective tools for controlling the vector, emphasising the importance of novel techniques. Engineering male mosquitoes that are functionally genetically sterile and releasing them to compete with wild males for a female mate is a modern alternative to the traditional sterile insect technique (SIT). Successful implementation of this approach in the field relies on efficacious release methods. One key objective of a sterile insect release is to obtain a well-balanced distribution of the released sterile insects at the required density across the targeted treatment area. Approaches that facilitate operational optimisation of releases are investigated and applied to a range of theoretical and real field scenarios. The distribution of sterile male release points in a theoretical arena is examined, leading to further investigation of the optimal placement of release points at a field site. For field releases, the primary objective of maximising the coverage of sterile males competes with secondary, limiting objectives such as the number of mosquitoes released or the number of release points that must be visited. Multiple objective particle-swarm optimisation (MOPSO) techniques are used to investigate the trade-offs between coverage and the cost of a release using a range of measures. For large-scale transgenic sterile insect programmes, releases may be conducted from moving vehicles. MOPSO is combined with an ant-colony optimisation procedure to estimate the most efficient driven route for sterile male releases at a field site.

3

INSIGHT ON OVIPOSITION CHOICE OF *ANOPHELES GAMBIAE* SENSU LATO PRESENTS A NEW FRONT FOR VECTOR CONTROL

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The two riskiest parts of a female mosquito's life are taking a blood meal and laying her eggs. Whilst there is a considerable body of work that has demonstrated that *Anopheles gambiae* is attracted to human subjects by the volatiles produced by humans, next to nothing is known about how they locate a breeding site. Here we presented the results of a case-control study of potential breeding sites to identify the characteristics of habitats associated with the presence of *Anopheles gambiae* sensu lato, the principal African malaria vector. A cross-sectional study of aquatic habitats with anopheline larvae (cases) and without larvae (controls) was carried out in Rusinga Island, on Lake Victoria, Kenya. Factors evaluated include biological characteristics of the sites, zooplankton, invertebrate fauna, physical parameters, nutrients, bacteria communities and volatile chemicals released from the water. Characteristics of 120 habitats (74 cases and 46 controls) were analyzed between March and July 2012. Data were analysed using a random forest model. The presence of early instar larvae was associated with habitats located within 100 meters of the lake. Preferred habitats of *An. gambiae* s.l. were characterised by increasing content of phosphates, conductivity and turbidity beyond 200 NTU. Late instar culicines and small crustaceans of the orders Cyclopoida and Cladocera were also abundant in these sites. Contrary to previous report invertebrate predators of the orders Odonata, Coleoptera and Heteroptera were common in anopheline habitats. Volatile chemicals released from the water headspace were less diverse and released in lower concentration from cases than from controls. This study demonstrates that *Anopheles gambiae* select breeding sites with specific characteristics These factors can potentially be used to target breeding habitats for larval control or manipulated to attract and kill gravid females.

4

HIGH LINKAGE DISEQUILIBRIUM IN *ANOPHELES GAMBIAE* FROM KILIFI, KENYA IS CONSISTENT WITH A RECENT REDUCTION IN POPULATION SIZE

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We used RADseq to genotype ~6000 SNPs in An. gambiae collected from Kilifi, Kenya and Muheza Tanzania. Linkage disequilibrium (LD) was higher but genetic diversity only slightly lower in Kilifi compared with Muheza. We investigated the relationship between rho (population recombination parameter) and theta (genetic diversity). In the Kilifi samples the ratio rho/theta departed from neutral expectations. Simulations showed that a recent, severe population reduction gives a significant reduction in rho without an equivalent change in theta. This is consistent with the rho/theta observed in Kilifi. It has been previously observed that abundance of An. gambiae has been reduced in the Kilifi district in the last 10-15 years; our results suggest that the population size change can be detected in the population genomics of the remaining mosquitoes. This result shows that monitoring linkage disequilibrium in vector populations may be an effective way of tracking recent changes in population size, due to mosquito control interventions or climate change.

5

UNBIASED CHARACTERIZATION OF *ANOPHELES* MOSQUITO BLOODMEALS BY TARGETED HIGH-THROUGHPUT SEQUENCING OF 16S RRNA GENES

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Understanding mosquito blood feeding behavior is important for assessing vector competence or identifying possible reservoir hosts. To date, there is no unbiased method of evaluating mammalian host bloodmeals from mosquitoes and most current molecular assays are designed to test whether a mosquito fed on *a priori* selected species. Here, we describe a targeted high-throughput sequencing method that utilizes a universal primer pair, which amplifies mammalian mitochondrial 16S ribosomal genes, to identify host bloodmeals from female mosquitoes. Our assay enables analyzing 96 mosquitoes simultaneously and, with more than 1,000 sequences generated per mosquito, provides a comprehensive and quantitative perspective on each blood meal composition. We applied our approach to 529 blood-fed female *Anopheles* collected from five villages in Papua New Guinea. 483 mosquitoes (91%) yielded a successful PCR product and, after sequencing, showed that these *Anopheles* mosquitoes fed almost exclusively on humans, dogs and pigs. Interestingly, 68 mosquitoes (14%) showed clear evidence of having fed on two or more species. In one village, where similar number of mosquitoes were collected on both sides of an erected barrier screen, we observed that mosquitoes fed more often on humans on the village versus the bush side of the net (p=0.001). Our analyses also showed that *An. farauti s.s* . fed more often on humans in the village of Matukar than in Mirap (p=8.7e-9), revealing potential behavioral differences between these populations. Overall, our study shows that this assay enables us to objectively identify host bloodmeals from female *Anopheles* mosquitoes and to discover feeding behavior differences. This approach is generalizable to any insects that feed on mammalian blood and can be applied to improve our understanding of a wide variety of infectious diseases.

6

SOUTH AUSTRALIA'S FIRST LARGE SCALE ARBOVIRUS SURVEY: SPATIAL ANALYSIS OF SENSITIVE FTA® CARD METHOD

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Every year in Australia, over 6000 people become infected with a mosquito-borne virus (arbovirus) including Ross River virus (RRV) and Barmah Forest virus (BFV), among others. The most prevalent of these viruses is RRV which causes prolonged symptoms of fatigue, muscle soreness and polyarthritic joint pain and costs Australians tens of millions annually in diagnosis, prevention and treatment. Approximately 250 cases of human infection are reported every year in the state of South Australia. Until recently, the methods for conducting arbovirus surveillance made large scale surveys logistically and economically difficult. A modern development has capitalized on the nectar-feeding behaviour of mosquitoes to collect abroviruses on nucleic acid-preserving cards (FTA® cards), thus streamlining the virus surveillance process. We adapted this technique to an existing mosquito surveillance program and conducted the first ever large-scale arbovirus survey in South Australia. During two months of the peak virus season (January/February 2014), we set CO2-baited EVS light traps at 100 locations around South Australia with a honey-baited FTA® card inside. After collection, mosquitoes were kept in a humid environment and given one week to feed on the honey-soaked card. Cards were then tested for arboviruses using a nested PCR. We made 23 virus detections from around South Australia including 14 of RRV, 6 of BFV and 3 of Stratford virus (not previously reported from South Australia). This level of detection suggests that our low-budget method is highly sensitive at detecting infectious mosquitoes. In this presentation, we report on our methods, the resulting detections (including pending results from March) and the mosquitoes associated with the detections. We also will present a spatial analysis of how these detections correlate with human case data from the last 20 years and explore the implications of our method and findings.

7

IDENTIFICATION OF PROXIMATE INDICATORS OF MALE MATING PERFORMANCE IN *ANOPHELES GAMBIAE*

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Releases of male mosquitoes for population control or replacement strategies rely on those males successfully mating with wild females, in competition with wild male counterparts. Currently performance of strains is tested in competition assays, in laboratory cages then in increasingly large and complex mating arenas. The extent to which these results reflect how well a strain will perform in the field is unclear. A quick, small-scale assay to predict which strains will fail to meet the required standard to compete with wild males would save time and cost in this lengthy process. I have optimised a method to measure the mating performance of single male *Anopheles gambiae*, allowing the characteristics of more and less successful males to be compared on an individual basis. Cohorts of males were were reared, selected or categorized according to characteristics thought to be important for the mating success or competitiveness of male mosquitoes, for example longevity or size. The relative performance of phenotypically distinct males was measured, and these correlations validated in competition assays on a larger scale, in an attempt to identify those male characteristics that are most predictive of male performance. I have also addressed through competition assays the question of whether assortative mating occurs based on, for example, adult size. If evidence for this is seen it would indicate that an understanding of the wild female population and the production of compatible males would give an advantage over the current strategy of simply producing the largest and nutritionally best prepared release cohort possible under mass rearing conditions.

8

CHIKUNGUNYA INCIDENCE AND CORRELATION WITH PROTECTION IN A PROSPECTIVE LONGITUDINAL COHORT IN THE PHILIPPINES

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Chikungunya is a re-emerging arboviral disease that has caused increasing epidemics and recently appeared for the first time in the Americas. However, the true incidence and clinical spectrum of chikungunya virus (CHIKV) infection have not been well established. In addition, although CHIKV antibodies have been suggested as being protective, no correlation with protection has been demonstrated in humans. In March 2012, we initiated a longitudinal prospective cohort of approximately 1000 subjects aged ≥6 months in Cebu, Philippines, which underwent community-based active surveillance for febrile episodes. Acute and 3-week convalescent blood samples were obtained and tested by CHIKV RT-PCR and CHIKV hemagglutination inhibition assay (HAI). Enrollment and 12-month follow up samples were tested by CHIKV HAI to identify subclinical

seroconversion. During one year of surveillance, the annual incidence of total and symptomatic CHIKV infection in the cohort was approximately 8% and 2%, respectively. The total and symptomatic incidence in the 6 month-5 year old age group was 7% and 3%; 6-15 years was 8% and 4%; 16-30 years was 9% and 1%; 31-50 years was 9% and 1.5%; >50 years was 5% and 0.5%. The total and symptomatic incidence among 672 subjects with negative CHIKV HAI titer (≤10) at enrollment was 9% and 2.5%; and among 181 subjects with positive CHIKV HAI titer (>10) was 0.5% and 0%. Subjects with negative CHIKV HAI at enrollment were more likely than those with positive HAI to have CHIKV infection (Fisher's exact, p=0.0001) and symptomatic CHIKV infection (Fisher's exact, p=0.019). Our results demonstrate that CHIKV infection is a common endemic infection across all age groups in the Philippines, but a greater proportion of those ≤15 years old are symptomatic. CHIKV HAI titer >10 is correlated with protection against both symptomatic and subclinical CHIKV infection. Our findings have important implications for assessment of CHIKV disease burden, understanding of virus transmission, and development of candidate vaccines.

9

HIGH RATES OF O'NYONG NYONG AND CHIKUNGUNYA VIRUS TRANSMISSION IN COASTAL KENYA

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Chikungunya virus (CHIKV) and o'nyong nyong virus (ONNV) are mosquito-borne alphaviruses endemic in Kenya that cause acute febrile illness and arthritis. The objective of this study was to measure the seroprevalence of CHIKV and ONNV in coastal Kenya and link it to demographics and other risk factors. Demographic and exposure questionnaires were administered to 1,862 participants recruited from two village clusters (Milalani-Nganja and Vuga) in 2009. Sera were tested for alphavirus exposure using standardized CHIKV IgG ELISA protocols and confirmed with plaque reduction neutralization tests (PRNT). Logistic regression models were used to determine the variables associated with seropositivity. G statistic and kernel density mapping were used to identify locations of high seroprevalence. Weighted K test for global clustering of houses with alphavirus positive participants was performed for distance ranges of 50-1,000 meters. 486 (26%) participants were seropositive (1-79 years, mean 27 years). Of 443 PRNT confirmed positives, 24 samples (5%) were CHIKV, 246 samples (56%) were ONNV, and 173 samples (39%) had equally high PRNT titers for both CHIKV and ONNV. Age was significantly associated with seropositivity (OR 1.01 per year, 95% C.I. 1.00-1.01). Males were less likely to be seropositive (p<0.05; OR 0.79, 95% C.I. 0.64-0.97). Adults who owned a bicycle (p<0.05; OR 1.37, 95% C.I. 1.00-1.85) or motor vehicle (p<0.05; OR 4.64, 95% C.I. 1.19-18.05) were more likely to be seropositive. Spatial analysis demonstrated hotspots of transmission within each village and clustering among local households in Milalani-Nganja, peaking at the 200-500m range. Alphavirus exposure is common in coastal Kenya with ongoing interepidemic transmission of both ONNV and CHIKV. Women, adults and those with higher socioeconomic status were more likely to be seropositive. Household may be one of the defining locations for the ecology of alphaviral transmission in this region, given our spatial analysis results and the fact that anophelines transmit ONNV. Human disease caused by ONNV and CHIKV in this region is being missed in clinical settings.

10

DETERMINANTS OF CHIKUNGUNYA EMERGENCE POTENTIAL IN SOUTH FLORIDA

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Arbovirus transmission is dependent on the transmission efficiency between infectious and susceptible hosts. Heterogeneity of virus acquisition from a viremic human to a susceptible mosquito is often assumed to be nearly perfect and almost always uniform across the infectious period. Chikungunya transmission models often do not account for the variation in infectiousness of a single individual, and subsequent infection of naïve mosquitoes. Understanding the contribution of human infectious heterogeneity is especially important in the context of introduction events where an infected individual carries the virus into a population(s) of competent vectors. We construct a stochastic, compartmental model to describe the heterogeneity of human viremia and calculate the probability of a successful introduction, taking into account the viremia level (and thus acquisition potential) of the index case on, and after, the day of introduction into a susceptible population. We inform our model based on the experience of dengue emergence in South Florida, 2009-2013. We further account for viral genotype and predominant vector (Ae. aegypti versus Ae. albopictus) by altering the transmission efficiency rate between the human and specific mosquito populations by exploring 1) differential vector competence between genotypes of chikungunya and 2) differential contact rates within and between the two mosquito populations. We find that the infectivity of the index case as well as the parameters affecting transmission efficiency affected the probability of emergence, but that the effects among these parameters were not equal. To predict the likelihood and magnitude of a potential chikungunya outbreak in the United States, it is critical to understand the interplay between individual human heterogeneity of infectiousness and the transmission efficiency of the virus and vector populations.

11

PRECLINICAL EVALUATION OF A LIVE ATTENUATED CHIKUNGUNYA VACCINE

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Recently, Chikungunya virus (CHIKV) has re-emerged to cause major epidemics in the Indian Ocean, India and Southeast Asia, infecting millions. Autochthonous transmission in Italy and France after CHIKV introductions via travellers and the recent outbreak in the Caribbean highlight the threat of wide spread chikungunya transmission. We used a molecular attenuation approach, inserting a picornavirus internal ribosome entry site (IRES) to replace the subgenomic promoter, to generate a live attenuated vaccine strain (CHIKV/IRES). The CHIKV/IRES vaccine provides robust immunity and protection in murine models, and is incapable of infecting mosquito vectors, an important safety feature for use in nonendemic locations. The CHIK vaccine elicited a robust memory $CD4^+$ and $CD8^+$ T cell response. However, adoptively transferred immune T cells did not protect against wtCHIKV-LR challenge. In contrast, passive immunization with anti-CHIKV/IRES immune serum provided protection, and a correlate of a minimum protective neutralizing antibody titer was established. Vaccination of cynomolgus macaques with a single dose produced no signs of disease but was highly immunogenic. After challenge with wild-type CHIKV, the vaccine prevented the development of detectable viremia and signs of clinical CHIKV disease. We also have established processes and assays for high-titer manufacture at a large scale. The CHIKV/IRES

vaccine candidate is safe, immunogenic and efficacious in multiple animal models, supporting its potential as a human vaccine to protect against CHIKV infection and reduce transmission and further spread. Current pre-clinical development efforts are aiming to complete the Investigational New Drug (IND)-enabling studies necessary to begin human clinical testing.

12

CLINICAL RESULTS OF NOVEL CHIKUNGUNYA VACCINE TESTED IN PHASE 1/2 TRIAL: NEUTRALIZING ANTIBODIES AND ANTI-VECTOR IMMUNITY

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We show here a first in man phase - 1/2 clinical trial to demonstrate the immunogenicity, safety and tolerability of a novel chikungunya virus vaccine. Chikungunya Virus is an emerging mosquito borne virus that causes a severe polyarthritis in humans. The virus is endemic in nearly 40 countries in Asia, Africa, Europe, and recently also in the Americas posing a major threat to public health. So far there is no vaccine approved for human use that prevents the disease. Nucleotide sequences encoding the Chikungunya virus capsid and envelope structural proteins were inserted into the Schwarz vaccine strain of measles virus to produce the candidate vaccine MV-CHIK. To evaluate the optimal dose of MV-CHIK in regard to immunogenicity, safety and tolerability we performed an observer blinded, block-randomized, active and placebo-controlled, dose escalation, phase 1 trial in 42 healthy volunteer subjects. All subjects received three i.m. injections (days 0, 28, 90), the first injection was a vaccine. Subjects were block-randomized to MV-CHIK or to the control-vaccine Priorix® (MV-CHIK: n=12, Priorix: n=2/ Cohort). All volunteers were additionally randomized to one of two treatment sequences, vaccination in day 0 and 28 or 0 and 90. The Immunogenicity on day 28 after vaccination was confirmed by the presence of functional antibodies as determined by the plaque reduction neutralization test (PRNT50). Preliminary findings point at excellent safety and immunogenicity profile in all three doses tested. Data are currently under final evaluation and auditing, and will be presented here for the first time.

13

A MULTIAGENT ENCEPHALITIC ALPHAVIRUS DNA VACCINE DELIVERED BY ELECTROPORATION ELICITS PROTECTIVE IMMUNITY AGAINST AEROSOL CHALLENGE WITH EASTERN AND WESTERN EQUINE ENCEPHALITIS VIRUSES IN NONHUMAN PRIMATES

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Next-generation vaccines that can safely and effectively protect against Venezuelan, eastern, and western equine encephalitis virus (VEEV, EEEV, and WEEV) are needed. Previously, we demonstrated that monovalent or a trivalent combination of DNA plasmids expressing codon-optimized envelope glycoprotein genes of VEEV, EEEV, or WEEV delivered by intramuscular (IM) electroporation (EP) elicited high levels of virus-neutralizing antibodies in multiple animal species and provided protective immunity against lethal aerosol homologous viral challenge in mice. Currently, we have completed studies to further assess the monovalent and trivalent DNA vaccines delivered by IM EP in nonhuman primate (NHP) models of aerosol EEEV and WEEV challenge. Although some neurological signs of disease were observed after aerosol EEEV challenge in three of four NHPs that received the monovalent EEEV DNA vaccine, these were of lesser severity than those observed in the negative control animals and all survived. Interestingly, no significant clinical signs of disease were observed after challenge in the NHPs that received the trivalent DNA vaccine. While one of four NHPs that received the monovalent WEEV DNA vaccine displayed clinical signs of disease similar to those observed in the majority of the negative control animals after aerosol WEEV challenge, the remaining animals from this group only displayed mild clinical manifestations. Similar to results observed for the EEEV NHP challenge study, no significant clinical signs of disease were observed in any of the NHPs that received the trivalent DNA vaccine. We are currently investigating the apparent synergistic protective effect achieved with the trivalent DNA vaccine in further detail. Taken together, the results of our current studies further demonstrate that IM EP delivery of a multiagent formulation of VEEV, EEEV, and WEEV DNA vaccines represents a potent means of protecting against aerosolized encephalitic alphavirus infections and support its continued development into a mature vaccine candidate suitable for future clinical testing.

14

A MURINE MODEL FOR ACUTE AND CHRONIC, FLARING CHIKUNGUNYA VIRUS-INDUCED DISEASE

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Chikungunya virus (CHIKV) has gained notoriety in recent years with the explosive pandemic outbreak that has afflicted of over six million people in Africa, Indian Ocean, Asia, southern Europe, and recently the Caribbean and South American mainland. CHIKV is a mosquito-borne, enveloped, single-stranded, positive-sense RNA virus that causes fever, rash, and chronic polyarthralgia/polyarthritis in humans, often accompanied by incapacitating joint and muscle pain that can last anywhere from weeks to years. Currently there are no licensed vaccines or antiviral therapies against CHIKV. The development of a murine model that mimics both the acute and chronic, flaring phases of disease is critical to our understanding of this disease but has proven difficult. Current murine models mimic features of the acute phase but overt chronic disease has not been observed. To improve the model, we adapted to mice a wild-type strain of CHIKV by *in vivo* passage in musculoskeletal tissue. During the acute disease, the mouse-adapted strain of CHIKV causes swelling of both the inoculated and opposite footpad, which does not occur with the wild-type CHIKV in immunocompetent mice. Furthermore, mouse-adapted CHIKV induced chronic disease in the mice with flaring of musculoskeletal disease and swelling around three weeks post infection with chronic swelling lasting for months. Preliminary data indicate that markers of human chronic CHIKV disease are also present in our model (e.g. CCL2 and CXCL8). Recent identification of a single nucleotide change that confers this phenotype suggests possible mechanisms by which chronic disease may arise which are currently being investigated. Importantly, this model will also aid in the testing of candidate vaccines and antiviral therapeutics targeting long-term sequelae of CHIKV infection.

15

A MOUSE MODEL OF HIV AND CEREBRAL MALARIA CO-INFECTION

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Experimental cerebral malaria (ECM) models are controversial because leukocytes and platelets accumulate in rodent brain vasculature, but sequestration of parasitized red blood cells as described in human cerebral malaria (CM) has been inconsistently observed. We have found that HIV is a risk factor for pediatric CM and that intravascular platelets and monocytes are prominent in the brains of children with fatal CM. We revisited the ECM model to study co-infection, infecting mice transgenic for a HIV-1 provirus and for the human cyclin T1 promoter with *Plasmodium berghei* ANKA. 56 mice (31 transgenic "HIVJR-CSF", 25 littermate controls) age 6 weeks-4 months were infected IV with 105

P. berghei ANKA. Mice were assessed for development of CM daily using a standard behavioral scale and were sacrificed when severely impaired. Mice that did not develop CM were sacrificed when they became less active and severely anemic. Brains were harvested for histopathologic analysis. All mice developed peripheral parasitemia. During early infection HIVJR-CSF mice had higher mean parasitemia than controls (0.79% vs. 0.32% on day 4 post-infection, p=0.014), but there was no difference by day 7 when signs of CM develop. HIV viral load increased during malaria infection, from 80,000 copies/ml at baseline to 1.7 million copies/ml on day 7 (p=0.05). Evaluation of H&E stained sections on a subset of mice with CM found that all control mice and only 41% of HIVJR-CSF mice had ≥2 microhemorrhages/100 high power fields while 33% of HIVJR-CSF mice had no visible hemorrhages, similar to pathology patterns noted in our pediatric CM studies. Quantification of intravascular platelets and monocytes is ongoing. While there was no difference in incidence of CM (68% for HIVJR-CSF mice and 52% for controls, p=0.23) in young mice, older adult HIV transgenic mice continued to develop CM, unlike age-matched littermates. The mouse model may be a powerful tool to understand the pathophysiology of pediatric CM and HIV co-infection.

16

CD47 MEDIATED PARASITE CLEARANCE IN *PLASMODIUM YOELII* MALARIA

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Many *Plasmodium* species, including a few human malarias that cause a relatively less severe infection have a strong preference to invade and grow inside young red blood cells (RBC). We utilized the murine GFP-*Plasmodium yoelii* 17XNL, which predominantly invades young RBC, to identify the host factors responsible for the preferential RBC infection and a possible mechanism for the potential cellular advantage for this preferential infection. CD47 or Integrin Associated Protein is a marker of self on most cells including RBC that, in conjugation with its receptor SIRP-α, prevents the phagocytosis of RBC. By injections of biotin in C57Bl/6 mice, we were able to differentiate between the young and old RBC by flowcytometry. On day 9 post infection (PI) with GFP-PyNL, young RBC had significantly higher parasitemia than old RBC (young RBC 45.1 ± 11.8 vs. old RBC 1.05 ± 0.8, p<0.001) and old RBC, young RBC had significantly higher CD47 (mean fluorescence intensity, young RBC 2373 ± 139 and old RBC 1156 ± 24, p<0.001). Importantly, infected RBC had higher CD47 levels than non-infected RBC throughout the course of GFP-PyNL infection and higher CD47 levels on RBC also associated with the higher parasitemia. C57Bl/6 $CD47^{-/-}$ mice resolved their infection at an accelerated rate (wt on 17 PI vs. $CD47^{-/-}$ on 15 PI) and developed significantly lower parasite burden (day 9, 2.3 ± 0.7 % vs. 26.48 ± 3.9 %, p<0.001). FACS analysis revealed a higher percentage of splenic F4/80 population in $CD47^{-/-}$ mice (day 7, 4.2 ± 0.4% $CD47^{-/-}$ vs. 2.3 ± 0.9 % wt , p< 0.05) that also had a higher percentage of phagocytized infected RBC (13.6 ± 1.7 % CD47-/- vs. 3.7 ±0.7 % wt, p<0.01). Furthermore, Injection of CD47 neutralizing antibody caused a significantly reduction in parasite burden in wt C57Bl/6 mice. Together, these results strongly suggest that CD47 is important for parasite growth and survival and, through the mechanism of lower phagocytic clearance of infected RBC, may be mediator of immune escape to avoid the splenic clearance of infected RBC.

17

PLASMODIUM SPOROZOITES DIRECTLY TARGET HEPATOCYTE EPHA2 RECEPTOR FOR SUCCESSFUL HOST INFECTION

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After mosquito transmission, selection of a suitable host hepatocyte to support the intracellular liver stage malaria parasite is an essential early step in infection. Yet, the molecular recognition conduits by which invasive *Plasmodium* sporozoites select a suitable target cell remain largely unknown. Here, we show that sporozoites engage the hepatocyte EphA2 receptor for infection, preferring $EphA2^{high}$ hepatocytes as their hosts. This is mediated by a direct interaction between the parasite ligand P52 and EphA2, which occurs at the point of invasion and is critical to establish a permissive intracellular replication niche. When EphA2 is deleted, liver stage infection is dramatically reduced in a mouse model of malaria. Deletion of P52 in the parasite mirrors this host phenotype resulting in the loss of selectivity for hepatocytes that express high levels of EphA2 and are permissive to productive parasite development. Taken together, the data provide mechanistic insights into host cell selection at the point of infection.

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GLUCOSE STARVATION REVEALS EVIDENCE OF A NOVEL FATTY ACID OXIDATION PATHWAY IN *PLASMODIUM FALCIPARUM*

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Plasmodium falciparum has finely tuned metabolic pathways to support growth in the various nutrient environments of its complex lifecycle. Despite its metabolic importance, relatively little is known about fatty acid (FA) metabolism in *Plasmodium*. Using ^3H hypoxanthine incorporation assays and SYBR Green-based FACS, we show reduced growth of 3D7 in limited FA media, and titrating glucose and glutamine to 40-45% of normal levels causes additional growth restriction. Knockdown of members of the acyl CoA synthetase (ACS) gene family further reduces growth in restricted glucose and FAs, an intriguing finding, as the ACS enzymes are known to mediate FA scavenging and activation. This observation prompted us to investigate a metabolic link between the exogenous FA substrates of ACSs and carbon sources used to generate ATP in the parasite. We determined that glucose-deprived parasites exhibit FA oxidation, as measured by release of $^3H_20$ from parasites grown in ^3H labeled FAs. Following two cycles of glucose restriction, there was an 18-24 fold increase in FA oxidation as compared to uninfected red blood cells. Parasites grown in the standard glucose concentration of RPMI1640 did not exhibit FA oxidation, suggesting that typical *in vitro* culture conditions mask this metabolic phenomenon. To our knowledge, this is the first reported experimental evidence for FA oxidation in *Plasmodium*. The canonical FA oxidation enzymes are not annotated in *P. falciparum*, though the pathway is believed to be present in *Toxoplasma gondii*. Our data suggests that glucose restriction redirects FAs into a beta-oxidation pathway to generate ATP. Furthermore, this important role for exogenous FAs offers a potential explanation for the previously reported expansion and positive selection of the ACS gene family in *P. falciparum*. Ongoing experiments will reveal the physiological importance of this catabolic pathway and shift in energy metabolism. Additional characterization of the molecular mechanisms may reveal novel targets for development of antimalarials.

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GENOMIC ASSESSMENT OF *PLASMODIUM FALCIPARUM* POPULATION STRUCTURE AND DIVERSITY DURING AN ENHANCED MALARIA CONTROL PROGRAM IN WESTERN KENYA

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Malaria Control and Elimination Partnership in Africa is working toward aggressive malaria transmission reduction in Siaya County (western Kenya). The application of population genetic approaches to understand and estimate transmission dynamics in the context of malaria elimination in this setting will be crucial. To monitor changes in *Plasmodium falciparum* population structure in this population, we are applying estimates of parasite genetic diversity and relatedness, and assessing key biomarkers, such as those related to drug resistance that may impact efforts to reduce the malaria burden toward eradication. Additionally, we aim to provide a genetic map of parasite movement between Siaya County, and other parts of Kenya (e.g., Nairobi and central regions) and eastern Uganda where substantial movement of parasites have been spatially quantified through population movement studies.

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SUSCEPTIBILITY WEIGHTED IMAGING IN PEDIATRIC CEREBRAL MALARIA AT 1.5T

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The pathogenesis of Pediatric Cerebral Malaria (PCM), a leading killer of children in sub-Saharan Africa, is unknown. The pathological hallmark of the disease is sequestration of parasitized red blood cells in brain microcirculation. Intravascular hemozoin, a ferromagnetic breakdown product of hemoglobin metabolism and microhemorrhages in various structures, including the white matter and grey-white junction, have been reported. We imaged Zambian children with cerebral malaria on a 1.5T magnet to investigate the distribution of iron breakdown products from hemoglobin metabolism and BBB breakdown. Twelve children with strictly defined PCM (peripheral blood slide positive and retinopathy positive) were scanned at 1.5T. Eleven received intravenous gadolinium enhancement. SWI (susceptibility weighted imaging), T2, T1 pre and post, DWI with ADC and FLAIR imaging sequences were obtained. The children had mild PCM: none died and none had severe brain swelling. Normal intravascular gadolinium enhancement was noted but there was no parenchymal enhancement to suggest BBB breakdown. SWI imaging confirmed the present of a ferromagnetic substance in distributions similar to those of parasite sequestration and hemozoin seen at autopsy. A ferromagnetic substance, possibly hemozoin, the metabolic end product of hemoglobin digestion, was identified. Additional studies using R2* and SWI will assist in quantifying hemozoin, and parasite sequestration distribution *in vivo*. In this small population of non-fatal PCM cases, extravasation of contrast indicative of BBB breakdown was not evident using 1.5T MRI. Additional evaluations with dynamic enhancement techniques are warranted to further assess the integrity of the BBB, as this has relevance to adjuvant treatment strategies.

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SEVERE AND CEREBRAL MALARIA IN CHILDREN LEADS TO A SIGNIFICANT IMPAIRMENT OF TRANSITORY OTOACOUSTIC EMISSIONS - A MULTICENTER PROSPECTIVE STUDY

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Lately, severe and especially cerebral malaria have been suspected to influence the inner ear function. Cerebral malaria has been associated with hearing loss in 9 of 23 patients, as reported previously. However, prospective studies evaluating the inner ear function in severe and cerebral malaria are missing. An objective way to quickly evaluate the inner ear function is to measure otoacoustic emissions - a technique which is used worldwide to screen newborns for potential hearing impairment. This prospective multicenter study analyses the function of the inner ear in patients with severe and cerebral malaria up to the age of 10 years. In three study sites (Ghana, Gabon, Kenya) 144 patients with severe and cerebral malaria and 108 age-matched local control children were included. All patients were treated with artemisinin combination therapies. Of the severe malaria patients 42 % failed the initial otoacoustic emissions testing at the baseline measurement, i.e. prior to therapy, on day 28 only 27.1%. The cerebral malaria group showed a comparable number of failures with 38 % at the baseline. In contrast to the severe malaria the number of fails increased up to 66 % at day 7 after diagnosis. A slight improvement to 55% fails could be noted on day 28. Negative transient otoacoustic emissions are associated with a threshold shift of 20 dB and above. The present data shows a pathologic involvement of the inner ear in severe and cerebral malaria of around 40% prior to the initiation of schizontocidal therapy, a figure which improves in severe malaria but deteriorates over the next weeks in cerebral malaria. Therefore, especially in children with cerebral malaria, hearing screening and post-malaria audiologic work up has to be recommended after severe malaria infection.

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ARTESUNATE-MEFLOQUINE VERSUS CHLOROQUINE IN PATIENTS WITH ACUTE UNCOMPLICATED *PLASMODIUM KNOWLESI* MALARIA: AN OPEN-LABEL RANDOMIZED CONTROLLED TRIAL IN SABAH, MALAYSIA (ACT KNOW TRIAL)

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Plasmodium knowlesi is the most common cause of malaria in Malaysia, and has been reported throughout South-East Asia. There are no recommendations in the current 2010 WHO malaria treatment guidelines for the optimal treatment of uncomplicated knowlesi malaria, which replicates every 24 hours and has the potential to cause severe disease and death. *P. knowlesi* is commonly microscopically misreported as other *Plasmodium* species, including *P. falciparum* and *P. vivax,* a high proportion of which are chloroquine-resistant in Malaysia. Artemisinin combination

therapies (ACT) and chloroquine have each been used to successfully treat *P. knowlesi* malaria in the past, however a unified ACT treatment protocol would support effective blood stage malaria treatment for all *Plasmodium* species. Malaysia's national policy for malaria pre-elimination involves mandatory admission for all patients with confirmed malaria, therefore a more rapidly acting anti-malarial agent also has health cost benefits. ACT KNOW, the first RCT ever performed in knowlesi malaria, is a 2-arm open label trial with enrolments over a 2-year period at 3 district sites in north-western Sabah, powered to show a difference in proportion of patients negative for malaria by microscopy at 24 hours between the treatment arms (clinicaltrials.gov #NCT01708876). Enrolments commenced in December 2012 and are expected to be completed by September 2014. As at March 2014 a total of 153 patients meeting inclusion criteria have been enrolled, with a total sample size of 228 required to give 90% power (alpha 0.05) to determine the primary endpoint using intention-to-treat analysis. Secondary endpoints include parasite clearance time, rates of recurrent infection/treatment failure to day 42, gametocyte carriage throughout follow-up, and rates of anemia at day 28, as determined by survival analysis. Results will be presented at the 2014 ASTMH annual meeting.

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REPEATED TREATMENT OF YOUNG CHILDREN 4-11 MONTHS WITH ARTEMETHER-LUMEFANTRINE AND DIHYDROARTEMISININE-PIPERAQUINE OVER A TWO-YEAR FOLLOW UP PERIOD IN REAL-LIFE SETTINGS: NEURO-OTOTOXICITY DRUG SAFETY ASSESSMENTS

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Artemisinin-combination therapies (ACTs) are widely seen as safe drugs. Early animal data of artemisinins raised the possibility of neurotoxicity with high doses of the lipophilic (oil-based) derivatives such as arte(m) ether in the form of selective damage of the brainstem involved in auditory and vestibular function, and recent animal data suggested the possibility of a cumulative effect. While the implication for human toxicity is controversial and audiology assessments in adults in Asia and single-use trials in older children over 5 years in Africa have been reassuring so far, this has never been confirmed in the most vulnerable group of young children during a phase of substantial brain maturity and high drug exposure. We conducted a cluster-randomized effectiveness trial in an area of high malaria transmission in Malawi. 814 children aged 4-11 months, recruited at community-level, were randomized at village level into either receiving artemether-lumefantrine (ArLu, lipophilic) or dihydroartemisinin-piperaquine (DHA-PPQ, hydrophilic), for any consecutive clinical malaria episodes during a 2 year follow-up period, in a 'real-life'setting. To explore any neuro-ototoxic adverse effect of repeated ACT exposure, two audiology safety components were conducted. Short-term and potentially reversible effects were assessed in a subset of 176 clinical malaria episodes with auditory brainstem response (ABR) tests before treatment on Day 0, and day 7 and day 42 post treatment. Long-term irreversible, cumulative effects were assessed with ABR readings at trial baseline, mid-point and end-of-study. Primary ABR endpoint was the interpeak latency between wave I and V(IPL). Overall trial analyses are ongoing and will be finalized by September. We will present the intention-to-treat and per-protocol analyses results, adjusting for treatment exposure, artemisinin intake dose, child age, concomitant treatments, and history of exposure of known determinants of audiological changes, including relevant birth and disease history, and other reported exposure to known ototoxic drugs.

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BOUTS OF MALARIA ILLNESS AS MEDIATED BY ANEMIA DIMINISHES COGNITIVE DEVELOPMENT IN VERY YOUNG UGANDAN CHILDREN

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We evaluated child development in an open-label RCT of anti-malarial chemoprevention in children living in an area of intense, year-round transmission in Eastern Uganda. Infants 4 to 5 months of age were enrolled and randomized to one of four treatment arms: no chemoprevention, monthly sulfadoxine-pyrimethamine (SP), daily trimethoprim-sulfamethoxazole (TS), or monthly dihyrdroartemisinin-piperaquine (DP). At 2 yrs of age chemoprevention was stopped and children were followed for 1 additional year. Number of malaria episodes and anemia (hgb<10) were summarized up to cessation of chemoprevention (2 yrs of age) and again from 2 to 3 yrs of age. The Mullen Early Learning Scales (MELS) was administered to 471 children at 2 yrs and 452 children at 3 yrs. 69% were HIV-unexposed and 31% were HIV-exposed, equally distributed across treatment arms. The number of malaria episodes differed at the end of chemoprevention by trial arm (P <.0001) with DP having a high protective efficacy against malaria and anemia; TS having a moderate protective efficacy against malaria only, and SP offering no protection against malaria or anemia. Following cessation of chemoprevention, all treatment arms displayed levels of malaria (~7.5 episodes per year) and anemia comparable to that of the no chemoprevention arm. To determine the effects of the number of malaria episodes on cognitive development, linear mixed effects model related 2 repeated measures of Mullen scores (at 2 and 3 yrs) to trial arm, HIV exposure status, sex, socio-economic status, weight for age z-score, duration of breast feeding, and the number of malaria episodes in each year. Trial arm was not significantly related to any of the Mullen development outcomes. The number of bouts of malarial illness was significantly predictive of Mullen cognitive outcomes both at 2 and 3 yrs of age (P = 0.02). This relationship was mediated by the number of anemia episodes. Anemia episodes over and above those related to malaria was independently associated with poorer Mullen working memory (P = 0.02). HIV exposure was associated with lower Mullen receptive language development over and above malaria and anemia. We are the first to document within an RCT study that early malaria illness and anemia can lead to adverse developmental outcomes in very young children. Concurrent intervention for both of these risk factors in early childhood is important for enhancing developmental outcomes in at-risk children.

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SEASONAL MALARIA CHEMOPREVENTION AND MICRONUTRIENT SUPPLEMENTATION IN EARLY CHILDHOOD: EFFECT ON ASYMPTOMATIC PARASITAEMIA, ANEMIA AND COGNITION

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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 trial 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 be associated with improvements in malaria morbidity, anemia and/or physical growth, the benefits for cognitive and linguistic development are comparatively unknown. The combined effect of these two interventions has not previously been examined. A cluster-randomized controlled trial was conducted in 60 rural communities with pre-school programs in southern Mali. Children aged less than 5 years living in the 30 intervention communities received two rounds of seasonal malaria chemoprevention in Oct and Nov 2013, followed by daily supplementation of micronutrients for four months from January-April 2014. Delivery of the two interventions at community-level was organized by pre-school management committees. The impact of the interventions will be evaluated in May 2014 through cross-sectional surveys to compare malaria infection, nutrition and cognitive performance in children aged 3 and 5 years living in intervention and control communities. Study outcomes will include asymptomatic parasitaemia, hemoglobin concentration, nutritional indices (height-for-age, weight-for-age), and cognitive foundation skills for early literacy. Findings on the combined effect of the malaria and nutrition interventions after the first year of implementation will be presented.

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POPULATION PHARMACOKINETICS OF PIPERAQUINE IN YOUNG UGANDAN CHILDREN TREATED WITH DIHYDROARTEMISININ-PIPERAQUINE FOR UNCOMPLICATED MALARIA

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Dihydroartemisinin-piperaquine (DP) is currently recommended as first-line treatment for malaria. Doses of DP to treat malaria in young children were initially chosen by scaling adult doses based on weight. This approach does not adequately address physiologic changes in early childhood that affect pharmacokinetics (PK). There is little information about the PK of piperaquine in children, and particularly those < 2 years of age. The objective of this study is to describe the PK of piperaquine in children 6 months to 2 years of age using a population approach that also explores covariate effects. We undertook a prospective population PK study of piperaquine (PQ) in 107 children 6 months to 2 years old who were treated with standard weight-based three-dose DP for uncomplicated malaria in Uganda. Participants underwent 5-7 finger sticks for 28 days following each treatment, providing 1282 evaluable capillary plasma samples from 218 treatments for a mixed effects model analysis with the program NONMEM®. Capillary plasma concentrations on day 7 in most patients, but especially in lower weight children 1-2 years of age, were below the value generally believed to be associated with a longer time to infection. PK data of piperaquine were well described by a 3-compartment open model with first-order absorption. Age, and not weight or other covariates, had a statistically significant effect ($p<0.005$) on clearance/bioavailability (CL/F) or on relative bioavailability (F) alone. This finding provides a possible contributing explanation for the low exposure in low-weight 1-2 year old children relative to higher-weight 1-2 year olds and 6 month-1 year olds, all of whom were dosed according to weight. Based on this population model we then simulated pharmacokinetic profiles of alternative treatment doses, as well as candidate PQ chemoprevention regimens, including monthly administration of standard therapeutic doses, a regimen currently being evaluated. Our results suggest that higher doses, especially in lower-weight 1-2 year olds, will increase the fraction of young children with piperaquine exposure greater than a predefined target, whether it is a previously published one or otherwise chosen. This study supports a growing body of evidence that there is a need to re-evaluate dosing of piperaquine in children.

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ARTEMETHER-LUMEFANTRINE EFFICACY: POTENTIAL FOR FURTHER DOSE OPTIMIZATION

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Artemether-lumefantrine (AL) is the first line antimalarial treatment in 49 countries, administered according to four weight bands. Patients at the margins of these bands can receive significant deviation from the target dose. To assess the impact of weight adjusted (mg/kg) dose variations in therapeutic efficacy, individual patient data were shared with the WorldWide Antimalarial Resistance Network (WWARN) and collated using standardised methodology. Risk factors associated with recrudescence were evaluated using Cox's regression model with shared frailty on study sites. Data from 14,327 patients (61 efficacy studies between 1996 and 2012 in Africa, Asia and South America) with uncomplicated *P. falciparum* malaria were included in the analyses. A total of 386 Polymerase Chain Reaction (PCR)-confirmed recrudescent infections were reported. The PCR adjusted cure rate was 97.6% [95% CI: 97.4-97.9%] at day 28 and 96.0% [95% CI: 95.6-96.5%] at day 42. After controlling for age and parasitemia, every unit increase in daily artemether dose reduced the risk of being parasitaemic on day 1 by 8% [95% CI:1-14%, p=0.024] and every unit increase in total artemether dose reduced the risk of gametocyte carriage by day 14 by 8% [95% CI:1-15%, p=0.037]. Overall the dose of AL did not correlate with recrudescence, however the risk of recrudescence was higher in Asian children weighing 10 to 15 kg who received a total lumefantrine dose less than 60 mg/kg (Adjusted Hazards Ratio of 2.73 [95% CI: 1.40-5.32]), accounting for 41% of all treatment failures; p=0.003. In Africa, the risk of recrudescence was greatest in malnourished children from 1 to 3 years old (PCR adjusted cure rate 94.3% [95% CI: 92.3-96.3%]). The currently recommended dose of AL provides reliable efficacy in most patients with uncomplicated malaria. However, cure rates were lowest in young children in Asia and young underweight children from Africa. A higher dose regimen should be evaluated in these groups.

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ARE PUBLIC OR RETAIL SECTOR PATIENTS MORE LIKELY TO COMPLETE TREATMENT? AN ANALYSIS OF PATIENT ADHERENCE TO ARTEMETHER-LUMEFANTRINE IN SOUTHERN TANZANIA

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Artemisinin combination therapies (ACTs) are first-line treatment for malaria in the public sector of most endemic countries and increasingly available in the private sector. Most studies on ACT adherence have been conducted in the public sector, with minimal data from private retailers. We conducted parallel adherence studies in Mtwara, Tanzania, in which patients obtaining artemether-lumefantrine (AL) at 40 randomly selected public health facilities and 37 private drug shops were followed up at home and questioned about each dose taken. The effect of health sector on adherence was assessed with random effects logistic regression controlling for potential confounders. Factors associated with adherence in each sector were examined in separate models. Of 572 health facility patients and 451 drug shop patients, 75% and 70% respectively completed treatment (p=0.2) , and 46% and 35% took each dose at the correct time (p=0.003). Drug shop patients were wealthier, more educated, older, sought care later in the day, and were less likely to test positive for malaria than health facility patients. Controlling for patient characteristics, the adjusted odds of drug shop patients completing treatment and taking each dose at the correct time were 0.63 and 0.67 times that of health facility patients (p=0.030 and p=0.044). Factors associated with adherence in drug shop patients were higher socioeconomic status and recalling correct dose instructions. Patients seeking care in the evening were half as likely to be adherent as those who sought care in the morning. In the public sector, having fever and recalling correct instructions were associated with completed treatment, while seeking care within two days, being tested for malaria, and taking the first dose at the facility were associated with timely completion. Patients attending drug shops differ from those at public health facilities, but when controlling for these characteristics, adherence was lower in drug shops. Better understanding is needed of which aspects of patient care are most important for adherence.

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IMMUNE REGULATION BY HELMINTH PARASITES, INVOLVES MODULATION OF HUMAN DENDRITIC CELL METABOLISM INCLUDING INDUCTION OF INDOLEAMINE 2,3-DEOXYGENASE, DOWNREGULATION OF MTOR SIGNALING, AND INDUCTION OF AUTOPHAGY

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Helminth infection has been associated with dysregulation of professional antigen presenting cells (APC) such as Langerhans cells (LC) and dendritic cells (DC) with DC being the cell type most affected by parasite exposure, particularly when exposed to the micrrofilarial (mf) stage of the *Brugia malayi* one of the 2 major species causing lymphatic filariasis in humans. To gain further insights into nature of the mf-induced alterations in DC function and viability (previously shown), we used LC-MS/MS to elucidate the entire proteome of mf-exposed monocyte derived human DC (n=4) in comparison to mf-unexposed human DC. Our proteomics data indicated that multiple components of the Mammalian Target of Rapamycin (mTOR) signaling pathway, including mTOR, and Eukaryotic Initiation Factor 4 (eIF4) A were downregulated by mf, suggesting that mf target this pathway for immune modulation in DC. Utilizing western blot analysis we showed that similar to rapamycin (a known mTOR inhibitor), mf downregulate the phosphorylation of mTOR regulatory proteins p70S6K1 and 4E-BP1, a process essential for DC protein synthesis. As active mTOR signaling regulates autophagy by inhibiting an autophagy induction complex, we examined whether mf exposure alters autophagy-associated processes. Expression of p62, a ubiquitin-binding protein that aggregates protein in autophagosomes and is degraded upon autophagy, was reduced dramatically by mf exposure ($P<0.05$), suggesting that mf induce autophagy in DC. Furthermore, as amino acid deficiency is one mechanism of mTOR activation and inhibition of autophagy, upregulation of kynureninase observed in our proteomics data suggested that mf also induce tryptophan catabolism in human DC; this was corroborated by showing that the expression of Indoleamine 2,3-deoxygenase (IDO) and activity was increased dramatically by exposure to mf ($p<0.05$) compared to mf-unexposed cells. Together, these results suggest that *Brugia malayi* mf employ mechanisms of metabolic modulation in DC to influence the regulation of the host immune response by downregualting mTOR signaling resulting in increased autophagy.

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HYPERREACTIVE ONCHOCERCIASIS IS CHARACTERIZED BY AN ANTIGEN INDEPENDENT BIAS TOWARDS TH17 COMBINED WITH STRONG TH2 IMMUNE RESPONSES

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Onchocerciasis or river blindness is the second leading infectious cause of blindness after trachoma. In human, the disease is caused by infections with the filarial nematode *Onchocerca volvulus* and presents two major polar forms: the hyporeactive form or generalized onchocerciasis (GEO) and the hyperreactive form (hyperreactive onchocerciasis: HO). The latter is associated with more aggressive disease manifestations and disfiguring dermatopathology and eye lesions that can lead to blindness, whereas GEO is characterized by tolerance to the parasite and mild skin disease. The immunological determinants of this polarization of the disease are still not fully clarified. Using multicolor FACS analysis, multiplex cytokine arrays and Real Time PCR, we compared the immune profiles of a group of 16 endemic normals (EN), individuals who had no clinical or parasitological evidence of infection despite ongoing exposure to the parasite, with a group of 16 individuals with GEO and a group of 6 individuals presenting the rare but sever hyperreactive form of the disease. We could show that individuals with HO presented higher frequencies of CD4+ cells expressing Th17 related cytokines (IL-17A, IL-17C, IL-6, IL-22) and markers (RORC, STAT3), while those with GEO presented significantly higher CD25+Foxp3+ and IL-10 secreting T cells. Concordantly, TCR activation using anti-CD3/CD28 induced high amounts of IL-17 in culture supernatants of peripheral blood mononuclear cells (PBMCs) from patients with HO, whereas IL-17 was barely detectable in PBMCs from EN and GEO individuals in same conditions. Strikingly, antigen specific stimulation using Onchocerca volvulus extracts did not elicit IL-17 secretion, either in PBMCs of GEO or in those of HO individuals. In contrast, a robust Th2 response was induced upon antigen specific activation of PBMCs from HO patients, as shown by prominent expression of GATA3, IL-4, IL-5 and IL-13 in PBMCs of these individuals. These findings suggest that, strong parasite specific Th2 responses combined with robust parasite-independent Th17 polarization in the absence of adequate immune regulation is associated with HO, revealing a novel Th17/Th2 axis as potential target in the development of new therapeutical strategies to prevent HO in human.

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HELMINTH INFECTIONS DURING PREGNANCY IS ASSOCIATED WITH IMPAIRED VACCINE EFFICACY IN KENYAN INFANTS

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Both animal models and human studies suggest that parasitic infections can result in decreased vaccine efficacy. Maternal parasitic infections during pregnancy prime the fetal immune response and induce an immunomodulatory phenotype at birth that may affect subsequent immune responses to childhood vaccines. We investigated whether prenatal exposure to helminth infections affect the pattern of infant immune response to standard vaccination against Haemophilus influenzae (Hib), hepatitis B (Hep B), tetanus toxoid (TT) and diphtheria toxoid (DT). 450 Kenyan women were tested for LF, urogenital schistosomiasis, malaria, and intestinal helminths during pregnancy. Their newborns were followed biannually to age 36 months and tested for levels of IgG against Hib, Hep B, TT, and DT at each time point. Overall, one third of the mothers were infected with LF, urogenital schistosomiasis, malaria or hookworm. Using a generalized estimating equation analysis, the presence of multiple maternal infections were associated with lower immune response to Hib PRP-specific IgG (p=0•001, 0•002, 0•045 with one infection; p=0•028, 0•022, 0•051 with two infections at 12, 18 and 24 months of age), compared to no maternal infection. There was a significant difference in response to DT in infants of mothers with three or more infections (p= 0•001 and 0•02 at 6 and 12 months) compared to no maternal infection. Response to Hib was also associated with immunophenotype; offspring putatively tolerized to filarial antigens (LF infected mothers but lacking filarial-specific Th1/Th2-type response in cord blood, N=94) compared to unexposed (no evidence of maternal LF infection nor antigen responsiveness in cord blood, N=119) had a lowest vaccine-induced antibody response to Hib-specific IgG (p= 0•052, 0•033 and 0•035 at 12, 18 and 24 months). Antenatal helminth infections are associated with lower immune response to Hib and DT vaccine antigens. Thus, in developing countries, eradication of chronic helminthic infections may be imperative to the success of future global vaccination efforts.

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BLADDER UROTHELIAL CELL CYCLE RESPONSES TO *SCHISTOSOMA HAEMATOBIUM* INFECTION ARE MODULATED BY IL-4 RECEPTOR-α

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The bladder urothelium, a normally quiescent epithelial organ, undergoes dynamic changes during *Schistosoma haematobium* infection (urogenital schistosomiasis). These alterations include hyperplasia, ulceration, dysplasia, and frank carcinogenesis, and likely involve shifts in urothelial cell cycle status. Defining the pathways underpinning these urothelial responses will contribute to a deeper understanding of how *S. haematobium* egg-induced expulsion, hematuria, and bladder cancer develop in humans. Akin to their responses to many different helminth infections, mammals mount an IL-4 and -13-associated type 2 immune response during *S. haematobium* infection. These cytokines share the IL-4 receptor-α subunit as one of their cognate receptor subunits. To determine whether IL-4 receptor-α plays a role in urothelial cell cycle alterations in urogenital schistosomiasis, we injected *S. haematobium* eggs or control vehicle into the bladder walls of wild type, IL-4 receptor-α-deficient, and myeloid-associated IL-4 receptor-α-deficient mice. Three weeks later, mice were sacrificed and their bladder urothelium isolated and prepared as single cell suspensions. These suspensions were stained with DAPI and antibodies to CD45 and EpCAM. CD45-EpCAM+ urothelial cells were gated and their DAPI staining analyzed to assess cell cycle status. Relative to vehicle controls, wild type mice injected with eggs demonstrated increased proportions of urothelial cells in S phase and decreased proportions in G2/M phase. Although egg-injected, macrophage-associated IL-4 receptor-α-deficient mice featured similar urothelial responses as their egg-injected wild type counterparts, egg-injected conventional IL-4 receptor-α-deficient mice exhibited fewer urothelial cells in S phase. Thus, IL-4 receptor-α signaling through non-myeloid cells indeed seems to affect urothelial cell cycle status; this effect may specifically target DNA synthesis, a crucial process in carcinogenesis. Ongoing work seeks to determine which IL-4 receptor-α-expressing cells are crucial to these phenomena, and how these cells mediate alterations in the cell cycle status of the bladder urothelium.

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IMMUNOGENICITY AND ANTI-FECUNDITY EFFECT OF NANOPARTICLE COATED GLUTATHIONE S-TRANSFERASE (SJGST) DNA VACCINE AGAINST MURINE *SCHISTOSOMA JAPONICUM* INFECTION

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There is still urgent need for a vaccine against schistosomiasis, especially in *Schistosoma japonicum* endemic areas where even a vaccine that will interrupt zoonotic transmission will be potentially effective as an intervention tool. We had developed a novel nanoparticle gene delivery system, which has proven efficacious in gene transfection to target immune cells with complementary adjuvant effect and high protective efficacy in several diseases. Here, we have applied this nanoparticle system in combination with *S. japonicum* glutathione S-transferase (SjGST) DNA vaccine to show improved immunogenicity and anti-fecundity effect of the nanoparticle coated vaccine formulation against murine schistosomiasis. The nanoparticle-coated DNA vaccine formulation induced desired immune responses with significantly increased humoral response, T-helper 1 polarized cytokine environment, higher proportion of IFN-γ producing CD4+ T-cells and the concomitant decrease in IL-4 producing CD4+ T-cells. Although there was no effect on worm burden, the proportion of immature worms was higher in the SjGST vaccinated groups with a lower proportion of mature paired worms. We recorded an unprecedented reduction in tissue egg burden. There was 71.9% decrease in liver egg burden, 71.6% decrease in intestinal egg burden, and 54.7% reduction in the fecundity of female adult worms. In conclusion, our data showed that the combination of nanoparticle gene delivery system with SjGST DNA vaccination significantly improved the characteristic anti-fecundity effect of SjGST, thereby proving this DNA vaccine formulation as a promising candidate for anti-pathology and transmission blocking application.

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THE MOLECULAR CONSEQUENCES OF INTRA-PATIENT PHAGE PREDATION ON *VIBRIO CHOLERAE* POPULATION DIVERSITY

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Vibrio cholerae is a globally important water-borne pathogen, which is the causative agent of the severe acute diarrheal disease cholera. We analyzed geographically disparate cholera patient samples from Haiti and Bangladesh and found that ICP2-like phage can impact the evolutionary trajectory of intra-patient *V. cholerae* populations. Evidence of intra-patient selection of phage-resistant mutants was found in one Haitian patient sample in which >99% of *V. cholerae* isolates harbored mutations in the major outer membrane porin, OmpU. Mutant *ompU* alleles were sufficient for ICP2 resistance when moved into a clean background, indicating that ICP2 uses OmpU as a receptor to initiate infection. Phage-resistant and phage-sensitive isolates from this sample were subjected to whole genome sequencing and were found to be isogenic except for single site mutations in *ompU*. The mutations within *ompU* between phage-resistant isolates were heterogeneous, indicating that phage predation and selection of resistant mutants occurred multiple independent times during infection. High levels of ICP2 were also observed in a Bangladeshi patient sample found to harbor a mixture of *toxR* mutants. Similar to what was observed in the Haitian patient sample; multiple unique *toxR* mutants were observed within this single sample. ToxR directly activates expression of *ompU* and critical virulence factors. ICP2-sensitivity was restored to the clinical *toxR* mutants by expressing *ompU* in trans, demonstrating that resistance is mediated through decreased OmpU expression. The clinically isolated *ompU* and *toxR* alleles had differing impacts on *V. cholerae* virulence, with the *toxR* mutants being severely attenuated *in vivo*. We demonstrate that *V. cholerae* faces phage-mediated predatory interactions during the natural course of infection that can impose a strong selective pressure. This work highlights the notion that host-pathogen interactions are often embedded in a diverse microbial ecosystem that can impact the virulence and transmission of the pathogen.

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CIRCULATING MUCOSAL ASSOCIATED INVARIANT T (MAIT) CELLS ARE ACTIVATED IN *VIBRIO CHOLERAE* O1 INFECTION AND ASSOCIATED WITH LIPOPOLYSACCHARIDE ANTIBODY RESPONSES

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Mucosal Associated Invariant T (MAIT) cells are innate-type T cells that account for up to 10% of circulating T cells and are found in the intestinal mucosa, liver, and mesenteric lymph nodes. MAIT cells are activated in response to the presentation of riboflavin metabolites, which are produced by various bacteria including Vibrio cholerae. MAIT cells are thought to play a role in bridging the innate-adaptive interface, and a recent study demonstrated that activation of MAIT cells is associated with B cell responses to a Shigella vaccine. We collected blood from patients presenting with culture-confirmed severe cholera to the icddr,b Dhaka Hospital at days 2, 7, 30, and 90 of illness. We characterized MAIT cells by multicolor flow cytometry, and assessed antibody responses to V. cholerae O1 lipopolysaccharide (LPS) and cholera toxin B subunit (CTB) by ELISA. We defined MAIT cells as $CD3^{+}CD4^{-}CD161^{hi}$ cells that are positive for the invariant T cell receptor segment $V\alpha7.2^{+}$, and used CD38 as a marker of MAIT cell activation. We found that MAIT cells were maximally activated at day 7 of cholera in all age groups. In adults, MAIT frequencies did not change over time, whereas in children, MAITs were significantly decreased at day 7 compared to day 2. This decrease persisted to day 90, the last time point examined. Notably, the magnitude of increase in MAIT cells from day 2 to day 7 of infection correlated with fold changes in LPS IgA and IgG responses. Such a correlation was not found with LPS IgM or antibody responses to CTB. In this study, we show that MAIT cells are activated during severe cholera, suggesting that they may play an important role in the innate response against V. cholerae infection. Given that increases in MAIT cells are correlated with increases in class-switched antibodies against LPS, but not CTB, we hypothesize that MAIT cells may be involved in mechanisms underlying class switching of antibodies to T-independent antigens. The persistent decrease in MAIT cells following cholera in children, not seen in adults, needs further investigation.

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IMMUNOGENICITY OF A KILLED ORAL BIVALENT WHOLE CELL CHOLERA VACCINE IN ADULTS WITH HIV INFECTION

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Cholera epidemics often occur in settings where human immunodeficiency virus (HIV) infection is prevalent. While some evidence suggests that individuals living with HIV are more susceptible to cholera than those that are HIV negative, little is known about how HIV alters the immune response to oral cholera vaccines. We evaluated immune responses following bivalent oral cholera vaccination (BivWC; Shanchol) in a cohort of adults in Haiti, some of which were known to have HIV infection. Blood samples were obtained prior to immunization and 7 days after each of two doses of BivWC vaccine were administered. A total of 25 adults with HIV infection were enrolled in the study; 23 of these individuals received both doses of BivWC and completed the three-week observation period including all blood draws. Compared to 22 adults without known HIV infection who completed the three-week observation period, HIV-infected individuals in this cohort had significantly lower vibriocidal antibody responses against both the *V. cholerae* O1 Inaba and Ogawa serotypes. Among the HIV-infected vaccinees, we found an inverse relationship between $CD4^{+}$ T-cell count and the subsequent vibriocidal antibody response following vaccination. However, despite lower vibriocidal antibody responses, there was still a substantial vaccine take rate in this cohort adults living with HIV infection. Both O- polysaccharide antigen specific IgA and vibriocidal antibody titers increased significantly after oral cholera vaccination in HIV-infected adults. Seroconversion, defined as four-fold or greater increase in vibriocidal antibody titer, occurred at a rate of 65% against the Ogawa serotype and 74% against the Inaba serotype in adults with HIV infection. These results suggest that killed oral cholera vaccines retain substantial immunogenicity in adults with HIV infection and may provide a significant benefit in a population that is otherwise vulnerable to cholera.

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EFFECTIVENESS OFAN ORAL KILLED BIVALENT WHOLE-CELL CHOLERA VACCINE IN RURAL HAITI

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Between April and June 2012, a reactive oral cholera vaccine campaign was conducted in a rural district of Haiti using the oral killed bivalent whole-cell vaccine, Shanchol®. We conducted a case-control study to estimate vaccine field effectiveness in the context of this campaign. Cases had acute watery diarrhea, sought treatment at 1 of 3 participating cholera treatment units between October 24, 2012 and March 9, 2014, and had a stool sample positive for cholera by Crystal VC® rapid test and culture. For each case, four controls (individuals who did not experience or seek treatment for acute watery diarrhea between the date of study initiation and the date of their corresponding case's symptom onset) were matched by neighborhood, calendar time, and age. We also conducted a bias-indicator case-control study to assess the likelihood of bias in the vaccine effectiveness case-control study by examining the relationship between vaccination and non-cholera diarrhea. In the absence of bias we expected no association between vaccination and non-cholera diarrhea in the bias-indicator study. During the study period, 115 individuals presented with acute watery diarrhea. After excluding 8 individuals who lacked specimen for culture, 22 with discordant rapid test and culture results, and 1 cholera case that lacked interview data, 41 were included as cases in the vaccine effectiveness case-control study and 43 as cases in the bias-indicator study. In univariable analysis, both self-reported vaccination and verified vaccination (via vaccination card or registry) were associated with a statistically significant reduction in the risk of cholera. In adjusted analyses vaccine effectiveness was 66% [95% confidence interval: 15%-86%; p-value: 0.02] by self-report and 60% [95% confidence interval: 14%-82%; p-value: 0.02] for verified vaccination. Neither self-reported nor verified vaccination was significantly associated with non-cholera diarrhea in univariable or multivariable analyses. The oral cholera vaccine is an effective component of the comprehensive response to cholera in Haiti.

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THE CASE FOR USING A SINGLE DOSE OF ORAL CHOLERA VACCINE IN EMERGENCIES

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A global stockpile of oral cholera vaccine (OCV) was recently established for emergency use, but with less than 2 million doses available worldwide officials will likely have to contend with limited vaccine. Previous work shows that speed of response is the strongest predictor of the impact of reactive vaccination campaigns. Two OCVs are internationally licensed for use as a two-dose formulation administered 1-6 weeks apart, and the delay between vaccination rounds may compromise the benefits of receiving the second dose. With evidence from clinical trials and immunological studies suggesting that a single dose may be moderately protective, there is a strong argument that more may be gained by distributing a single dose to more people quickly, than a full course to a smaller population, thereby administering as many doses of vaccine as quickly as possible. Whether this is the case will be driven by the efficacy of a single dose, the mechanism of vaccine protection, and local cholera transmission dynamics. Using computational models of cholera transmission with varying assumptions about the epidemiologic context and vaccine mechanism, we explore the minimum single-dose efficacy needed to avert at least as many cases as a two-dose regimen in a reactive vaccination campaign with limited vaccine. In a simple model not taking into account indirect effects (i.e. herd protection), we find that early vaccination with a single-dose that confers about at least 42% protection against clinical disease would avert more cases than a two-dose regime with the same quantity of vaccine. As vaccination is delayed and indirect effects are taken into account, using a single dose with even lower efficacy will avert more cases than a two-dose campaign. When considering logistical delays and epidemic specific transmission dynamics from recent large outbreaks in Haiti, Zimbabwe, and Guinea, we find that with realistic quantities of vaccine, use of a single-dose regimen would have averted more cases than a two-dose regimen. Through synthesis of clinical and immunological evidence with data from recent cholera outbreaks, we show that a single dose of OCV may be an important tool for emergency response. If future empirical studies are consistent with current single-dose efficacy evidence, our results help create a case for the international licensing of a single-dose OCV regimen.

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SINGLE-DOSE LIVE ATTENUATED ORAL CHOLERA VACCINE (CVD 103-HGR) PROTECTS AGAINST CHOLERA AT 10 DAYS FOLLOWING VACCINATION: RESULTS OF A *VIBRIO CHOLERAE* O1 EL TOR INABA CHALLENGE STUDY

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No cholera vaccines are currently licensed and available in the U.S.A; two killed oral cholera vaccines licensed in other countries require 2 spaced doses. A single-dose oral cholera vaccine that rapidly elicits protection is needed for immunization of short-notice travelers to high risk areas and for use in explosive outbreaks, where practical administration and accelerated onset of protection are also desirable. Attenuated *Vibrio cholerae* O1 classical Inaba live oral vaccine strain CVD 103-HgR harbors a deletion of 94% of the gene encoding the cholera toxin A subunit. CVD 103-HgR, manufactured by PaxVax Inc., typically elicits vibriocidal antibody seroconversion (a correlate of protection) within 10 days of vaccination. To evaluate vaccine efficacy, we challenged healthy volunteers, 18-45 years old, with virulent *V. cholerae.* Consenting healthy cholera-naïve adults (n= 98, including n=50 of blood group O, high risk for cholera gravis), were randomly allocated 1:1 to receive vaccine (10^8 CFU) or placebo. Ten days after vaccination, 68 subjects ingested 10^5 CFU wild type *V. cholerae* O1 El Tor Inaba strain N16961. Subjects were observed on a Research Isolation Ward for measurement of stool output and management of cholera illness. The vaccine was well tolerated: adverse events, generally mild to moderate, were reported in 13% vaccine and 14% placebo recipients. The primary endpoint, moderate (≥3.0 liter cumulative purge) to severe (≥5.0 liter cumulative purge) diarrhea occurred in 20/33 (61%) placebo and 2/35 (6%) vaccine recipients (point estimate of vaccine efficacy [VE] =91%, p<0.0001). 13/33 (39%) placebo recipients and 1/35 (3%) vaccinees had severe diarrhea (point estimate of VE =93%, p=0.0002). Additional subjects will be challenged at 90 days post-vaccination and overall VE will be calculated in May 2013 with pooled placebo recipients serving as the control. The encouraging preliminary analysis of VE observed 10 days after receipt of a single 10^8 CFU dose of CVD 103-HgR supports an accelerated clinical development path towards FDA licensure.

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OPTIMAL ALLOCATION OF ORAL CHOLERA VACCINE IN ENDEMIC AND EPIDEMIC SETTINGS

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In 2013 the World Health Organization (WHO) established a global stockpile of two million doses of oral cholera vaccine (OCV) to be deployed reactively during cholera epidemics. With an estimated 3--5 million cases, 100,000 deaths annually, and 1.4 billion people at risk of infection, the current cholera vaccine supply can only prevent a small percentage of cases. Due to the limitations in vaccine supply, it is important to determine how to best allocate existing vaccine between populations in endemic settings versus stockpiling the vaccine for reactive use during epidemics, where attack rates and case fatality ratios are often higher. We used a mathematical model of cholera transmission to examine how the allocation of a limited OCV supply can maximize the total number of cholera cases prevented in both epidemic and endemic settings. We found that under a broad range of expected epidemic sizes the number of cases prevented is maximized by allocating a fairly high proportion of the existing OCV supply to reactive vaccination in the epidemic setting. This optimal OCV allocation is sensitive to the timing of the reactive vaccination campaign. For less explosive epidemics with lower initial growth rates the optimal allocation to reactive vaccination increases as the start of the reactive vaccination campaign is delayed (up to a limit of 120-150 days). However, in the case of larger epidemics with high initial growth rates, moderate delays to the start of reactive vaccination (>50 days after the start of outbreak) lead to allocating OCV doses away from reactive vaccination and towards endemic populations because the reactive vaccination campaign will miss the peak of the epidemic and prevents relatively few cases. Our results indicate that the strategy of maintaining the stockpile for reactive campaigns rather than using these doses in an endemic setting is optimal with the current limited supply as long as vaccine deployment is timely.

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MODELING THE COST EFFECTIVENESS OF MALARIA CONTROL INTERVENTIONS IN THE HIGHLANDS OF WESTERN KENYA

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Tools that allow for in silico optimization of available malaria control strategies can assist the decision making process for prioritizing interventions. The OpenMalaria stochastic simulation modeling platform can be applied to simulate the impact of interventions singly and in combination as implemented in Rachuonyo South District, western Kenya, to support this goal. Combinations of malaria interventions were simulated using a previously-published, validated model of malaria epidemiology and control in the study area. An economic model of the costs of case management and malaria control interventions in Kenya was applied to simulation results and cost-effectiveness of each intervention combination compared to the corresponding simulated outputs of a scenario without interventions. Uncertainty was evaluated by varying health system and intervention delivery parameters. While an intervention with long lasting insecticide treated net (LLIN) use by 80% of the population, 90% of households covered by indoor residual spraying (IRS) with deployment starting in April, and intermittent screen and treat (IST) of school children using Coartem® with 80% coverage twice per term had the greatest simulated health impact, the current malaria control strategy in the study area of LLIN use of 56% and IRS coverage of 70% was the most cost effective at reducing DALYs over a five year period. All the simulated intervention combinations can be considered cost effective in the context of available resources for health in Kenya. Increasing coverage of vector control interventions has a larger simulated impact compared to adding IST to the current implementation strategy, suggesting that transmission in the study area is not at a level to warrant replacing vector control to a school-based screen and treat program. These results have the potential to assist malaria control program managers in the study area in adding new or changing the implementation of current interventions.

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THE IMPORTANCE OF HISTORICAL DATA IN SIMULATING MALARIA EPIDEMIOLOGY AND CONTROL INTERVENTIONS: APPLICATION TO MULTIPLE SITES IN MADAGASCAR

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Planning malaria interventions requires prediction of likely impacts of different intervention strategies. Simulation models can provide such predictions but weak information about pre-control levels of transmission, intervention coverage and access to care often makes it challenging to correctly parameterize them. We consider a number of low malaria transmission sites in Madagascar, with available historical prevalence and entomological inoculation rate (EIR) estimates (by mosquito sampling), giving disparate estimates of historical exposure. Information about implementation of Long Lasting Insecticide Nets (LLINs) and Indoor Residual Spraying (IRS), and access to healthcare, collated from malaria surveys and on-going cross-sectional studies were used to parameterise simulations of malaria transmission, prevalence and burden within the OpenMalaria platform. Multiple parameterisations were considered using various sources of data for pre-intervention transmission level, intervention coverage and access to healthcare. In some sites the simulated impact of existing vector control programs matches reasonably well the malaria prevalence measured in a recent national survey. In others it predicts lower than observed prevalence, very likely because the models do not capture residual local transmission foci. The simulations suggest that the most cost-effective vector control strategy would be to scale-up LLINs or IRS only, depending on the transmission level. Indeed, preliminary results show no additional benefit of IRS where LLINs were used. These preliminary results suggest that historical prevalence data, combined with current coverage information are potentially adequate for planning intervention strategies. The outcome of intervention scale-up is essentially unpredictable if baseline information is poor. Reproducing the observed epidemiology of malaria through simulations both provides confidence in the use of the model but serves as a basis for prospective studies that support decision-making, including cost-effectiveness analyses.

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ASSESSING THE POTENTIAL IMPACT OF ARTEMISININ RESISTANCE IN SUB-SAHARAN AFRICA

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Artemisinin and partner drug resistant malaria parasites have emerged in Southeast Asia. If resistance were to emerge in Africa it could have a devastating impact on the morbidity and mortality associated with ongoing malaria transmission. We estimate the potential impact of artemisinin and partner drug resistance on disease burden in Africa if it were to emerge. Using data from Asia and Africa, we characterise five possible artemisinin and partner drug resistance scenarios. Artemisinin

resistance is characterised by slow parasite clearance (SPC). Partner drug resistance is associated with late clinical failure (LCF) or late parasitological failure (LPF). An individual-based malaria transmission model is used to estimate the impact of each resistance scenario on clinical incidence, severe incidence and parasite prevalence across Africa. We find that scenarios with high levels of recrudescent infections (LCF/LPF) resulted in far greater increases in clinical incidence compared to scenarios with high levels of SPC. Across Africa, we estimate that partner drug resistance at levels similar to those observed in parts of Africa for AS+SP could result in 39 million additional cases over a five year period, a 2.7% increase compared to a scenario with no resistance. Artemisinin resistance similar to levels observed in Pailin, Cambodia could result in an additional 29 million cases over the same period. Artemisinin resistance is potentially a more pressing concern than partner drug resistance due to the lack of viable alternatives. However, widespread partner drug resistance, characterised by SP resistance in parts of Africa, would result in greater increases in malaria morbidity than if widespread artemisinin resistance were to develop at levels currently observed in Pailin, Cambodia.

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MODELING THE IMPACT OF COINFECTION ON PERSISTENCE AND INFECTIVITY OF *PLASMODIUM FALCIPARUM*

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Each year nearly 200 million people are infected with the malaria parasite, *Plasmodium falciparum*. One of its most notable features is the variable course and duration of infection experienced by different individuals, ranging from high parasite density, acute and often severe infections to persistent, chronic infections that are often undetectable by microscopy. Levels of acute and chronic infections vary across different transmission settings, and what disturbs the delicate balance between parasite growth and immune control, leading to bursts of parasite growth or clearance of an infection, remains an open question. Field studies examining persistence of infection have used a variety of different genotyping methods, but due to limitations, it is difficult to determine the extent of mixed infections, and nearly impossible to determine if the reemergence of parasitemia is due to a new infection or recrudescence of an existing one. Mathematical models, despite limited knowledge of mechanistic details of host-parasite interactions, have qualitatively reproduced single parasite dynamics observed in patient data. Here we adapt a discrete model by Recker et al. (PLoS Pathogens, 2011) of blood-stage parasite dynamics including innate and adaptive immune responses. We analyze simulated output to examine how coinfecting strains, particularly from similar clones that elicit overlapping immune responses, impact infection length and infectiousness. We find that the level of both innate and adaptive immune responses present at the time of coinfection as well as the similarity of the coinfecting strains significantly alters the duration of both the resident and coinfecting strains, particularly during chronic infections. Timing of coinfection also influences the infectivity of the coinfecting strains, likely altering transmission patterns at a population level. Duration of infection and infectivity are critical epidemiological parameters for predicting the efficacy of control strategies, particularly with the looming problem of emerging drug resistance.

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MEASURING THE PATH TOWARDS MALARIA ELIMINATION

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As an area approaches local elimination it becomes increasingly difficult to quantify transmission accurately. This is because countries that manage to control local transmission to relatively low levels but receive large numbers of imported cases from outside its borders are likely to see a considerable number of autochthonous cases. The reproduction number R (the average number of persons infected by a malaria case) is a new method of measuring malaria transmission though, to date, its use has remained largely theoretical and it has had limited practical impact. Here, we present an operational framework to determine whether programs are successful at controlling local transmission of falciparum malaria. It is based on a new and simple method to test the hypothesis R≥1 from standard surveillance data consisting of the numbers of local and imported malaria cases. We apply this approach to Swaziland, a country which embarked on an elimination campaign in 2008. Thirty six percent (52/143), 45% (170/377) and 67% (153/229) of investigated cases were imported in 2010, 2011 and 2012 respectively. This indicates that the status of controlled non-endemic malaria was reached in 2011 and 2012 but not in 2010. This provides evidence that since 2010, Swaziland has halted endemic transmission at the national level, and that malaria would be eliminated if the current level of control was continued and importations ceased. The method offers a simple and practical solution to quantify transmission as the disease becomes increasingly rare. Instead of just aiming for zero local cases, programs should use these metrics to set a series of intermediate milestones which are easy to test and show progress towards disease elimination. Evidence like this will be essential to enable the successes of countries like Swaziland to be maintained and replicated elsewhere.

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SPATIAL DYNAMICS OF MALARIA TRANSMISSION IN THE EMOD MODEL FOR CAMPAIGNS TARGETING SUSTAINED REGIONAL ELIMINATION IN SOUTHERN ZAMBIA

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As malaria control programs move towards regional elimination campaigns, it will be increasingly important to understand the dynamics of malaria transmission within and between adjacent regions with different characteristics. Within the framework of the EMOD model, we have constructed a network of 116 interconnected simulated populations corresponding to the spatial distribution of households in Gwembe and Sinazongwe districts in southern Zambia. We use a gravity model that includes local and regional roadways to infer the relative rates of migration between nodes. For each simulated population, we use geographically-specific values for larval habitat, acquired immunity, ITN usage, case management rates, drug-regimen compliance, and mass-screen-and-treat (MSAT) coverage levels to the extent that they may be inferred from surveillance data. Our simulations are able to reproduce the geographically variable pattern of reduced rapid diagnostic test (RDT) detectable parasite prevalence from 2012 to 2013. Finally, we extend this spatial-simulation framework to predict the impact of different drug-delivery, vector-control, and case-management parameters on the potential to sustain regions of local elimination. When anti-malarial treatment, with both curative and short-term prophylactic effects, is distributed regardless of RDT status, substantial regions of the simulated geography are parasite-free within a few years. However, sustained success almost certainly requires significant improvements in case-management and vector-control activities.

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THE MATHEMATICAL EPIDEMIOLOGY OF *PLASMODIUM VIVAX* MALARIA - INSIGHTS INTO THE SLOW TIMELINE TO ELIMINATION

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There is a rich history of using mathematical models of malaria transmission to inform the design of programmes for the control and elimination of *Plasmodium falciparum* malaria, building on the pioneering work of Ross and MacDonald. However, the valuable lessons learned

from these models do not always apply to *P. vivax* due to the occurrence of relapse infections months to years after the primary infection. These models are extended to consider relapsing malaria using intuitive, high-school level mathematics to illustrate the qualitative relationships between parameters of epidemiological importance: *P. vivax* parasite prevalence, the basic reproduction number R0, the proportion of infections due to relapses, and the density of *Anopheles* mosquitoes. These relationships are illustrated via examples from malaria control campaigns in The Solomon Islands, Vanuatu and Papua New Guinea where notably different trends were observed in *P. vivax* and *P. falciparum* transmission following the introduction of vector control and mass drug administration. Evidence is presented for three key results, and their implications are discussed with examples from past malaria control programmes. (i) At similar parasite prevalence, *P. vivax* has a greater basic reproduction number than *P. falciparum* ensuring more stable transmission and greater difficulty in reducing R0 < 1. (ii) Although vector control is effective at reducing the transmission of all malarias, it is predicted to be approximately twice as effective at reducing *P. falciparum* prevalence compared to *P. vivax* prevalence, depending on the expected number of relapses. (iii) In the absence of drugs such as primaquine for preventing relapses, *P. vivax* elimination timelines are predicted to be substantially longer than *P. falciparum* timelines due to the reduced effectiveness of vector control and the extended time to relapse infections. As a relapsing malaria, *P. vivax* presents a number of epidemiological challenges that must be considered if it is to be successfully controlled and eliminated.

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BREATHING MY NEIGHBOR'S AIR: AIR POLLUTION DISPERSION FROM BIOMASS COOKSTOVES IN MIRPUR, DHAKA, BANGLADESH

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Exposure to high indoor concentrations of fine particulate matter (PM2.5) is a major risk factor for pneumonia and other respiratory disease. Biomass cookstoves emit high concentrations of PM2.5. However, high concentrations of PM2.5 (>1000 µg/m3) have been observed in Dhaka homes that do not use biomass cookstoves. We aimed to describe dispersion of PM2.5 from biomass cookstoves into nearby homes in a low-income urban area of Dhaka, Bangladesh. We recruited 10 clusters of homes consisting of one biomass-burning (index) home, and 3-4 neighboring homes that used electricity or kerosene cookstoves and had no obvious major sources of PM2.5. We administered a questionnaire and recorded physical features of all homes. We recorded PM2.5 concentrations inside each home, near each stove, and outside one neighbor home per cluster for 24 hours; during 8 of these 24 hours, we directly observed daily activities, such as cooking. We calculated geometric mean PM2.5 concentrations at 5-6am (baseline), during biomass cooking times, and during the entire monitoring period, for each monitor. We recruited a total of 44 homes from 10 clusters. Geometric mean PM2.5 concentrations for all monitors were near the limit of detection (50 µg/m3) at baseline. During biomass cooking in the index home, median geometric mean PM2.5 concentrations were highest in monitors near the biomass stove (341 µg/m3), followed by those inside the index home (174 µg/m3), then neighbor homes that share a wall with the index home (132 µg/m3), then neighbor homes that do not share a wall with the index home (78 µg/m3). Biomass burning in one home can be a source of indoor air pollution for several homes, increasing geometric mean PM2,5 concentrations to over 5 times the World Health Organization standard of 25 µg/m3. The effect of biomass cookstoves is greatest in homes that share a wall with a biomass-burning home. Eliminating just one biomass cookstove can potentially improve air quality for several households in a community.

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SOLAR-POWERED OXYGEN DELIVERY

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Hypoxemia is a grim prognostic sign in pediatric pneumonia and other potentially lethal conditions, for which oxygen therapy may be life-saving. In resource-limited settings, where the majority of pneumonia deaths occur, capacity to deliver oxygen may be limited. Compressed oxygen cylinders are subject to frequent stock-outs and oxygen concentrators require a reliable electrical power source. Here we describe a strategy using solar energy to drive an oxygen concentrator, a technological innovation that could be applied in remote settings off the hydro-electric grid. The system design consisted of commercially available products: 26x80W solar panels, a bank of 8x220Ah batteries, and a 300W oxygen concentrator capable of delivering up to 5L/min of pure oxygen (total cost USD$18,000). Pilot testing of the equipment indicated that the solar panels generated a median (range) of 7.0 (3.6 to 9.0) kWh of energy daily (47% of maximum theoretical output). Between September 2013 and February 2014, 28 critically ill patients with hypoxemia presenting to the emergency ward at Jinja Regional Referral Hospital were treated with solar-powered oxygen. The median (range) age was 6 months (3 months to 3 years) and 45% were female. Common symptoms at presentation included difficulty breathing (89%), cough (82%), fever (74%), and inability to eat/drink (36%). At presentation, all were hypoxemic (SaO2<90%), 74% were tachypneic, and 33% had temperature >37.5°C. Diagnoses included pneumonia (79%), malaria (21%), and sepsis (14%). Treatment with solar-powered oxygen increased the peripheral saturation to >95% in 25 patients (89%); for the remaining 3 patients, oxygenation improved to >90%, but the patients died before recovery of lung function. Duration of hospitalization was median (range) 3 (1-28) days. Outcomes were as follows: 19 patients discharged without disability, 1 discharged with sequellae (cerebral palsy), 2 transferred to another facility, and 6 (23%) died. These data demonstrate proof-of-concept that solar energy can be used to concentrate oxygen from ambient air and oxygenate critically ill patients with hypoxemia using unlimited and freely available inputs, the sun and the air.

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BIOMARKERS DIAGNOSE THE PATHOGEN OF FEBRILE RESPIRATORY DISTRESS

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Blood-markers that rapidly and accurately differentiate the bacterial, viral or malarial etiology of febrile acute respiratory distress could reduce the morbidity of respiratory disease, by facilitating diagnosis and appropriate treatment. Using 56 markers measured in a multiplex immunoassay, we sought to identify proteins and protein combinations that could discriminate these three diagnoses. We selected 80 patients with clinical pneumonia (according to the World Health Organization) meeting criteria for bacterial, viral or malarial disease based on clinical, radiographic, and laboratory criteria, including bacteria blood cultures. Patients were subdivided into a training set (17 malaria, 20 virus, and 15 bacteria) and a validation set (10 malaria, 10 virus, and 8 bacteria).

Markers that accurately differentiated any two of the three groups included haptoglobin, IL-10, IL-6, and TNF-alpha. Among markers that accurately classified bacterial disease in the training set, only haptoglobin (AUC-ROC > 0.85 in all comparisons) performed well in the validation set, where it misclassified only one bacterial patient. The overall sensitivity of haptoglobin (> 0.995 mg/mL) for bacteria was 96% with a +Likelihood-Ratio of 3. In a Classification Tree (CT) signature, TNFR2 (or equivalently IL-10) and TIMP-1 were added to haptoglobin to further classify non-bacterial patients into malarial or viral etiologies. Signatures based on Support Vector Machine (SVM) and regression models showed comparable performance and included haptoglobin, IL-10, MMP9, and CK-MB. The overall sensitivity of the CT/SVM signatures for bacteria were 96%/91%, for malaria 81%/96%, and a total of 20%/17% virus patients were misclassified. Blood-proteins can constitute biomarkers for pediatric respiratory disease amenable to deployment as rapid diagnostic tests. These biomarkers are sensitive for the crucial diagnosis of bacterial infection while achieving moderate to good specificities for viral and malarial infections. These markers should allow diagnosis in malaria endemic and non-endemic areas.

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LOW-COST NON-IMAGING ULTRASOUND (LOCONIUS) FOR PNEUMONIA DETECTION IN RESOURCE-CONSTRAINED SETTINGS

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Pneumonia is the leading cause of child mortality. Its diagnosis is currently based on chest X-rays (CXR) and clinical/laboratory criteria, which require trained professionals and a complex infrastructure. In low-resource settings clinical criteria are the only available resources, but these lack sensitivity and specificity. New, simple, low-cost methods for diagnosis of pneumonia are urgently needed. Ultrasound (US) has been increasingly used to diagnose pneumonia in children. Although US has a high sensitivity and specificity when compared to CXR, its major limitation is the lack of specialists to interpret US images in resource-poor settings. The goal of our study is to develop a specialist-independent system that uses real time US information interpreted through mathematical algorithms for diagnosis of pneumonia in children. Pulmonary US data were obtained from 30 pneumonia and 30 healthy children presenting to local hospitals in Lima, Peru. Images from US videos were processed to obtain vectors of illumination density (10 pixels per vector) along the depth-axes (475 pixels per image). Vectors skipping one column and corresponding to pulmonary infiltrates on US images were classified as pneumonia as our gold standard. 76,964 vectors were used to train and test a single layer neural network classification system. This neural network was used to classify 38,482 vectors from images not used in developing the algorithm, with an observed accuracy of 92% for recognizing pneumonia. The same data were analyzed in a logistic and multinomial multiple statistical regression. On preliminary analysis, we found 94% accuracy in the classification by the neural network. A preliminary multiple regression model achieved a 93.5% sensitivity and 91.4% specificity, with an accuracy of classification expressed by the area under the ROC curve of 0.97 (i.e. 97% of probability to correctly classify a pair of random samples). We expect that further refinement of this model will lead to an automated algorithm for pneumonia that will permit accurate diagnoses using US in low resource settings.

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RISK FACTORS FOR SEVERE OUTCOMES AND IMPACT OF VACCINATION ON PNEUMONIA AND INFLUENZA WITHIN ACTIVE US MILITARY POPULATIONS 2000-2012

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Respiratory infections are responsible for up to 350,000 of medical encounters each year among US military personnel. Our objective in this study was to quantify risk factors associated with severe acute respiratory infection (SARI) in a US Military Cohort admitted to hospital with respiratory illness. We obtained data on 15,210 hospitalizations for pneumonia and influenza (P&I) between 01/01/00 and 31/12/12. From these, we identified 335 SARI episodes using standard case definitions. We evaluated the effect of demographic and occupational characteristics, comorbid conditions, and history of influenza vaccination on the risk of a hospitalized P&I case becoming a SARI case. We also evaluated the risk of SARI and the length of time since vaccination. The median age of subjects was 22 years (range, 17-64) and subjects were predominantly male (89.5%). Risk factors for developing SARI included age (≥45 years, RR=1.8, 95% CI 1.2-2.7), American Indian or Alaskan Native ethnicity (RR=3.0, 95% CI 1.0-8.7), and service in the Air Force (RR=1.7, 95% CI 1.3-2.3). Being male, born in mainland US (vs outside of mainland US) and recent vaccination (within 180 days of episode) were protective against developing SARI. Among comorbid conditions, risk factors for SARI included chronic liver disease (RR=5.7, 95% CI 4.5-7.1), diabetes (RR=2.8, 95% CI 1.7-4.6) and, immune disorders (RR=2.6, 95% CI 1.1-6.1). Male gender (RR=0.7, 95% CI 0.5-0.9) and influenza vaccination (RR=0.7, 95% CI 0.5-0.97) remained significantly protective. Our results also suggest that the closer the vaccination was to hospitalization, the less likely the individual was to develop SARI, with substantially reduced odds of SARI for delays of 1 vs. 6 months). These data suggest that timing of influenza vaccination is critical in reducing severity of P&I hospitalizations; additional analysis of delays in vaccination may impact future military immunization policy.

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IMPACT OF INTERMITTENT PREVENTIVE TREATMENT IN PREGNANCY WITH AZITHROMYCIN-CONTAINING REGIMENS ON MATERNAL NASOPHARYNGEAL CARRIAGE AND ANTIBIOTIC SENSITIVITY OF *STREPTOCOCCUS PNEUMONIAE*, *HEMOPHILUS INFLUENZAE* AND *STAPHYLOCOCCUS AUREUS* AT DELIVERY

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Sulfadoxine-pyrimethamine (S/P) combined with azithromycin (AZI) has the potential for use as intermittent preventive treatment in pregnancy (IPTp) to prevent malaria, treat sexually transmitted infections, and reduce low birthweight. However, the intervention may increase circulation of antibiotic-resistant bacteria associated with severe paediatric infections. We evaluated the effect of IPTp with S/P+AZI compared to a single course of S/P plus chloroquine (S/P+CQ) on maternal nasopharyngeal carriage and antibiotic susceptibility of *Streptococcus pneumoniae, Haemophilus influenzae* and *Staphylococcus aureus* at delivery amongst 854 women participating in a randomised controlled trial in Papua New Guinea. Serotyping was performed, and susceptibility to azithromycin and other antibiotics tested by disk diffusion and Etest. Potential risk factors for carriage were examined. Significantly lower proportions of women who received S/P+AZI had nasopharyngeal carriage of S. pneumoniae (S/

P+AZI: 7.2% [30/418] vs S/P+CQ: 19.3% [84/436], P < 0.001) and H. influenzae (2.9% [15/418] vs 6.0% [26/436], P = 0.028), but not S. aureus (23.7% [99/418] vs 24.7% [105/436], P = 0.892). The number of macrolide-resistant pneumococcal isolates was small, but increased in the S/P+AZI group (13.3% [4/30]) compared to S/P+CQ (2.2% [2/91], P < 0.033). The proportion of S. pneumoniae isolates with serotypes covered by the 13-valent pneumococcal conjugate vaccine was not statistically different by IPTp arm (S/P+AZI: 10.3% [3/29] vs S/P+CQ: 17.6% [16/91], P = 0.352). Many pneumococcal isolates exhibited reduced sensitivity to penicillin (59.5% [72/121]) and trimethroprim-sulfamethoxazole (42.1% [51/121]), irrespective of treatment arm. Although numbers were small, S/P+AZI increased the proportion of macrolide-resistant pneumococci, whilst significantly reducing maternal carriage of *S. pneumoniae* and *H. influenzae*. Future studies on S/P+AZI should evaluate the persistence of maternal macrolide-resistant *S. pneumoniae* and assess the clinical significance of their circulation.

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UNEXPECTED HIGH FREQUENCY OF VIRAL AGENTS IN ASYMPTOMATIC CHILDREN AND LOW INFLUENZA REPRESENTATION IN PNEUMONIA CASES AMONG CHILDREN IN RURAL NORTH PAKISTAN

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Pneumonia is a major cause of child mortality and morbidity in developing countries; the contribution and dynamics of various viral pathogens is not clear, affecting the implementation of optimal prevention and treatment strategies. Oshikhandass, a rural village in NE Pakistan, was previously studied from 1989-1996, when pneumonia was the main cause of mortality in children <5 years (disease incidence 44/100 child years). This study was repeated (after HIB vaccine introduction) with weekly surveillance of children <5 from Apr. 2012-Mar. 2014 to determine changes in epidemiology and describe the frequency of viral agents in nasopharyngeal swabs. PCR from Apr. 2012-Nov. 2013 used the Luminex® platform to detect 20 viruses (RSV, influenza, enterovirus/rhinovirus, parainfluenza, adenovirus, corona virus, metapneumovirus, bocavirus), while the Taqman® method was used Dec. 2013-Mar. 2014 to detect 4 viruses (RSV, influenza). From Feb. 2013, age, sex and neighborhood matched controls with no respiratory symptoms were added to the study. On average, 809 children were followed monthly with 238 pneumonia episodes detected (14.7/100 child years, average age 22.5 months). Pneumonia incidence was highest during winter months with significant variation between the 2012-13 and 2013-14 winters (54.6 and 10.1 episodes/100 child years, respectively). Among all 232 cases tested, 77% had a detectable virus. Detection rates were (cases/controls): RSV 25.0/15.6, influenza 3.4/1.6, enterovirus/rhinovirus 70.3/41.9, parainfluenza 8.8/7.0, adenovirus 4.4/9.3, corona virus 1.1/4.7, metapneumovirus 1.1/0.0, bocavirus 3.3/2.3. Unexpected findings include: 1) a substantial portion (54.7%) of asymptomatic children carried viruses, only slightly below the rate for their matched counterparts with pneumonia (69.0%); 2) the low prevalence of influenza viruses despite high sensitivity of the assay used. Further investigation on severity of disease is underway, but it seems that public health interventions aiming to reduce pneumonia burden should not focus on influenza vaccination as a priority.

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SCHISTOSOMIASIS HAEMATOBIA AND INFERTILITY IN COAST PROVINCE, KENYA

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We examined reproductive patterns, cultural practices surrounding reproduction, and the potential effects of childhood urogenital *Schistosoma haematobium* infection and treatment on adult subfertility among women in Coast Province, Kenya. Previous research has documented an increased risk of subfertility in areas of sub-Saharan Africa due to high rates of pelvic infection, as well as an ecological association between urogenital schistosomiasis prevalence and decreased fertility. This project analyzed findings from 162 interviews with women of childbearing age in a rural, coastal community, linking them to their individual treatment records from a previous 30+ year longitudinal study of parasitic infections. Both quantitative and qualitative findings were included. Reproductive histories suggested a much higher rate of subfertility (43.8%) than worldwide averages (8-12%). Qualitative analysis regarding reproductive practices demonstrated a high saturation of public health messages regarding proper pregnancy care, co-existing with continuing ethnomedical beliefs. Although no significant relationship was demonstrated between Schistosoma infection history and adult subfertility due to the high regional prevalence of schistosomiasis, significant associations were found between age at first treatment and fertility in adulthood, with those treated before age 21 less likely to have subfertility (p=0.001). The high subfertility rate documented in this study suggests the importance of public health programs to prevent and treat pelvic infections in their early stages to prevent reproductive tract damage. The qualitative findings suggest the successful saturation of some public health messages regarding pregnancy care, such as the importance of sleeping under bednets in malaria-endemic regions. However, other messages, such as the importance of seeking prenatal care, were less frequently mentioned. Finally, the findings suggesting the importance of early treatment to prevent the fertility-damaging effects of urogenital schistosomiasis lend further support for programs for universal treatment of children in endemic regions.

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THE IMPACT OF PRAZIQUANTEL GIVEN AT 12-16 WEEKS GESTATION ON PREGNANCY OUTCOMES: RESULTS OF A DOUBLE BLIND, PLACEBO CONTROLLED TRIAL

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Praziquantel was released in 1979, but was never studied in pregnant or lactating women, necessitating its designation as an FDA Pregnancy Class B drug. Given this, and the lack of well-controlled trials evaluating its safety and efficacy in this population, pregnant and lactating women are excluded from treatment programs in many countries where schistosomiasis is endemic. In 2002, based on post-market experience and a concern that Praziquantel would never be formally evaluated in human pregnancy, a WHO informal conference concluded that all schistosomiasis infected pregnant and lactating women should be considered a high-risk group and be offered treatment. Though many nations, particularly in sub-Saharan Africa, adopted this approach, many continue to withold treatment pending more data on safety and efficacy. The objectives of this randomized, double blind placebo controlled trial were to evaluate the safety and efficacy of Praziquantel given to pregnant women infected with *S. japonicum* at 12-16 weeks gestation. Women were enrolled into the study if they provided informed consent and were over age

18, infected with *S. japonicum*, otherwise healthy, and pregnant at 12-16 weeks gestation. Women (N=380) were enrolled and treated with overencapsulated Praziquantel (60 mg/kg in split dose) or placebo and admitted for 24 hours. The following efficacy outcomes were ascertained: birthweight (primary), maternal hemoglobin and iron status at 32 weeks gestation, maternal gestational weight gain, newborn hemoglobin and birth weight. In addition, safety data were collected including toxicology pre and post dosing, abortion and miscarriage rates, and congenital anomalies. Though all analyses for these efficacy and safety outcomes are complete and data locked, we cannot report these until pharmacokinetic studies are completed (expected summer of 2014). We will present the impact of treatment on the aforementioned outcomes. Results from this trial will provide important data from a well controlled study to inform policies regarding treatment of this high risk group.

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IMPACT OF SCHISTOSOMIASIS AND SOIL TRANSMITTED HELMINTHS ON VACCINE-INDUCED IMMUNE RESPONSES

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In light of recent global health initiatives to increase vaccine rates for the world's most vulnerable populations, there is an urgent need to address the issue of vaccine efficacy in populations where helminth infection rates are high. Helminths such as schistosomes are remarkable in their ability to modulate host immune responses, which helps to promote their survival. Immunoregulation begins early in schistosome infection and is characterized by hyporesponsiveness to parasite antigens and other bystander antigens, suggesting that a schistosome infection at the time of vaccination could negatively impact the induction of a protective response to the vaccine. To investigate the impact that concurrent helminth infection might have on how individuals respond to vaccine antigens, we recruited participants from Kisumu Polytechnic College in Kisumu County, western Kenya. At study enrollment participants were screened for schistosomiasis and soil transmitted helminths (STHs) and assigned to a group based on helminth status. The vaccines were then administered: tetanus toxoid (single dose), hepatitis B (doses at 0, 1 and 6 months), and meningococcus A+C (doses at 0 and 2 months). Helminth infections were treated a week after the second hepatitis B boost. A baseline blood draw, a blood draw 2 months after the start of vaccinations and 2 months after the final hepatitis B boost were obtained for evaluation of humoral and cellular immune responses to the vaccine antigens. CD3+/CD4+/CD25high T regulatory cell levels were also determined at each time point to assess their impact on vaccine responsiveness. Preliminary data analysis shows that participants with schistosomiasis had significantly higher proportions of circulating CD3+/CD4+/CD25high T regulatory cells compared to uninfected controls at baseline. At the second blood draw a week after treatment, CD3+/CD4+/CD25high T regulatory cell levels in the schistosomiasis group were significantly elevated compared to baseline levels.

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EVALUATION OF ORGANOMEGALY AND OTHER MORBIDITIES FOLLOWING TWO ROUNDS OF PRAZIQUANTEL MASS DRUG ADMINISTRATION AMONG KENYAN SCHOOL CHILDREN. THE SCORE PROJECT

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One of the goals of schistosomiasis control among school age children is to reduce morbidity. However, monitoring and evaluation of the impact of deworming projects usually is limited to parasitological outcomes as there are not well defined markers of schistosomiasis morbidity other than for persons with severe hepatosplenic disease. In a subset of a larger study comparing mass drug administration approaches in school children, we measured organomegaly and anemia at baseline and after two years to determine whether praziquantel treatment approaches made a difference in morbidity outcomes. Children from 12 schools were enrolled; children from 6 of the schools received school-based treatment (SBT) once after baseline assessment while children from the other 6 schools lived in villages that received community-wide treatment (CWT) once a year. Children were examined for *Schistosoma mansoni* infection using the Kato-Katz method, venous blood was collected to test for malaria parasites and hemoglobin levels, and abdominal ultrasound was performed by a trained ultra-sonographer. The differences between baseline and follow up schistosomiasis prevalence and intensity were not significant for either SBT or CWT schools, or between the treatment approaches. However, ultrasound-detected liver pathology ($P< 0.001$) and anemia ($P< 0.001$) were significantly higher at the 2 year follow up for both groups, as was the percent of students with malaria infection ($P<0.001$). Because anemia and the type of liver pathology we detected are both associated with malaria, we believe that the increased morbidity that we observed at follow-up was likely due to the increased malaria and could have masked any benefits of the schistosomiasis treatments. Our results indicate a need for continued monitoring of morbidity markers in deworming projects, including assessment of co-infecting organisms that can affect morbidity, to determine the best intervention strategies to control *S. mansoni* infection as a public health problem among school age children.

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GLYCAN MICROARRAY-ASSISTED ANALYSIS OF ANTI-GLYCAN ANTIBODY RESPONSES UPON INFECTION OR VACCINATION WITH *SCHISTOSOMA MANSONI*

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Schistosomiasis is a chronic and potentially deadly parasitic disease that affects millions of people in (sub)tropical areas. Immunity to *Schistosoma* can be acquired, but this takes many years of exposure, multiple infections and treatments, and maturation of the immune system. Most antibodies generated are directed against the numerous schistosome glycans, but the precise structure of the glycan antigens and the relation to immunity are poorly understood. Anti-glycan antibodies can be studied efficiently using glycan microarrays. We have generated a microarray containing hundreds of naturally occurring glycans isolated from different life stages of *S. mansoni*. To study the specificity and nature of anti-glycan antibodies in schistosomiasis, we have applied this microarray to the analysis of anti-glycan IgG and IgM in sera from different age groups within an *S. mansoni*-endemic community in Uganda. The most intense IgG responses are against highly fucosylated glycans associated with cercarial and egg glycoproteins and glycolipids. We observed age-dependent differences in anti-glycan responses, especially when considering changes induced by treatment with PZQ. Also within age groups we observed differences between groups with high and low infection intensities. In addition we have used sera from baboons protected against challenge infection after vaccination with irradiated *S. mansoni* cercariae to study longitudinally the development of anti-glycan responses. The strong responses against cercarial lipid-derived glycans as well as cercarial O-glycans are gradually induced by repeated vaccination raising the question if these responses contribute to the high level of protection observed in the vaccinated baboons. Shotgun glycan microarrays allow the definition of groups of schistosome-infected individuals as well as glycan element clusters to which antibody responses are generated in different cohorts and settings.

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MULTIPLEX SEROLOGICAL ASSESSMENT OF SCHISTOSOMIASIS IN MBITA DISTRICT, WESTERN KENYA

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Schistosomiasis control programs centered on mass drug administration (MDA) reduce parasite prevalence and morbidity in treated individuals. Currently, program impact in areas endemic for *Schistosoma mansoni* is measured primarily by assessing changes in infection prevalence and intensity as measured by stool examination. Antibody-based tests potentially have an advantage over currently used antigen-based tests, and a newly developed multiplex bead assay allows for the analysis of multiple antigens at one time. A total of 935 serum samples collected from individuals (1-85 years) living in communities on the shores of Lake Victoria in western Kenya were tested for antibody responses to 36 antigens, including two *Schistosoma* spp. antigens (soluble egg (SEA); adult worm microsomal (Sm25)). Stool and serum samples were collected at three time points: May-July 2012; December 2012; May-July 2013. One MDA was conducted in August 2012. Parasitological data were available for 916 (98.0%) of the samples. Overall prevalence of *S. mansoni* at baseline was 35.6% by stool examination, and modestly decreased to 29.6% 9-11 months after MDA with praziquantel. However, intensity of infection significantly decreased after treatment ($p=0.005$), and importantly, heavy intensity infections decreased from 16.1% to 7.5% ($p<0.05$). Antibody responses to both SEA and Sm25 were associated with age ($p<0.001$), and older individuals were more likely to have higher antibody responses than children under five years. Antibody responses to both antigens were significantly associated with intensity of infection, with responses increasing as egg burdens increased ($p<0.001$). Overall responses to Sm25 significantly decreased after one round of MDA ($p<0.001$). The observed decrease in Sm25 response after treatment has not been previously reported. Additional samples from this study area will be tested to further evaluate this observation. Detection of antibodies to Sm25 or other defined antigens have the potential to be a useful tool for monitoring the impact of schistosomiasis treatment programs.

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THE ADIPOSE TISSUE DERIVED STEM CELLS (ASC) CHANGED THE ACTIVATION PROFILE OF IMMUNE SYSTEM CELLS IN *SCHISTOSOMA MANSONI* ACUTE INFECTION

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Mesenchymal stem cells (MSC) have the ability of self-renewal and differentiation into various mesodermal cell lineages. Recently, it has been observed that MSC have potent anti-proliferative and anti-inflammatory effects in autoimmune and inflammatory diseases as a new strategy for immunosuppression. The control of inflammation in infectious and parasitic diseases by MSC was not evaluated so far. The present study aimed to evaluate if the adipose tissue derived stem cells (ASC) could be able to regulate the inflammation in an experimental acute *Schistosoma mansoni*-infection model by immune system activation analysis. The ASC were isolated from C57BL/6 mice, expanded *in vitro* and characterized phenotypic and functionally. These cells were injected by the tail vein into C57BL/6 mice (n=5) in two, four, six and eight weeks post-infection with *S. mansoni*. Fifteen and thirty days after the ASC injection, the splenocytes were obtained and lymphocytes activation evaluated by the expression of CD25, CD69, CD28 and CTLA-4 molecules. The IL-2, IFN-γ, TNF-α, IL-17, IL-4, IL-6 and IL-10 cytokine levels were measured in the serum blood by flow cytometry. The results showed a decrease ($p<0.05$) in TCD4+ regulation as determined by CD25, CD69 and CTLA-4, mainly six weeks post-infection and after fifteen or thirty days post-injection of ASC. Interesting, the cytokines analysis revealed a different profile in the IL-2 and IL-4 levels. Both cytokines showed significant levels after eight weeks post-infection and fifteen days post-injection of ASC. In conclusion, our results shows that the ASC can modulate the immune response in *S. mansoni*, mainly after six weeks post-infection, and suggest that ASC can be evaluated for the control of the granulomatous reaction in this disease.

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ESTIMATING ACCURACY OF PARTICIPANT RECALL FOLLOWING AN INTEGRATED MASS DRUG ADMINISTRATION FOR NEGLECTED TROPICAL DISEASES

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Coverage Surveys (CS) assess quality of implementation of mass drug administration (MDA) and provide feedback for ministries of health and drug donation programs. CS independently determine if individuals in targeted areas received medication during previous MDA, and rely on individuals' correct recall of medications taken. As neglected tropical disease programs integrate, multiple medications may be offered during MDA, potentially diminishing the strength of CS as a tool. We assessed individuals' ability to recall multiple medications received in integrated MDA, over time. Niger's 2012 MDA distributed ivermectin (IVM), albendazole (ALB), praziquantel (PZQ), and azithromycin (AZM) to eligible individuals. During MDA, observers accompanied distributors and created a Gold Standard Register (GSR), independent from the distributor register. All persons living in households in villages were registered, and it was noted whether or not they took medications, regardless of their eligibility. Households were systematically selected to be revisited for CS at 2, 6, or 12-months post-MDA. During CS, respondents were shown pills. During CS, surveyors and respondents were blinded to MDA register responses. Of 4423 individuals registered from 806 households at GSR, 3455 (78.1%) responded at CS. Response rate at 2, 6, and 12-months post-MDA was 84.4%, 80.7%, and 68.3%, respectively. At GSR, 93.9% (3243 of 3455) ingested one or more medications, while 93.5%, 93.5%, 88.7%, and 91.2%, ingested IVM, ALB, AZM, and PZQ respectively. At CS, 95.1% (3287 of 3455)recalled having ingested one or more medications, while 86.2%, 88.7%, 89.0%, and 82.0%, recalled ingesting IVM, ALB, AZM, and PZQ, respectively. IVM concordance (% agreement between GSR and CS) at 2, 6, and 12-months post-MDA was 82.1% (95% CI: 79.1-85.1%), 86.6% (CI 83.6-89.6%), and 80.8% (CI 77.2-84.4%), respectively. ALB concordance at 2, 6, and 12-months was 85.2% (CI 82.5-87.9%), 87.3% (CI 84.5-90.0%), and 85.0% (CI 81.8-88.3%), respectively. AZM concordance at 2, 6, and 12-months was 79.9% (CI 76.7-83.2%), 82.9% (CI 79.2-86.5%), and 82.5% (CI 79.2-85.8%), respectively. PZQ, concordance at 2, 6, and 12-months was 74.5% (CI 70.9-78.2%), 82.6% (CI 79.6-85.7%), and 80.3% (CI 77.0-83.6%), respectively. CS correctly measured overall MDA coverage, though it is less reliable for drug-specific coverage. We confirm the strength of CS as an MDA evaluation tool.

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THE SOCIO-ECONOMIC IMPACT OF CONTROL OR ELIMINATION OF FIVE NEGLECTED TROPICAL DISEASES

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There is renewed commitment to control and eliminate neglected tropical diseases (NTDs) as defined by the WHO and endorsed by the London Declaration. We estimated the economic impact of meeting the targets for the five diseases eligible for mass drug administration: lymphatic filariasis, onchocerciasis, schistosomiasis, soil-transmitted helminthiasis, and trachoma. We performed a systematic literature review to identify information on costs related to the diseases or related health outcomes, considering out of pocket payments (OPP), productivity loss, and impoverishment. Cost estimates per person were combined with projections of the number of people suffering from clinical manifestations per NTD, country, and year, for the periods 2011-2020 and 2021-2030, comparing the ideal scenario in which the targets are met with a counterfactual scenario (assuming that the prevalence (%) of clinical manifestations remains constant at its pre-control level). The averted burden is calculated as the difference between the two scenarios. The limited information available on the costs of illness shows that the productivity loss associated with clinical manifestations are about 3% for infestation and mild symptoms, about 15% for more severe manifestations like lymphedema and hydrocele, and as high as 38% and 79% for severe vision loss and blindness. The total productivity costs averted globally by reaching the goals for the five above-mentioned diseases may amount to US$240 billion in the period 2011-2020 and US$390 billion in the period 2021-2030, even excluding OPPs as well as productivity loss due to premature mortality. Soil-transmitted helminthiasis accounted for approximately half of this amount. There is considerable uncertainty in these estimates because of scarce literature. Nevertheless, even with conservative assumptions, the averted costs of achieving the London Declaration targets are high. Results will be discussed with respect to the impact on poverty.

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SHOULD SCHOOL BASED DEWORMING BE SUBSIDIZED? LONG RUN EVIDENCE FROM A RANDOMIZED CONTROL TRIAL IN KENYA

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Recent research suggests that preventative and non-acute health investment in the developing world are often highly responsive to subsidies, but what level of subsidy should governments provide? Using data from a deworming randomized control trial in Kenya this paper shows that by combining data on take up elasticities with data on labor market impacts and tax rates one can establish the optimal subsidy. To derive this optimal subsidy we first develop a framework where individuals may generate a positive fiscal externality, through consumption or labor income tax, by investing in human capital. Applying this framework to the case of deworming in Kenya, we find that fully subsidizing child deworming raised adult earnings generating sufficient future gains in government revenue to make possible Pareto improving reductions in tax rates. Consistent with previous reports, we estimate differing labor market impacts of child health investments by gender. Ten years after the start of the deworming program, men who were eligible to participate as boys work 3.5 more hours each week, spend more time in entrepreneurship, are more likely to hold manufacturing jobs with higher wage earnings, and have higher living standards. Women, who were eligible as girls, have better educational outcomes, are more likely to grow cash crops, and reallocate labor time from agriculture to entrepreneurship. These results suggest that health interventions that are too late in life to affect cognition or height can still have long-run impacts on labor market outcomes by affecting the amount of time people spend in school or work.

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THE IMPACT OF MASS DRUG ADMINISTRATION WITH ALBENDAZOLE ALONE ON LYMPHATIC FILARIASIS IN THE REPUBLIC OF CONGO

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Implementation of mass drug administration (MDA) with ivermectin plus albendazole (Alb) for lymphatic filariasis (LF) has been delayed in Central Africa, because ivermectin sometimes causes severe adverse events in people with very high *Loa loa* microfilaremia (Mf). Albendazole has activity against *Wuchereria bancrofti*, and it is safe for use in patients with loiasis, and WHO has recently recommended use of Alb MDA together with vector control for control of LF in areas with co-endemic loiasis. We are presenting early results from a planned 3-year community trial that is assessing the impact of MDA with Alb on LF in a village of the Republic of Congo. Baseline results (September 2012) from 773 subjects revealed a filarial antigenemia rate of 17.3% (ICT) and a Mf rate of 5.3% (140 µl night blood smear). The population was offered 400 mg of Alb at baseline and 6 months later. Therapeutic coverage for the population > 2 years of age was ~ 85%. A second cross-sectional evaluation at 12 months (741 tested) showed that Alb MDA had not yet reduced ICT or Mf rates in the community (16.6 and 4.2%); however, Mf counts in Mf-positive subjects were reduced by 60% (geometric mean reduced from 199.4 to 79.6 Mf/ml, $P = 0.01$). The effect of Alb was more dramatic in those 38 people who were Mf positive at baseline and retested at 12 months: 37% had total Mf clearance, and Mf counts in those with >10 Mf/ml at baseline were reduced in average by 82.1% (range: 4.3-100%). In addition, ICT scores (a semi-quantitative measure of filarial antigenemia) were also reduced after Alb MDA. MDA also dramatically reduced the hookworm infection rate from 6.5% to 0.6%, with less impressive effects on *Ascaris* and *Trichuris*. These preliminary results suggest that semiannual community MDA with Alb is a useful tool for control of LF and STH that is feasible for use in areas where loiasis is co-endemic. Evaluation at 24 months is planned for October 2014.

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COST-EFFECTIVENESS OF COMMUNITY-WIDE, INTEGRATED PREVENTIVE CHEMOTHERAPY FOR SCHISTOSOMIASIS AND SOIL-TRANSMITTED HELMINTHS

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World Health Organization (WHO) recommendations for preventive chemotherapy against helminth infections emphasize treatment of school-age children. We aimed to evaluate the cost-effectiveness of expanding integrated preventive chemotherapy to adults in high-burden settings. We developed a dynamic, age-structured transmission and cost-effectiveness model that simulates integrated preventive chemotherapy programs

for schistosomiasis and soil-transmitted helminths (STH). We utilized data on prevalence and intensity of infection with *Schistosoma mansoni* and STH from surveys in Côte d'Ivoire, and simulated a community of 15,000 children and adults. Transmission parameters were calibrated according to prevalence and dispersion of each worm. We simulated drug administration with praziquantel+albendazole among: (i) school-age children only or (ii) school-age children and adults. We assumed 75% coverage for the intervention. Treatment costs for a child and adult were estimated at US$1.09 and US$1.94, respectively. The incremental cost-effectiveness ratio (ICER) was calculated in 2014 US$ per disability-adjusted life year (DALY) averted, comparing expanded treatment of both child and adult populations against current WHO guidelines that target children alone. We defined strategies as highly cost-effective if the ICER was less than the GDP per capita of Côte d'Ivoire (US$1,244 in 2014). An integrated preventive chemotherapy program for schistosomiasis and STH was highly cost-effective in treatment of children alone (ICER: US$620/DALY averted) compared to no treatment. Expanded coverage of both children and adults (ICER: US$653/DALY averted) was highly cost-effective compared to treatment of children alone, and remained highly cost-effective even if treatment costs for adults were 4-fold greater than school-based strategies. Expanded treatment of adults yielded a 50% increase in DALYs averted for children, suggesting that treating adults can lower the prevalence and infection intensity in children. Integrated, community-wide preventive chemotherapy programs for schistosomiasis and STHs may be highly cost-effective. These results support re-evaluating global guidelines for helminth control programs and possibly expanding coverage to adults.

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SOCIOECONOMIC INEQUALITIES IN THE BURDEN OF NEGLECTED TROPICAL DISEASES

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It is generally assumed that neglected tropical diseases (NTDs) are concentrated in poor populations, but evidence remains scattered. We describe between and within country poor-rich inequalities in burdens of London Declaration NTDs, including NTDs controlled through preventive chemotherapy (onchocerciasis, blinding trachoma, lymphatic filariasis, schistosomiasis, soil-transmitted helminths (STH)) and those controlled through intensified disease management (Chagas disease, leprosy, visceral leishmaniasis, human african trypanosomiasis (HAT)). First, using data from the global burden of disease 2010 study, we examined to which extent each NTD is concentrated in low and lower-middle income countries based on Gross National Income per capita. Second, we conducted a systematic literature review on the socioeconomic distribution of NTD prevalence within countries, including publications between 2004 and 2013. The vast majority (84-100%) of the global burden of each NTD in 2010 is concentrated in low and lower-middle income countries, with the exception of STH (63% of the global burden) and Chagas disease (8%). The concentration in low income countries is strongest for HAT (92% of the global burden), followed by onchocerciasis (55%) and schistosomiasis (45%). For many NTDs the burden per 100.000 population has declined since 1990, but low income countries have benefitted less than lower-middle and upper-middle income countries from control initiatives. Evidence on the socioeconomic distribution of NTD prevalence within countries is scarce for some NTDs like onchocerciasis and HAT, but more readily available for schistosomiasis and STH. Most studies report a gradient in NTD prevalence, with highest prevalence among lower socioeconomic groups. The magnitude of inequalities varies, but often prevalence is at least twice as high in lower compared with higher socioeconomic groups. Future NTD control as pursued by the London Declaration will benefit the poorest countries and within these countries the poorest populations.

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AN INTEGRATED SENTINEL SITES APPROACH TO EVALUATING THE IMPACT OF MASS DRUG ADMINISTRATION ON NEGLECTED TROPICAL DISEASES

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Preventative chemotherapy for the Neglected Tropical Diseases (NTDs) is provided through mass drug administrations (MDA). Although the interventions for different NTDs are often integrated, the protocols to evaluate MDA impact are disease specific. We field-tested an integrated impact survey to evaluate feasibility and determine if this low resource method could be used by the trachoma program to decide when to conduct their more resource-intensive impact surveys. We validated the trachoma data with the WHO recommended cluster survey. Impact evaluations were conducted in Gwadabawa district, Nigeria and Dô district, Burkina Faso (BF). We implemented sentinel sites over a 3-year period as currently done by the lymphatic filariasis (LF) program but added schistosomiasis and soil-transmitted helminthes (STH). Four sites per district were selected based on NTD prevalence and geographic representativeness. In each district, 2000 children aged 1-9 years in 4 sites were clinically examined for signs of trachoma using Trachomatous Inflammation - Follicular prevalence (TF) as indicator. In addition, 1600 children (80 per cluster, aged 1-9 years) were examined using the WHO cluster survey method. In Nigeria, TF in year 1 was 29.8%, 14.3%, 21.0%, and 16.3% per site, and cluster survey prevalence was 16.4% [95% CI: 14.8-18.0]; for year 2, TF was 4.75%, 9.25%, 6.00%, and 8.75% and 10.9% [95% CI: 9.5-12.5]; for year 3, TF was 10.0%, 16.0%, 9.0% and 15.0%, and 17.2% [95% CI: 14.8-19.7]. In BF, TF in year 1 was 9.6%, 10.2%, 15.2% and 7.9%, and cluster survey prevalence was 5.9% [95% CI: 3.8-7.6]; for year 2, TF was 1.6%, 2.6%, 5.0%, and 1.4% and 1.6% [95% CI: 0.0-3.71]; for year 3 TF was 0.8%, 3.0%, 2.8%, and 0.8%, and 1.8% [95% CI: 0.0-3.9]. Schistosomiasis prevalence for sites in BF in year 1 was 0.3%, 0.0%, 0.0%, and 0.0%; for year 2, 0.3%, 0.0%, 0.0%, and 0.0%; for year 3, 0.0% in all sites. STH prevalence for sites in BF in year 1 was 0.0%, 0.0%, 0.0%, and 0.3%; for year 2, 0.3%, 0.0%, 0.3%, and 0.0%; for year 3, 0.7%, 0.3%, 2.3%, and 0.3%. LF prevalence for sites in BF in year 1 was 0.0% in all sites and in year 3, 0.3%, 0.0%, 0.5%, and 0.0%. We conclude that integrated sentinel sites are feasible. Prevalence trends between sentinel sites and trachoma cluster surveys in both countries indicated that using sites might be a reliable indicator to determine when the prevalence is low enough to conduct the WHO recommended cluster survey.

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CAN WE IGNORE THE INFORMAL PROVIDERS FOR THE TREATMENT OF CHILD DISEASES IN INDIA?

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Diarrhea is one of the two leading child killers in India. When asked about care seeking behavior for a child with diarrhea, 83% of caregivers in Uttar Pradesh, India, reported going to a private "doctor". Due to the dearth of qualified health providers in rural India, the private doctor (called Rural Health Provider – RHP) is generally an unqualified provider who nevertheless, has gained the trust of the community. Childhood diarrhea is optimally treated through a short course of ORS and zinc. However, the RHP routinely dispenses unnecessary antidiarrheals and antibiotics. Under the Diarrhea Alleviation through ORS and Zinc (DAZT) project, FHI 360 partnered with local NGOs to identify the RHPs and educate them on the improved treatment of diarrhea through ORS and zinc. The trained NGO

health worker, emulating the marketing strategy of the pharmaceutical industry, repeatedly visited the RHP to provide him with the benefits of ORS and zinc treatment. The partners initially listed 27 000 RHPs and drug sellers in 12 districts representing a population of 34 million people. After categorizing the RHPs according to their work load and potential, the list was reduced to 22 000 RHPs. A tracking study in August 2013 showed that 54% of RHPs dispensed ORS and zinc treatment from a baseline of 23% ORS use and 1% zinc use in 2010. With the increasing demand for ORS and zinc, the pharmaceutical companies became motivated to supply to the rural areas. The market became large enough to attract several companies who are maintaining the demand and providing uninterrupted supply to the RHPs. As a sign of a sustainable market, 42 brands of zinc were inventoried in the area of intervention, up from seven at the start of the intervention three years ago. Changing RHPs' treating behaviors towards rational treatment of critical childhood diseases shows potential to improve health outcomes. This simple approach could be replicated for other MNCH interventions in an integrated way aiming at reducing maternal, newborn and child mortality.

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PREVALENCE OF ANEMIA AND FACTORS ASSOCIATED WITH SEVERE ANEMIA AMONG UNDER FIVE CHILDREN ADMITTED AT BUGANDO MEDICAL CENTRE, MWANZA, NORTHWESTERN TANZANIA

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Anemia is a major public health problem in developing countries, contributing significantly to morbidity and mortality among under-fives. About 43% of under-fives are anemic worldwide, and two-thirds reside in Sub Saharan Africa. Even where blood transfusion is available for treatment there is still a significant case fatality rate of 6-18%. This study aimed to determine the prevalence of anemia, factors associated with severe anemia and morphological types of anemia among anemic under-fives admitted at Bugando Medical Centre (BMC). This hospital-based, cross-sectional study was conducted between November 2012 and February 2013. Selected laboratory investigations were done using standard operating procedures. A total of 448 under-five children were eligible but detailed history taking and physical examination was available on 309. The overall prevalence of anemia was 77.2% (346/448). Mild, moderate and severe anemia were 16.5%, 33% and 27.7% respectively. Of 239 children with moderate and severe anemia 22.6% (54/239) had iron deficiency. Majority of the anemic children (37.5%) had microcytic hypochromic anemia. The factors associated with severe anemia included malaria parasitaemia [OR = 4.0 95% CI (2.1 - 7.8); p-value <0.001], presence of sickle haemoglobin [2.0 (1.1 - 3.5); 0.018] and unemployment of the parent [2.2 (1.2-4.0); 0.007]. In conclusion, the prevalence of anemia among underfives admitted at BMC was high. Iron deficiency anemia was the leading type. Factors strongly associated with severe anemia were malaria parasitaemia, presence of haemoglobin S and unemployment among caretakers.

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EFFECT OF LOW BIRTH WEIGHT AND INTRA-UTERINE GROWTH RESTRICTION ON STUNTING AND WASTING IN A COHORT OF INFANTS IN THE PHILIPPINES

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The proportion of babies born low birth weight (LBW) and intra-uterine growth restricted (IUGR) has increased over the past few decades in both high and low-income nations. A recent meta-analysis in Europe found that after controlling for socio-economic factors, LBW had a negative effect on health outcomes during childhood. In other studies, LBW infants had lower mean weight, length, and head circumference at one year of age. However, these studies have been conducted in higher income settings, and it is expected that infants in lesser-developed settings may be even less likely to experience catch up growth. The aims of this study were to compare rates of stunting and wasting at six and 12 months of age among LBW, IUGR, and normal weight infants born in Leyte, Philippines. Pregnant women were recruited at 12-16 weeks gestation as part of an NIH-funded trial. Newborns were weighed within 48 hours of delivery, and LBW was defined as weight < 2.5 kg, and IUGR as weight <3rd percentile for gestational age. WHOAnthro was used to derive height-for-age (HAZ) and weight-for height (WHZ) Z-scores at six and 12 months old. We found that overall 20.6% (72/349) of infants were LBW, 24.6% (86/349) were IUGR, and 2.3% (8/349) were premature. These findings suggest that the primary cause of LBW was IUGR rather than prematurity. Overall, LBW and IUGR infants had significantly lower mean HAZ and WHZ at six and 12 months of age than normal infants. The odds ratio (OR) for stunting (HAZ< -2.0) at 6 months of age among LBW infants compared to normal infants was 3.56 (P = 0.0002), and for wasting (WHZ < - 2.0) was 2.13 (P= 0.0002). At 12 months of age, the ORs for stunting and wasting were 2.09 (P = 0.061) and 1.16 (P= 0.6217), respectively. These results suggest that in this low-resource setting where infants often lack sufficient macro and micronutrients required for the rapid growth of infancy, catch up growth may be significantly delayed if it occurs at all. Further follow up of these children will be conducted to determine whether these effects persist throughout childhood.

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LOW QUALITY EVIDENCE OF EPIDEMIOLOGICAL STUDIES ON LEISHMANIASIS IN BRAZIL: THE LESS OBVIOUS OBSTACLE FOR CONTROL INITIATIVES

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According to recent estimates for Brazil, leishmaniasis accounted for 16,233 DALYs and 280 deaths in 2010, representing a higher disease burden than malaria or dengue. Currently, no implemented control efforts have proven effective given leishmaniasis incidence in the country has not declined and new foci continue to emerge. As observational epidemiological studies are a key component for developing control measures, we reviewed a decade of Brazil's scientific publications on leishmaniasis epidemiology and evaluated the quality and evidence of the studies. In a PRISMA structured literature search of PubMed, MedLine, ScieLO and LiLACS databases, we obtained 2,011 articles from the search terms "leishmaniasis" and "Brazil" published from 2002 to 2012. Only 14% (n=283) of articles were found to be epidemiological studies that incorporated human subjects. Predominant study type was descriptive (53.4%, n=151), followed by cross-sectional (20.8%, n=59), case-control (8.5%, n=24) and cohort (6.0%, n=17). Study design was not stated in 46.6% (n=120) of publications; in addition, 24 (17.5%) studies incorrectly reported study design. Majority of studies were conducted in a single municipality (62.9%, n=178) and did not include healthy controls (78.1%, n=221). Only 66.4% (n=188) of publications had an English version of the full text. Mean journal impact factor of all publications was 1.9 (± 1.6), with only 6% (n=17) of articles being published in journals with an impact factor higher than 4.0. Our findings demonstrated the majority of epidemiological studies reviewed here did not provide sufficient evidence for creating population-based interventions. The abundance of descriptive studies conducted in Brazil is not cost-effective, creates bad habits in the next generation of researchers and hinders the development of novel control strategies. Solutions include encouraging epidemiology courses

in biology and medical programs that emphasize how to evaluate quality of epidemiological studies and discouraging graduate projects based on describing leishmaniasis cases in an area.

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PAPER-BASED YEAST BIOSENSORS FOR ANTIBIOTIC DETECTION

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Paper-based tests have the potential to serve as inexpensive tools to address analytical questions both inside and outside of the laboratory setting. In this study, Saccharomyces cereviscIae were used to construct the first example of a yeast whole-cell, paper-based biosensor device. This test is sensitive to antibiotics in the tetracycline family and could potentially address questions of pharmaceutical quality as well as antibiotic contamination in liquids. This biologically-based paper analytical device or "BioPAD" can qualitatively discriminate the presence/absence of doxycycline over a range of 30 - 10,000 µg/mL. Using a BioPAD, a doxycycline dosage form (tablet) commonly used for malaria prophylaxis, was confirmed to contain the antibiotic with 92% and 95% success, evaluated by eye and computer-assisted image analysis respectively, with no false positives by either method. Stored at 4°C these tests were found to remain viable for greater than a year. This research demonstrates the utility of whole yeast cells in paper-based pharmaceutical testing, while highlighting the potential for the development of yeast-based BioPADs to address a range of qualitative analytical questions, especially in low resource settings.

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EFFECT OF COOKING METHODS ON THE CONCENTRATION OF OXYTETRACYCLINE RESIDUE IN CHICKEN

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Antibiotics are used in poultry industry to obviate disease, enhance growth and increase production. However, the use of these veterinary drugs often results in the accumulation of violative levels of residues in tissues. Consumption of such poultry meat would potentially adversely affect human health through the development of resistant pathogenic microorganisms and hypersensitivity reactions in sensitized individuals. Although meat is always heat treated before consumption, which should ordinarily render the residues innocuous, some drugs are heat stable and therefore would persist at residue violative levels even after heat treatment. Since tetracyclines are the most frequently used antibiotics in poultry production in Nigeria, this study was therefore embarked up on to find out the effect of cooking methods (boiling, microwaving and roasting) on the concentration of oxytetracyclines (OTC) in poultry meat and organs. Muscle and liver tissues were harvested from birds that were treated with OTC either by intramuscular injection or orally in drinking water and analysed for residues using the three plate test (TPT) and enzyme-linked immunosorbent assay (ELISA). TPT at two different pH levels reduced the inhibition zones of raw muscles between 34-49%, 67-69.6% and 53-56% for microwaving, boiling and roasting respectively but the difference in the means were not statistically significant (P>0.05). TPT however, significantly (P < 0.05) reduced the inhibition zones of raw liver between 79- 80.9%, 57-60.29% and 88-89.71% for microwaving, boiling and roasting respectively, at both pH levels. ELISA determined a slight increase in mean OTC concentration in microwaved (1.2%) and roasted (0.3%) muscle tissues with a slight decrease by boiling (3.5%) but the differences were not statistically significant. A significant (P<0.05) decrease in OTC concentration was however noticed between raw microwaved and roasted liver samples by ELISA at microwaving (1.85%), boiling (2.83%) and roasting (3.17%) respectively. Roasting has a higher reduction effect on oxyteracycline residue.

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EXPLORING THE EFFECTIVENESS OF AN EDUCATION INTERVENTION DELIVERED THROUGH LOCAL GROCERIES TO IMPROVE CHILD FEEDING PRACTICES IN RURAL COMMUNITIES. A CASE CONTROL STUDY

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Malnutrition is one of the most life-threatening conditions in early childhood. It causes about 35% of all deaths in children below 5 years and 50% of child mortality in sub-Sahara Africa. The condition can be effectively addressed by local solutions such as using local food grocers who on daily basis make contacts with child caregivers. Using case-control design in 5 villages in rural south eastern Tanzania, we evaluated an intervention involving training local grocers to select and provide appropriate meals to caregivers with children below two years. The trained grocers also trained their client caregivers on appropriate feeding of children. Ten local food grocers (2 per village) were recruited and each grocer was assigned to 5 randomly selected households with children <2 years. The grocers were retrained monthly, and were asked to help select appropriate foods for and advice their clients on appropriate feeding practices. Monitoring was done monthly. Primary outcome was performance of caregivers on key feeding indicators: breastfeeding, caloric density, nutrients density and food safety and feeding style. Main secondary outcome was growth, measured by length and weight and Z-scores for weight-for-age and length-for-age. We enrolled 27 children from intervention and 27 from control groups and all caregivers in intervention group received recommended training from the assigned grocers. Children in intervention group had higher breastfeeding scores than controls (25 [93%] vs 20 [74%], p = 0.068), higher caloric density scores (22 [81%] vs 21 [78%], p=0.735), higher scores on nutrient density/diversity (22 [67%] vs 14 [52%], p = 0.268) and higher scores on recommended food safety and feeding style targets than control (23 [85%] vs 12 [44%], p = 0.002). Overall, intervention group performed better in all indicators of good feeding practices than controls (19 [70%] vs 8 [29%], p=0.003). Local food groceries in rural areas, if empowered with knowledge on proper feeding practices and malnutrition can improve child feeding practices and nutritional status of children. Since mothers and caregivers make more contact with food grocers in their communities than they do to health facilities, this approach could significantly improve coverage with appropriate nutritional education.

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NUTRACEUTICALS: THE WAY FORWARD TO PREVENT ADVERSE HEALTH EFFECTS OF A CASSAVA-DOMINATED DIET

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More than 600 million people rely on drought-tolerant cassava as staple food, mostly under the tropics. Farming systems are dominated by harsh environmental conditions that mostly allow growing bitter cyanogen-containing cassava (manioc a.k.a tapioca or yuca) as staple crop. In times of war or agro-ecological crisis (and food shortages), populations are forced to adopt shortcuts in cassava processing , exposing themselves to cyanide poisoning and outbreaks of konzo, a permanent and irreversible paralytic disease. Cyanide is a mitochondrial toxin known to induce oxidative damage as evidenced by ongoing research that indicates high serum levels of isoprostanes F2 in proportion to motor and cognitive deficits in children relying on insufficiently processed cassava as staple

food. In anticipating interventional trials, we asked whether local food crops may be used as nutraceuticals (food with health benefits) to mitigate the neurotoxicity effects of cassava. We analyzed methanolic extracts of green vegetables (n=12), mushrooms (n=8), yams (n=2) and herbal teas (n=2) varieties that are consumed in a konzo-affected area using the 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS•+) free radical-scavenging assay. Scavenging actvities of methanolic extracts (2 - 40µg/ml) were compared using trolox and quercetin as references. The phytochemical screening was done by Thin Layer Chromatography (TLC) and High-Performance Liquid Chromatography with Diode-Array Detection (HPLC-UV/DAD) using Quercitrin, hyperoside, rutin and chlorogenic acid as references. Most extracts displayed efficient concentration-dependent inhibitory effects. The scavenging activity of extracts (mean ± SEM as reported) aligned as follows: herbal teas > green vegetables > yam > mushrooms. TLC and HPLC-UV/DAD analysis has demonstrated the presence of polyphenolic compounds, which may explain the observed free radical-scavenging effects. In conclusion**,** green vegetables contain rutin (cassava leaves and hibiscus), hyperoside (in hibiscus), and chlorogenic acid (in raphia) lookalike compounds, which may explain their antioxidant activities. Select food crops may be promoted as nutraceuticals in campaigns to mitigate the health effects of cassava neurotoxicity when adherence to modern interventional trials is not guaranteed.

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THE BURDEN OF RABIES IN TANZANIA AND ITS IMPACT ON LOCAL COMMUNITIES

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Rabies remains a major public health threat in many parts of the world and is responsible for an estimated 55,000 human deaths annually. The burden of rabies is estimated to be around US$20 million in Africa, with the highest financial expenditure being the cost of post-exposure prophylaxis (PEP). However, these calculations may be substantial underestimates because the costs to households of coping with endemic rabies have not been investigated. We therefore aimed to estimate the household costs, health-seeking behaviour, coping strategies, and outcomes of exposure to rabies in rural and urban communities in Tanzania. Extensive investigative interviews were used to estimate the incidence of human deaths and bite exposures. Questionnaires with bite victims and their families were used to investigate health-seeking behaviour and costs (medical and non-medical costs) associated with exposure to rabies. We found that a large proportion of bite victims do not obtain PEP (28%) and that compliance is relatively poor (~10% drop out rate) amongst those who do obtain PEP. The average costs incurred by bite victims was ~US$40 but varied from US$0 (for ~16% of bite-victims who were provided PEP free-of-charge and the further 28% of victims who did not seek PEP) to over US$300 (for patients with complicated dog bites). We calculated that an average patient in rural Tanzania, where most people live on less than US$1 per day, would need to spend over US$100 to complete WHO recommended PEP schedules. High costs and frequent shortages of PEP led to poor compliance with PEP regimens, delays in presentation to health facilities, and increased risk of death. The true costs of obtaining PEP were twice as high as those previously reported from Africa and should be considered in re-evaluations of the burden of rabies.

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PATHOGEN REDUCTION COMBINED WITH RAPID DIAGNOSTIC TESTS TO REDUCE THE RISK OF TRANSFUSION TRANSMITTED INFECTIONS IN SUB-SAHARAN AFRICA

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A safe and adequate supply of blood is critical to improving health care systems in Sub-Saharan Africa. The region is burdened by a high prevalence of diseases transmissible by transfusion including HIV, HBV, HCV, and malaria. Current strategies to reduce transfusion-transmitted infections include screening with either more sensitive, but more expensive enzyme immunoassays or less expensive, but generally less sensitive rapid diagnostic tests (RDTs). Pathogen reduction, a developing strategy to improve blood safety, uses a nucleotide binding additive and ultraviolet light to irreversibly inactivate pathogen nucleic acids, effectively reducing pathogen load. It is possible that pathogen reduction in combination with RDTs could be both more effective and more cost-effective in reducing transfusion-transmitted infections compared to current practices. A proof of concept analysis was performed to determine the efficacy of pathogen reduction combined with RDTs compared to current screening methods used by the Uganda Blood Transfusion Service (enzyme immunoassays for HIV, HBV, and HCV; no testing for malaria). For HIV, HBV, HCV, and malaria, probability models were created to determine the risk of an infectious unit being released into the blood supply, accounting for RDT test performance, pathogen reduction capability, pathogen infectivity, and disease prevalence in the donor population. RDTs were chosen based on their availability in Uganda. Terumo, the producer of the Mirasol pathogen reduction system, provided data on the effectiveness of pathogen reduction for each pathogen. Probability models of RDT screening and pathogen reduction yielded a calculated risk of an infectious unit entering the blood supply of 0.006, 5, 0.6, and 150 per 10,000 units collected for HIV, HBV, HCV, and malaria, respectively. The relative risk reduction compared to current methods in Uganda is 88%, 20%, 95%, and 83% for HIV, HBV, HCV, and malaria, respectively. Proof of concept analysis reveals that screening with RDTs combined with pathogen reduction could be effective in reducing the risk of infectious units entering the blood supply. This could vastly reduce the incidence of transfusion-transmitted infections, decreasing economic and social costs of new infections.

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UNDERSTANDING DISEASE AND ACCESS TO HEALTHCARE IN ISOLATED COMMUNITIES OF THE PERUVIAN AMAZON

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Isolated Peruvian Amazon communities have limited healthcare access and infrequent visits from boat-clinics. Research concerning the health and health beliefs of such communities is scarce. This pilot project assessed disease understanding and pain burden, use of traditional and modern medicine, and healthcare access. Whilst accessing healthcare from a mobile boat-clinic, 85 participants from 13 Peruvian Amazon communities completed a locally-adapted questionnaire detailing socioeconomic position, medical history, understanding of diagnosed illnesses, pain, and use of traditional and modern medicine. 21% of respondents had completed secondary school, 87% lived in crowded houses, and 35% had gone to bed hungry in the past month. 73% had received a prior diagnosis from a healthcare professional: the most common being hypertension, malaria and urinary infections. 17%(13/78) felt they fully understood their diagnosed illness, 43%(34/78) had no understanding at all, and 80% (62/85) wanted further education. 98%(82/84) reported pain, mainly head or musculoskeletal. Most pain (69%) had not received formal diagnosis,

was experienced daily (53%) and treated successfully with modern rather than traditional medicine (72% [95%CI 63-82] vs 19% [95%CI 11-28], p<0.001). Respondents were more likely to have used (89% [95%CI 83-96] vs 71% [95%CI 61-80], p=0.002) and less likely to have refused (5% [95%CI 2.1-9.3] vs 27% [95%CI 17-36], p<0.001) modern rather than traditional medicine. 21% cited long waits and distance as reasons to avoid local healthcare posts. 95% had previously attended a medical boat. In conclusion, amongst pilot project respondents, there was a lack of understanding of diagnosed illnesses and a desire for further education. There was a high burden of undiagnosed pain, likely linked to agricultural work. While traditional and modern medicine were both used, more patients had taken modern medicine, especially analgesics. Individuals avoided using local healthcare posts due to limited access and almost all had had prior contact with mobile boat-clinics. Mobile boat-clinics may have a unique opportunity to target locally relevant healthcare issues (sun-exposure and headaches, self-physiotherapy for musculoskeletal pain) with simple educational interventions (pamphlets, community-advocate training) whilst healthcare infrastructure improves.

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PAPER ANALYTICAL DEVICES FOR THE SCREENING OF SUBSTANDARD MEDICATIONS IN WESTERN KENYA: A COMPARISON OF TEST RESULTS

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Low quality medications are a global health challenge that has been associated with death and the development of drug resistance. The Paper Analytical Device (PAD) combines liquid chromatography and color reactions on a patterned paper to form an inexpensive method to screen for substandard drugs. The PAD overcomes challenges encountered, mostly in developed countries, in monitoring the quality of drugs, that is, cost of analysis and inadequate technical expertise. We aim to compare the interpretation of test results obtained in the optimization and validation of the PAD. The project's study site is Moi Teaching and Referral Hospital - Academic Model Providing applies Access To Healthcare (AMPATH). Samples of amoxicillin, ampicillin, amoxicillin-clavulanic acid tablets, azithromycin, ciprofloxacin and acetaminophen tablets were purchased using secret shoppers from registered and unregistered pharmacies in western Kenya. To determine the medication content, the user applies each sample onto the PAD and dips one edge of the PAD into water. After development, the user takes a picture of the utilized PAD and employs a color based key to interpret the results. The interpretation of the PAD test results is carried out by Kenyan pharmacy personnel and University of Notre Dame collaborators and the outcomes reported. The PAD outcomes are reported as "Pass" for samples which displayed similar color patterns to the standard or "Suspicious" for samples which did not. These drugs samples are also being analyzed using high performance liquid chromatography (HPLC) in order to validate the PAD. Of the 139 samples tested in Phase 1 and number of outcomes reported and compared, we found a reader agreement of > 90% in the interpretation of PAD test results. The high reader agreement indicates that the PAD test results can easily be interpreted and therefore may be used to address the challenge of inadequate technical expertise in resource limited settings. We also, anticipate having HPLC results for tested drug samples over the next few months.

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DETERMINANTS OF COVERAGE AND AVAILABILITY OF ZINC AND ORAL REHYDRATION SALTS (ORS) IN THREE NIGERIAN STATES

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Globally, diarrhoea is responsible for 11% of all under-five deaths in 2010, and Africa accounts for over 40% of the global diarrhoea mortality burden. In Nigeria, it is the third largest cause of death in children under age five after malaria and pneumonia. While it is well established that WHO recommended treatments such as Oral Rehydration Salts (ORS) and Zinc can prevent the majority of diarrhoea associated deaths, the coverage of these treatments is not ubiquitous in Nigeria. It is estimated that only 25% of children with diarrhoea receive ORS and less than 1% receive zinc. In light of this, Clinton Health Access Initiative (CHAI) and Nigeria's Ministry of Health (MOH) established a 4-year program with the goal of scaling up the use of ORS and zinc for treatment of childhood diarrhoea in 8 Nigerian States. By increasing the use of Zinc and ORS to 80% in four years, it is estimated that the scale-up will save over 228,000 lives. The program aims to create a competitive market for ORS and Zinc by the end of 2015 by 1) securing conducive policy environment; 2) ensuring availability and affordability of zinc and ORS 3) increasing provider awareness about Zinc and ORS; and 4) generating awareness and demand among caregivers. The purpose of the study is to evaluate the efficacy of CHAI strategies and identify other potential predictors of Zinc and ORS uptake that occur during the period of the program. The evaluation employs a pre-post design using cross-sectional surveys of both households and health providers in the intervention areas. A multi-stage cluster sampling technique was employed to systematically sample households and healthcare providers (primary healthcare providers, private patent medicine stores, chemists and pharmacies) across 3 Nigerian states. Baseline data were collected between November 2013 and March 2014, with a total of 2,700 caregivers and 900 health providers surveyed. Questionnaires collected data on caregivers' care seeking attitudes and perspectives, provider knowledge and attitudes, medicine audits, and mystery patient information. Results from this baseline work will provide valuable insight on the determinates of coverage and availability of Zinc and ORS and can inform program implementation and policy at both the National and Global level.

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ESTIMATING THE IMPACTS OF ROTAVIRUS VACCINATION ON GENDER DISPARITIES IN INDIA

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This study aims to assess the gender disparities in rates of vaccination coverage amongst girls and boys in India over time,and estimate their potential impacts on the introduction of rotavirus vaccination, across various geographic and household economic settings. A Microsoft Excel based spreadsheet model is used to model the expected health and economic outcomes disaggregated by gender of the child, for one annual birth cohort of children during the first five years of life. Three data sets have been used in the model: National Family Health Survey-3 2005-6,District Level Health Survey-3 2007-8 and Coverage Evaluation Survey 2009 to estimate the changes over time, in 3 highest mortality states and 6 regions of India. There is an overall increase in vaccination coverage in India. The gap between boys and girls in vaccination coverage over time is estimated to have reduced. Increase in gender parity is estimated to increase in benefits and decrease in cost effectiveness ratio per DALY. It is of immense importance to sustain the efforts of bridging the gender gap in health care utilization and creating enabling conditions to introduce the proposed universal rotavirus vaccination. Preferential treatment to boys increases the risk of mortality amongst girls along with increasing the cost of implementation of vaccination programs.

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QUANTIFYING THE IMPACT OF ACCESSIBILITY ON PREVENTATIVE HEALTHCARE IN SUB-SAHARAN AFRICA

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Poor physical access to health facilities has been identified as an important contributor to reduced uptake of preventative health services, and is likely to be most critical in low-income settings. However, the relationship between physical access, travel behavior, and the uptake of healthcare remains difficult to quantify. Here we analyze individual travel patterns of 14,816,521 people across Kenya and show that long travel times to health facilities are strongly correlated with increased mobility in geographically isolated areas. We provide regional and localized estimates of the disparity between estimated travel times to facilities, as a standard measure of access, and observed mobility patterns, and compare these measures to data on the uptake of two preventative healthcare interventions in an area of western Kenya: childhood immunizations and antenatal care. We show that even in areas with equal physical access, mobile phone-derived measures of mobility predict which regions are lacking either type of care, highlighting the potential utility of this approach to map the uptake of preventative healthcare.

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PERCEPTIONS ABOUT PROVIDERS AS DETERMINANTS OF APPROPRIATE TREATMENT SEEKING BEHAVIOR FOR SUSPECTED MALARIA IN CAMBODIA

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Seeking treatment for suspected malaria from an appropriate provider facilitates correct case management, contributing to malaria control and elimination and protection of antimalarial drug efficacy. Analysis of data from a nationally representative cross-sectional household survey in Cambodia was conducted to identify individual perceptions about providers associated with appropriate malaria treatment seeking behavior. 1012 individuals (334 age 0-15, 678 age 15+) were identified through screening members of randomly selected households for symptoms of malaria fever occurring in the past 2 weeks. Seeking treatment from an appropriate provider (public health facility or village malaria worker) was the main outcome. Nineteen percent of adults age 15+ and 18% of children <15 sought care at an appropriate provider. Potential covariates included individual preference for appropriate providers on measures of access and quality of care (e.g. cost and convenience of care, respectful and knowledgeable providers, effective medicines). Adjusting for age, gender, education, wealth, and artemisinin-resistance zone in a full logistic regression models, significant determinants of appropriate treatment seeking among adults at 15+ were viewing appropriate providers as: most convenient (AOR=2.9, 95% CI=1.0-4.6); most respectful (AOR=2.9, 95% CI=1.1-7.8), and most knowledgeable (AOR=4.0, 95% CI=1.2-13.6) relative to adults who did not view an appropriate provider as convenient, respectful, and knowledgeable. Relative to adults with fever only, people with additional symptoms were less likely to visit an appropriate provider (AOR=0.24, 95% CI=0.09-0.7). Determinants of appropriate treatment seeking for children <15 included viewing appropriate providers as possessing: lowest cost of transport (AOR=15.3, 95% CI=2.8-82.5); and lowest cost treatment (AOR=20.6, 95% CI=1.9-218.3) relative to caregivers who did not view an appropriate provider as having the lowest cost for treatment and transport. Interventions to improve access to appropriate providers that address cost of transport and treatment may improve appropriate malaria treatment seeking behavior for children. Promoting appropriate providers as knowledgeable and respectful, and encouraging provider practices that reflect these qualities are interventions that may improve appropriate treatment seeking behavior among adults.

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PERFORMANCE AND RELIABILITY OF DYNEX M² MULTIPLEXED ASSAY SYSTEM FOR IMMUNITY ASSESSMENT FOR THE DEMOCRATIC REPUBLIC OF THE CONGO - DEMOGRAPHIC HEALTH AND SURVEY

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Childhood vaccine- preventable diseases continue to be a major public health problem in terms of global morbidity and mortality. In endemic regions the only available tools of diagnosis are based on clinical and serological criteria. In support of the 2014 Democratic Republic of Congo -Demographic Health Survey (DRC-DHS) and in collaboration with University of California, Los Angeles, Fielding School of Public Health (UCLA-FSPH) an immunity assessment is being undertaken using dried blood spots (DBS). The Dynex Technologies M² ®multiplex immunoassay platform with a Measles, Mumps, Rubella, Varicella and Tetanus (MMRVT) panel is being used in which 10 polystyrene beads have been coated and immobilized within 54-well M² plates: separate assay beads with MMRVT antigens; positive controls with horseradish peroxidase, total human IgG, goat anti-human IgG; negative controls with MRC-5 and E6 cell lysate. Positive control DBS are 5-donor normal defibrinated serum and negative control DBS are pooled normal IgG-stripped serum. DBS are extracted into 1ml PBS, 0.5% tween20, 5.0% dried milk and processed on a modified Dynex DS2® automated ELISA system. DBS from 32 reference sera and 7-point dilutions of pooled sera were independently processed in DRC and USA. Assay cutoffs were set by reference to 13 singleplex and 1 multiplex FDA-approved ELISA kits. 423 DBS were collected as a pilot study from children visiting Kinshasa health centers and 9906 DBS during the principal DRC-DHS survey. Including optimization runs data from 60 plates comprising 32,400 data points have been collected including 423 pilot DBS and 1150 DHS samples, with the remaining to be completed by MMDDYY. Having been deployed to a substantially resource-limited environment the Dynex M² multiplex immunoassay system has proven to be a very robust assay platform with excellent sensitivity and specificity. This system in conjunction with DBS processing offers a very cost-effective reliable multiplexed immunoassay processing system for use in country-wide assessment of immunity status in challenging environments.

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FACTORS ASSOCIATED WITH FREQUENT SICK VISITS FOR FEBRILE ILLNESS AMONGST CHILDREN ENROLLED IN A MALARIA VACCINE TRIAL IN SIAYA DISTRICT, WESTERN KENYA

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Prompt care-seeking is essential to prevent severe disease and death from malaria. We investigated whether social and demographic factors affected number of sick visits for febrile illness including malaria among

children participating in a malaria vaccine trial in Kenya. From January 2010 to January 2011, 800 infants aged 6-12 weeks were recruited into the vaccine trial. Of these 446 with complete social and demographic data were analyzed. The association between number of sick visits, dichotomized as high (>18) and low (<18), and child age, sex, maternal age, maternal education, wealth quintile, sex of household head, distance from health facility, and religion was measured using bivariate and multivariate models. Wealth index was created using a list of household assets and principal component analysis was used to divide households into quintiles, poorest, poor, middle, rich and richest. The number of sick visits per participant during the 12 month period of follow-up ranged from 0-45 (median = 12, mean = 12.9). In multivariate analysis, children from the poor and middle income quintiles were significantly more likely to have a high number of sick visits than those from the wealthiest quintile (OR=3.13, 95% CI 1.12-8.74; OR=2.80, 95% CI 1.02-7.71, respectively). Compared with households practicing Catholicism, Protestant and Indigenous religion households were significantly more likely to have a high number of sick visits (OR=4.36, 95% CI 1.34-14.19; OR=3.14, 95% CI 1.11-8.88, respectively). No significant associations were found between other variables and number of sick visits, including distance to health facility (OR=1.06, 95% CI 0.42-2.67). Even though prior studies have shown wealth to be associated with increased care-seeking for children (Taff 2005, Chuma 2007), we found in this trial setting, where transport reimbursement and free health care were provided, a high number of sick visits was associated with the lower income quintiles. Distance to health facility was not associated with number of sick visits. It might be that in this resource poor setting, removing the barrier of transport through reimbursement and providing free health care could increase health seeking.

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EPIDEMIOLOGY OF LABORATORY CONFIRMED RUBELLA CASES IN ETHIOPIA, 2013

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Rubella is a contagious rash illness. Trans-placental infection leads to serious fetal disorder called Congenital Rubella Syndrome (CRS). Worldwide, more than 110,000 infants are born with CRS each year; most of these occur in developing countries where information is limited on the epidemiology and vaccine not introduced. This study was conducted to see the annual prevalence, seasonal and geographic variation and age distribution of rubella infection. Serum/plasma samples and demographic data were collected from measles/rubella suspected cases from all the 9 regional states and 2 city administration of Ethiopia, January-December 2013. The samples were tested for rubella IgM by ELISA. The data was analysed by Epi Info software version 3.5.4. Among 3,587 patient samples tested, 858 (23.9%) were positive for rubella IgM. The positivity rate was higher among females (26%) than males (22.2%). The highest rubella positivity rate, 27.5% (287/1043), was seen among children 5-9 years. During March and August, the highest 33.6% (179/533) and lowest 10.7% (17/159) prevalence rates were seen respectively. The highest positivity rate was seen in Amhara 36.7% (301/821) followed by SNNPR 36.1% (227/628), 29.4% (35/119) of Tigray, 15.3% (64/417) of Addis Ababa and the lowest 13.4% (190/1416) in Oromiya. In conclusion, I\ in Ethiopia, rubella become an increasing non-reportable public health problem and the infection is seasonal mostly affecting children. The current prevalence of rubella cases calls for conducting CRS surveillance in infants, surveillance among pregnant mothers, reporting and necessitates the introduction of rubella vaccine into the national routine immunization services.

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INTERNATIONAL AID AND NATURAL DISASTERS: A PRE AND POST-EARTHQUAKE LONGITUDINAL STUDY OF THE HEALTHCARE INFRASTRUCTURE IN LEOGANE, HAITI

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Little is currently known about the interaction between international aid donors and local healthcare systems in the context of the healthcare recovery following natural disasters. Using data collected prior to and following the 2010 Haiti earthquake, we detail the response of aid agencies and their interaction with local healthcare providers in Leogane, the town closest to the earthquake's epicenter. We collected geocoded data in Leogane, Haiti during three time periods: five months prior to the earthquake, one year after the earthquake, and three and a half years after the earthquake. During each visit we collected the same census data, including healthcare facility services, funding source, and receipt of aid. Our findings demonstrate that both one and three years after the earthquake there were marked increases in the total number of healthcare facilities, inpatient beds, and surgical facilities as compared with the pre-earthquake period, and that international aid has been a driving force behind the recovery. Twelve out of thirteen new healthcare facilities that have opened since the earthquake have been aid-financed, and seven out of eight healthcare facilities that were rebuilt after the earthquake were aid-financed. Despite increases in free, aid-financed healthcare following the earthquake, private Haitian healthcare facilities have remained at a constant number. Reconstruction efforts have not been fully sustainable, as the planned phase-out of several aid-financed facilities will leave Leogane with fewer inpatient-beds and emergency medical services as compared with the pre-earthquake period. We hope that our assessment of the recovery effort thus far in Haiti will help frame policy decisions regarding how best to support local healthcare systems in both the acute and long-term phases of disaster recovery efforts.

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SAMUEL LAPSLEY AND WILLIAM SHEPPARD: MISSIONARY MEDICINE, MEDICAL ENTOMOLOGY AND REALPOLITIK IN THE CONGO, 1890-1917

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The dawn of the 20th century marked the height of European colonialism on the African continent. By 1900, the major European powers had carved the continent into sectors of social, cultural, political, and military control. Christian missionaries, Protestant and Catholic alike, soon followed by the thousands. Although many were European, some hailed from the United States. Among the best-known Americans were William Sheppard and Samuel Lapsley, Presbyterian missionaries who worked in the Belgian Congo from the early 1890s to the era of The Great War. As tales of Dr. David Livingstone spread widely in the late 1800s, it is likely that the Reverend Mr. Sheppard and the Reverend Mr. Lapsley would have expected the usual missionary tribulations. They would have been all too familiar with the experiences of the faithful who had preceded them: suspicious--and possibly warlike--natives, linguistic and cultural barriers, fierce animals, and sweltering tropical climates. As such, they arrived in the Belgian-controlled Congo in the early 1890s armed with hundreds of pounds of equipment and supplies, not least, Bibles. The work of Sheppard and Lapsley, however, soon took them not only into the changing world of late 19th-century tropical medicine but also into the grim underside of Congolese life under Belgian rule. This paper will examine only their medical and scientific efforts amidst the changing paradigms in tropical medicine but human-rights efforts in the Congo.

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WOMEN'S PERCEPTION OF ANTENATAL CARE SERVICES IN PUBLIC AND PRIVATE CLINICS IN THE GAMBIA

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The main objective of this study was to assess women's preferences and perception of antenatal healthcare services in public and private healthcare facilities. Descriptive cross-sectional study using a face-to-face interview based on the standardized World Health Organization questionnaire. Six public and six private health facilities in the Gambia. Five hundred and two pregnant women. Patient's perception of antenatal services received was main outcome variables and measured in three aspects: willingness to come back, willingness to recommend to others and level of satisfaction. The satisfaction rate with antenatal services was 79.9% for public facilities and 97.9% for private facilities. Pregnant women's poor perception with public facilities (after adjustment) included their unhappiness, with the following dimensions of antenatal care (ANC): inadequate privacy, inadequate space and neatness and inadequate communication with care providers. We found that although women tended to be highly satisfied with both private and public ANC facilities, those attending public clinics were significantly less satisfied than those attending private clinics. The main complaints were related to the physical environment, technical process and provision of information or reassurance. Because public facilities constitute the main care providers for the general population and particularly for disadvantaged women, better management of public clinics and better training in communication skills for public care providers may help to retain women patients and improve the quality of ANC in the public sector.

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DEVELOPMENT OF ADVANCED SERO-ASSAYS TO BROADEN DIAGNOSTIC AND SURVEILLANCE CAPABILITY IN WEST AFRICA

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West Africa is home to a wide variety of viral infectious diseases, many of which go untreated in the local populations. Lassa virus causes a hemorrhagic fever that occurs throughout the region, with fatality rates approaching 30%. It is endemic in Sierra Leone, where the Kenema Government Hospital (KGH) houses a Lassa fever ward and laboratory. Approximately 600 suspected Lassa fever cases are received here annually from throughout the country, however of these, only 30-40% of samples test positive for Lassa virus; therefore, there is a large number of patients presenting with diseases of unknown origin. Severely limited resources at KGH hinder treatment and diagnostic capacity of Lassa fever, as well as of the additional undiagnosed infections that present there. Here, we have approached these two issues - diagnostic capability for acute Lassa infection, and surveillance of additional circulating viruses in west Africa- by developing an array of diagnostic assays for detection of hemorrhagic fever and arthropod-borne viruses utilizing Magpix technology (Luminex, Austin, TX) and evaluating their clinical performance on site at KGH. Magpix assays detecting Lassa-specific IgM and Lassa antigen in serum were significantly faster and more sensitive compared to traditional ELISAs, which are the current standard at KGH. Identifying past exposures to possible circulating viruses using IgG detection was carried out in a multiplex format, detecting antibodies to Ebola, Marburg, Rift Valley fever, Crimean Congo hemorrhagic fever, Lassa, and an array of flaviviruses and alphaviruses in one well. These assays have shown to be a valuable asset in a clinical setting, significantly improving upon current acute Lassa fever diagnostics at KGH as well as generating a large pool of data that gives insight into the scope of viral diversity and prevalence in West Africa.

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USING ITERATIVE PRACTICE FOR DEVELOPING PUBLIC HEALTH ICONOGRAPHY TO PREVENT SOIL-BORNE HELMINTHS

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Shoes are a proven method for disrupting the transmission of soil-borne helminths (STH). However, shoe-wearing practice is often uncommon in areas where STH are prevalent, largely because the health benefits of wearing shoes may not be apparent to local populations. This is especially true in non-literate populations where information dissemination through traditional means (e.g. posters, billboards) is difficult. We launched a public health intervention that combines an animated image (a "lenticular image") depicting the efficacy of shoes in preventing STH infection with "flip-flop"-style sandals. The image adheres directly onto the shoe, so that health information is linked to the primary means of intervention. Here, we describe the multi-phased, iterative design process used to develop the image and the process of its deployment and social uptake in rural communities in Uganda. We gathered standard iconographies that depicted specific elements of the image to show that shoes can interrupt STH. Elements included abstract constructs such as "healthy" and "don't," alongside concrete concepts such as "hookworm" and "sandals". Iconographies and color schemes were evaluated, deconstructed, and reconstructed over five focus group sessions. A resulting suite of "infection" and "prevention" messages were tested with 30 individuals across three study communities. The final versions of 'infection' and 'prevention' were selected based on the frequency of "correct" comprehension. Shoes with the image were then deployed to individuals in communities with high rates of hookworm infection. We found participants' overall shoe-wearing practices improved, but varied by activity and individual characteristics. This study demonstrates that holographic iconography can be an effective means of disseminating public health information to non-literate populations. It also demonstrates the importance of incorporating culturally appropriate images into pictorial depictions of public health messages. Interactive design processes are an efficient way to develop such messages.

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FEASIBILITY OF A ONE-WEEK COURSE TO TEACH BASIC ULTRASOUND SKILLS TO PHYSICIANS IN RESOURCE-LIMITED SETTINGS

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Portable ultrasound is an inexpensive and highly effective tool. Ultrasound provides real time data that can assist in diagnosis and therapeutic procedures, and may contribute to better outcomes in resource limited settings. However, the training required before a physician gains competency remains controversial. The optimal method of teaching, and which ultrasound views to teach are also questionable. This study aimed to assess whether a one-week intensive course would result in physicians ability to use ultrasound in daily clinical practice competently. The project was conducted at Bugando Medical Centre in Mwanza, Tanzania. The curriculum involved learning basic ultrasonography skills, and the ability to obtain and interpret views of the heart, perihepatic space and liver, perisplenic space, inferior vena cava, pelvis, and thorax. The curriculum involved daily lectures on theory, followed by practical sessions with healthy subjects and in-patients from medical wards. Informed consent was obtained from all participants. Candidates were required to complete a minimum of twenty-five total scans on different patients. Candidates submitted several still-images, which were evaluated for adequacy and

demonstration of normal or abnormal anatomy. Competency was also assessed by a practical examination at the end of the course involving an ultrasound examination of a standardized patient. All participants completed a minimum of twenty-five ultrasounds of each of the ten views. All candidates' passed the formative examination, with a median of 96%. Adequate images were submitted by all candidates, with a demonstration of a variety of pathology. Self-reported confidence was shown to be significantly greater after the completion of the course. The steep but rapid clinical learning curve demonstrates that an intensive training course to attain basic bedside ultrasound skills is feasible in a resource limited setting. Integration of a similar curriculum may be possible to enhance medical education and diagnostic accuracy in resource-limited settings.

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ACCURACY OF ULTRASOUND AS A TELE-MEDICINE COMPONENT USING A COMMERCIALLY AVAILABLE TELE-CONFERENCE SYSTEM

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Point-of-care (POC) ultrasound has been shown to have many benefits in patient care. Many international providers are not trained to perform or interpret US results. As a component of the telemedicine (TM) system, POC US has the potential to provide diagnostic capability in locations with limited diagnostic services. Our hypothesis is that US transmission using a commercially available teleconferencing (TC) system will yield similar results to DICOM image transmission as well as comparable picture quality. Similarly, the accuracy of diagnosis will be unchanged. This was a prospective observational trial of transmitted US video using an internet based peer-to-peer communication system (Skype) compared to images transmitted via a DICOM system. US images were obtained from patients presenting to the ED. After consent, limited, POC US exams were performed using a SonoSite US. The images were stored using a DICOMM protocol and transmitted using a TC system. The TC system consisted of a USB to VGA image capture device (Epiphan) connected to the VGA output in the minidock of the US system. The image capture device connected to a Dell laptop and using Skype, images were transmitted to a Mac Pro. Video recordings were made of the transmitted image and stored as mp4 files. Images of the transmitted and non-transmitted images were of the same patient and were of the same US exam. Three ED physicians with hospital credentials for ED US reviewed the US exams using both methods in random order. 148 examinations (74 transmitted and 74 non-transmitted) were reviewed. The kappa statistic was calculated separately for each diagnostic test, and for all tests combined, to examine agreement between each of three reviewers and the gold standard. Kappa for Reviewer #1 was consistently in the "almost perfect agreement" range ($0.8 < \kappa < 1$). For the Gallstones and IUP diagnostic tests, all reviewers had κ values in the "almost perfect agreement" range ($0.8 < \kappa < 1$). There were statistically significant differences between the scores for the transmitted vs. non-transmitted for all three types (image detail, image resolution, and image quality) for Reviewers 1 and 3 only. In particular, non-transmitted scores were significantly higher than transmitted scores. POC US transmitted via commercially available systems may be a vital and accurate diagnostic option for international providers where other diagnostic radiological resources are unavailable.

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TIME TRENDS FOR CHILD DIARRHEA PREVALENCE AND ORS USE IN A DOMINICAN REPUBLIC PERI-URBAN COMMUNITY

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Reducing child diarrhea prevalence and increasing oral rehydration solutions (ORS) use are important national policy aims for diarrhea control in low- and middle-income countries. Available national level data for some countries has identified gaps in attaining these aims. While informative, national level data may obscure variation across sub-national levels and may miss patterns within high-risk communities. Low-income peri-urban communities are particularly important given that they contain rapidly expanding populations and have conditions that may place children at high-risk for diarrhea (e.g., overcrowding, inadequate water and sanitation infrastructure). The aim of this study was to determine whether the prevalence of child diarrhea is decreasing and ORS use is increasing over time within a low-income peri-urban community of the Dominican Republic. The study community has received several health interventions initiated by non-governmental organizations over the last several years that included water, sanitation and health education projects. Information on child diarrhea and ORS use between 2009 and 2013 was extracted from a dataset derived from an ongoing child growth-monitoring program (GMP) located in the study community. All children in the community under four years of age were eligible to participate. The prevalence of child diarrhea among attendees at the GMP was determined within different time blocks over the 4 year study period. ORS rate was based on reported use within diarrhea cases over time. Data were available for 293 children from one or more appointments. Diarrhea prevalence remained relatively constant at 26 (SD: 2.6)% over the 4-year period with no reduction over time. The mean rate of ORS use over the 4-year period was 38 (SD: 3.4)% with no evidence of increase over time. Despite various health initiatives in this community, the burden of child diarrhea remained high and ORS use remained low. Examination of potential factors impeding progress on diarrhea control is still required, particularly in high-risk communities.

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DEVELOPING SUSTAINABLE CAREER DEVELOPMENT SUPPORT FOR AFRICAN RESEARCHERS

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Developing global health researchers to proficiency is crucial to support the strengthening of health research capacity, yet researchers based in low and middle-income countries in Africa face a disadvantage in their career development to proficiency through limited access to research training, resources and appropriate support and mentorship. Currently much of this support comes from externally funded capacity development programs who invest in developing individuals, strengthening research training and enhancing research environments. However, continued support post program ends remain a challenge. Led by senior African researchers from each of the Malaria Capacity Development Consortium's (MCDC) African partner institutions, structured and supported Career Development Groups (CDGs) have been set up to advance the career development of researchers within their institutions, in addition to developing sustainable research training and systems of support for researchers within the institution. Oversight of the CDGs sits within the offices of the provost, deans of faculty and schools of postgraduate studies to ensure integration within institutional processes and policies, and sustainability beyond the MCDC program. The CDGs have undertaken a baseline assessment of existing institutional career development support and in wider consultation drawn up plans for development. Plans are specific to institutional need and context, aligned to strategic institutional objectives and incorporate the needs of other capacity development programs based at the institution. Activities and strategies of support will vary but with Personal Development Planning (PDP), formal mentoring and support in postgraduate supervision being key strategies that will be embedded within institutional career development support for researchers and research staff. An evaluation will be undertaken on the impact of the career development support as well as the process of using CDGs as a new way of working within African institutions to support the sustainable development of global health researchers in LMICs in Africa.

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THE FIRST REPORTED CASE OF BABESIOSIS IN A RESIDENT OF OREGON

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Babesiosis is a tickborne infection caused by intraerythrocytic parasites of the genus *Babesia*. Most U.S. zoonotic cases have been caused by *B. microti*, which is endemic in the Northeast and upper Midwest. Sporadic cases caused by other *Babesia* species have been documented in various U.S. regions. The first reported case caused by *B. duncani* (the WA1-type parasite) occurred in Washington State in 1991. Here we describe the first known case of babesiosis diagnosed in Oregon—a parasitologically confirmed case of *B. duncani* infection, in a 69-year-old otherwise healthy asplenic man, who lived in a rural forested area of Deschutes County in central Oregon. He did not recall tick exposures or have a history of transfusions. On June 30, 2009, he was evaluated in an urgent-care center because of a several-day history of a febrile illness (maximum temperature, 40°C); his hematocrit was 37.3%, and his platelet count was 39 x10^9/L. The marked thrombocytopenia prompted manual review of a blood smear. Intraerythrocytic forms were noted, which were confirmed by a hospital pathologist as *Babesia* parasites; the parasitemia level was 3%. On July 1, he was hospitalized and began combination therapy with clindamycin plus quinine. When he was discharged on July 6, he had been afebrile for >48 hours and his parasitemia level was <1%. During a follow-up evaluation on July 9, no parasites were found on blood-smear examination and antimicrobial therapy was discontinued. In reference diagnostic testing at CDC, molecular and serologic analyses were negative for *B. microti* but were positive for *B. duncani* (the indirect fluorescent antibody titer was 4096); *B. duncani* also was isolated by inoculation of jirds (Mongolian gerbils). Clinicians and laboratorians should be aware that babesiosis can be caused by agents that are not detected by assays for *B. microti*. To date, parasitologically confirmed cases of *B. duncani* infection have been documented in Washington, California, and Oregon. The many unknowns about *B. duncani* include its geographic distribution, tick vector, and reservoir hosts.

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DETECTION OF *ANAPLASMA PHAGOCYTOPHILUM*, *BABESIA MICROTI* AND *BORRELIA BURGDORFERI* IN *IXODES SCAPULARIS* COLLECTED FROM LOCATIONS SURROUNDING THE RESIDENCE OFA LOCALLY-ACQUIRED CASE OF HUMAN BABESIOSIS IN MARYLAND, USA

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The Delmarva Peninsula has long been recognized as home to populations of *Ixodes scapularis* infected with *Borrelia burgdorferi*, although reports of tick surveillance are few. *Babesia microti* has never been reported from *I. scapularis* there, although there is only one published investigation of *Ba. microti* in the area. In 2009, Maryland Department of Health and Mental Hygiene (DHMH) reported the investigation of an autochthonous case of human babesiosis on the Maryland Eastern Shore of the Delmarva Peninsula. The patient was also infected with *B. burgdorferi*. Subsequently, a team from DHMH and U.S. Army Public Health Command (USAPHC) performed a preliminary investigation of vector ticks in locations surrounding the patient's residence. Sixteen *I. scapularis* adults were collected by flagging at the patient's yard and a nearby site. PCR testing at the USAPHC detected *B. burgdorferi* in 8/16 of the ticks; 2 of these 8 were coinfected with *Ba. microti*, and 1 of the 8 was coinfected with *A. phagocytophilum*. PCR positives were reconfirmed with second PCR at USAPHC, and *Ba. microti* positive tick samples were sent to Division of Parasitic Diseases and Malaria, Centers for Disease Control and Prevention, Atlanta, Georgia, for reconfirmation with nested primers targeting the ss-rDNA gene. To our knowledge, these are the first *Ba. microti* positive *I. scapularis* collected in Maryland, and the human case is the first locally acquired babesiosis infection reported from Maryland. *B. burgdorferi* infection can be quite robust in the mid-Atlantic region, so finding 50% of these ticks positive for *B. burgdorferi* was not completely unexpected. However, the regional prevalence of infection with *A. phagocytophilum* is low (2-3%), and *Ba. microti* has never been reported from *I. scapularis* in Maryland, so detection of these pathogens in a small sample of ticks was remarkable. Further investigation at this location of nymphal *I. scapularis,* the stage most likely to transmit human diseases, is warranted.

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IMPACT OF CLIMATE CHANGE ON TICK-BORNE RELAPSING FEVER BORRELIOSIS DISTRIBUTION IN WEST AFRICA

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Tick-borne relapsing fever (TBRF) borreliosis due to *Borrelia crocidurae*, is a major cause of morbidity in most rural areas of Senegal where its distribution, previously limited to the Sahel, spread to Sudan savannah areas during the 1970s. We report, here, studies conducted in 12 West African countries to investigate the distribution of ticks *Ornithodoros sonrai*, the occurrence of *B. crocidurae* infections in vectors and small mammals and their relationship with climatic change. From 2002 to 2012, we investigated the occurrence of *O. sonrai* in rodent burrows in 210 study sites from West African countries. Ticks collected in each site and their Borrelia infections were genetically characterized by sequencing. We collected small mammals in Mauritania, Senegal, Mali, Niger and Benin. They were tested for Borrelia infections by thick blood film, blood or brain inoculation into white mice and/or by PCR. Out of 8,716 burrows examined, 973 (11.1%) were found colonized by Ornithodoros ticks identified as *O. sonrai.* Only sites in Senegal, Mali, the Gambia and Mauritania were found positive for this vector. B. crocidurae infections were highlighted in 245/1,121 (21.8%) of ticks tested by nested PCR and 70/842 (8.3%) of rodents and insectivores. All infected mammals were collected in areas where we found the vector. The southern limits of *O. sonrai* and *B. crocidurae* corresponded approximately to the 750-mm isohyet. *O. sonrai* ticks and *B. crocidurae* are massively distributed north of latitude 13°30'N. Although climatic factors are clearly associated with the distribution and the recent spread of TBRF in the westernmost part of West Africa, the vector appears absent from climatically suitable areas of Burkina Faso and Niger. It is only in central and eastern Mali that the distribution of the vectors was associated with the principal riverbed of Niger River and its mains inflows.

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SPILLOVER AND GENETIC DIVERSITY OF *RICKETTSIA PARKERI* IN NORTHERN VIRGINIA

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Spotted Fever group *Rickettsia* (SFGR) cause a spectrum of disease worldwide, with symptoms ranging from mild to severe febrile illness. In the United States the most notable is *R. rickettsii*, the causative agent

of Rocky Mountain Spotted Fever (RMSF). Several other SFGR have been linked to human infection, many of which cause similar clinical symptoms and contribute to misdiagnosis. Previously thought to cause only zoonotic infections, *R. parkeri* was recognized as a human pathogen in 2002, and has subsequently been linked to twenty cases of spotted fever rickettsiosis in the U.S. and Argentina. The primary vector of *R. parkeri* is *Amblyomma maculatum*, which has spread beyond its historic range to occupy foci across the central and southeastern U.S. In 2010, *R. parkeri* -infected *A. maculatum* was discovered at a landfill in Fairfax County, Virginia, where sympatric populations of many tick species exist. Initial findings discovered *R. parkeri* infection of other ticks near the landfill, including *Dermacentor* variabilis, *Rhipicephalus sanguineus*, and *Haemaphysalis leporispalustris*, which are documented to bite humans. This is significant, and highlights the potential for spillover into vectors not typically associated with *R. parkeri*. Preliminary analysis of a relatively conserved outer membrane protein (gene *rompB)* indicates 97-99% sequence homology among *R. parkeri* samples. Low diversity implies that infection of novel vectors may be attributable to spillover from sympatric populations co-feeding on infected hosts. Further sequence analysis of more variable gene targets is ongoing; preliminary findings indicate that genetic variation may exist, which, if confirmed, will provide evidence for the introduction of multiple *R. parkeri* variants. This work represents a novel approach to understanding the ecology of *R. parkeri* in North America. Pathogen spillover of multiple genetic variants into novel vectors could lead to increased transmission and propagation of the disease cycle, resulting in an increase in spotted fever rickettsiosis cases.

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CONTROL OF TUNGIASIS (SAND FLEA DISEASE) IN EAST AFRICA: SELF-DIAGNOSIS AS A MEANS TO TARGET TREATMENT IN ENDEMIC AREAS

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In East Africa, tungiasis (sand flea disease) has re-emerged in epidemic proportions. This has prompted Ministries of Health and non-governmental organizations to conceive control measures. To target treatment in a cost-effective manner, diagnostic tools are required allowing a rapid assessment of the prevalence and severity of tungiasis at the community level.
We investigated the sensitivity, specificity and predictive values of self-diagnosis in rural Uganda and Kenya and determined factors which might have an impact on the validity of this diagnostic approach. The study was carried out in the Makoma Primary School, Bugiri County, Uganda, and in Shivakala village, Kakamega County, Kenya. All the primary school pupils (n = 589) and all the inhabitants of Shivakala village (n = 386) present on two consecutive days were individually asked whether they thought they had tungiasis on at least one foot. Immediately after the self-diagnosis, the individual was carefully examined for the presence of embedded sand fleas, and tungiasis-associated morbidity was assessed semi-quantitatively. In Makoma Primary School, the prevalence of tungiasis was 71.9% (95% CI 66.6 - 76.6). In Shivakala village, the prevalence was 70.3% (95% CI 62.2 - 77.3) in school-age children and 56.5% (95% CI 51.5 - 61.3) in the general population. At both locations, the ratio of infected males to females was 1.2. Whereas in the Ugandan primary school the sensitivity of self-diagnosis was very high (85.1%; 95% CI 80.0 - 89.1), the specificity was rather low (69.6%; 95% CI 59.5 - 78.0). In contrast, in the Kenyan village the specificity of self-diagnosis was high (97.1%; 95% CI 92.8 - 98.9), but the sensitivity was low (66.5%; 95% CI 59.3 - 73.0). In school-aged children the positive predictive value was 98.5 (95% CI 92.0 - 99.7), and the negative predictive value was 56.3 (95% CI 44.8 - 67.3). It was concluded that self-diagnosis can be relied upon to enable targeted community based control measures against tungiasis, but that the validity of this diagnostic approach has to be determined in different settings.

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EFFECTIVE TREATMENT OF TUNGIASIS WITH DIMETICONE: A TARGETED TOPICAL APPLICATION IS SUPERIOR TO WETTING THE SKIN OF THE WHOLE FOOT

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Tungiasis (sand flea disease) is a neglected tropical disease associated with debilitating acute and mutilating chronic morbidity. In the endemic areas patients attempt to remove embedded sand fleas with inappropriate sharp instruments, such as safety pins, thorns or sharpened pieces of wood - a hazardous procedure by itself. Recently, we demonstrated that a single topical application of a mixture of two dimeticones with low viscosity onto the skin of the feet effectively killed embedded sand fleas and significantly reduced local inflammation within seven days. We attempted to increase the efficacy of the new therapeutic approach by targeting the dimeticone to the abdominal rear cone of the parasite which protrudes over the corneal layer of the epidermis. 59 children from Bulidha sub-county, Bugiri District, Eastern Uganda, aged from 5 to 12 years, with a total of 311 embedded sand fleas were included in the study. The left and the right foot were randomized to either receive the "simple" or the targeted application with the dimeticone, respectively. The viability of the embedded parasites was assessed by a handheld digital video microscope and the degree of inflammation was determined by means of an inflammation score. The lesions were observed daily during 7 days. After the "simple" treatment 87.3% (95% CI 81-92%) of the embedded fleas lost all viability signs within seven days, and after the targeted treatment 97.5% (95% CI 94-99%; p = 0.008) giving a difference of 10.2% (95% CI 7-14%) in efficacy. We conclude that the targeted application is superior to wetting the skin of the whole foot with dimeticone. The targeted treatment requires less dimeticone, can be performed by the patient himself and is a promising tool for control measures aiming at the reduction of tungiasis-associated morbidity. In view of the high efficacy and safety of the topical application of dimeticone, the hazardous extraction of embedded sand fleas with sharp instruments is no longer warrantable.

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A MODEL OF INVESTIGATION OF HOST IMMUNITY DURING *ORIENTIA TSUTSUGAMUSHI* INFECTION

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Scrub typhus, a long neglected but important tropical disease, is caused by a Gram-negative obligately intracellular coccobacillus, *Orientia tsutsugamushi*. Scrub typhus is a serious global public health problem that causes illness in one million people each year, the majority in the Asia-Pacific region. Without appropriate diagnosis and treatment, the disease can cause severe multiorgan failure with a mortality rate of 7-15%. However, the mechanisms behind the interactions between *O. tsutsugamushi* and host immunity are largely neglected and unknown. With our newly developed intravenous (i.v.) mouse model, we demonstrated that there were significant changes in the host immune responses after *Orientia* infection. We discovered that host immunity leaned towards T_h1 responses from 12 days post infection (dpi) until 3 months post infection. Our flow cytometry data determined that more $CD8^+$ T cells than $CD4^+$ T cells appeared in the spleen of infected mice after 12 dpi. We also found that $CD4^+CD25^+Foxp3^+$ T_{reg} cells and IL-10 levels significantly increased from 6 dpi, which was in parallel with the body weight changes and bacterial loads. The mice that received a lethal dose (4.5×10^6 PFU/mL) began losing weight as early as 3 dpi. These mice died on 12 dpi. The mice inoculated with a sublethal dose (4.5×10^5 PFU/

mL) began losing weight at 7 dpi, reached a nadir on 9 dpi, and regained weight from 13 dpi. The spleen, lung and liver from both groups, and the kidney from lethal dose group had highest bacterial loads on 6 dpi, except the kidney in the sublethal dose group which peaked on 12 dpi. Further studies, especially study of human immune responses after *Orientia* positive mite bites in endemic areas, will benefit the understanding and control of scrub typhus well as development of vaccine and other prevention measures.

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THE EFFECT OF *AMBLYOMMA MACULATUM* FEEDING ON *RICKETTSIA PARKERI* INFECTION IN RHESUS MACAQUES

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Rickettsia parkeri is an emerging pathogen in the spotted fever group of *Rickettsia* that is transmitted by the bite of the Gulf coast tick, *Amblyomma maculatum*. The immune response to spotted fever group *Rickettsia* has been studied extensively in mice via intradermal, intraperitoneal, and subcutaneous routes of infection. However, these studies did not evaluate the effect of tick infestation on the immune response and rickettsial transmission. Since hard ticks feed on vertebrate hosts for several days, they must be able to counteract the host immune response. Several studies have shown that tick saliva alters several aspects of both the innate and adaptive arms of the immune system. However, the effect of this immunomodulation on *Rickettsia* transmission and pathology in the vertebrate host has not been examined. We hypothesize that by modulating the host immune response, tick feeding enhances infection and pathology of pathogenic SFG *Rickettsia* sp. in non-human primates. In order to assess this interaction *in vivo*, we will use rhesus macaques to compare intradermal needle inoculation of *R. parkeri* alone to inoculation during *Rickettsia*-free *A. maculatum* feeding and *A. maculatum* feeding without *Rickettsia* administration. For up to one month post inoculation, skin and lymph node biopsies, and blood will be collected to evaluate pathology, quantification of rickettsial load, and assess the acute phase response and cytokine concentrations. Comparison of the disease course, pathology and immune responses to infection in the presence or absence of tick feeding, will help define the role of the tick in spotted fever group *Rickettsia* transmission and develop a primate model of *Rickettsia parkeri* rickettsiosis.

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CHARACTERIZATION OF THE BACTERIAL COMMUNITY OF SPECIES AND POPULATIONS OF *DERMACENTOR* TICKS

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Our metagenomic analysis (*rrs* fragment pyrosequencing) of the bacterial communities present in 23 adult *Dermacentor variabilis* (Dv) ticks from Atlanta, Georgia and southeastern Ohio identified 27 taxonomic groups; however, only 6 were present in at least 3 ticks - 2 types of *Rickettsia*, 3 types of *Francisella* endosymbiont (FE) and a *Rickettsiella*. *Dermacentor* ticks are classic vectors for *Rickettsia* (7 ticks) while the FE was found in higher abundance and in all ticks; 15 (both sites) had two types of FE. In order to select other tick samples for further metagenomic analyses and to determine whether there was any relationship between the presence of *Rickettsia* and FE agents, additional Dv adults from 8 sites on a coastal barrier island in Georgia and 6 ecologically variant sites in Ohio, and *D. occidentalis* (Do) from 6 sites in southern California were collected. Their DNA was analyzed by semiquantitative EVAGreen assays (qPCR) for fragments of *omp*A (*Rickettsia*) or *rrs* (FE). The *Rickettsia* species were identified by sequencing *omp*A, *omp*B, and/or 17 Kd antigen gene amplicons. Nearly all samples again contained FE while *Rickettsia* was found in lower prevalence and abundance irrespective of species. Primers for 11 genes commonly used in multi-locus sequence typing (MLST) for pathogenic *Francisella* (PF) were tested by PCR with the Dv FE but products were obtained for only 4 of them. Three MLST targets exhibited no genetic variation between Dv populations but they had <90% homology to PF; however, a small number of 16S genotypes were detected at each site including some new variants. While the quantity of FE detected by qPCR varied significantly between ticks and tick locations for both Dv and Do, it did not correlate with either tick sex or the presence of a *Rickettsia*. The physiological importance of the widespread dominant prevalence of different *Francisella* endosymbionts to *Dermacentor* ticks is unknown; if (and how) their presence may alter these ticks' responses to acquisition, growth and transmission of *Rickettsia* also needs experimental investigation.

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CHARACTERIZATION OF VERTICAL AND HORIZONTAL TRANSMISSION OF PATHOGENIC AND NONPATHOGENIC RICKETTSIA WITHIN THE TWO TICK HOSTS *DERMACENTOR VARIABILIS* AND *AMBLYOMMA MACULATUM*

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Ticks act as vectors for an array of rickettsial species with primary pairings existing between specific tick and rickettsial species. However, transmission of pathogenic *Rickettsia* species by newly recognized vectors, coinciding with a rise in tick-borne rickettsial diseases has raised the need to consider variations in known tick/*Rickettsia* relationships. To fully understand the infection potential between several tick and rickettsial species, two sympatric species of ticks, *D. variabilis* and *A. maculatum,* were exposed to the rickettsial pathogens *Rickettsia rickettsii* or *Rickettsia parkeri*; non-pathogenic *Rickettsia montanensis* or *Rickettsia amblyommii;* or, flea-borne *Rickettsia felis* Adult, female *D. variabilis* and *A. maculatum* were capillary fed with either *Rickettsia* or delivery control and allowed to feed to repletion. Fitness-related metrics such as engorgement weight, egg production index, nutrient index, and egg hatch percentage were then assessed. Subsamples of egg clutches for each treatment group were assessed for transovarial transmission (TOT) of rickettsiae by qPCR. Results show that fitness of *D. variabilis* was not influenced by rickettsial exposure, excepting a decrease in egg production for *R. montanensis*-exposed ticks. TOT was observed for all groups except *R. rickettsii*. In contrast, engorgement weight for *A. maculatum* was reduced for all groups except *R. rickettsii*. Egg production was decreased in *R. montanensis*-exposed *A. maculatum*, which was also shown in nutrient index measurements. TOT was demonstrated only for *R. amblyommii* and *R. parkeri*-exposed *A. maculatum*, with the nutrient index of *R. parkeri*-exposed ticks significantly decreased. Results of this study will lead to a better understanding of the ecology and epidemiology of rickettsial diseases.

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CLINICAL VALIDATION OF NEW AND EXISTING *ANAPLASMA PHAGOCYTOPHILUM* REAL-TIME PCR ASSAYS

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Anaplasma phagocytophilum, an obligate intracellular bacterium, is the causative agent of human granulocytic anaplasmosis (HGA). Characterized by fever, headache, myalgia, leukopenia, and thrombocytopenia, HGA is a vector-borne disease carried by *Ixodes* tick species. While HGA has a low fatality rate, the risk increases with delayed treatment or when the elderly or immunocompromised are infected. Currently, our laboratory uses a SYBR Green assay that targets the 16S gene of *Anaplasma* and *Ehrlichia*, which requires sequencing confirmation to distinguish the species. The msp2 gene encodes an outer membrane protein unique to *Anaplasma*. Depending on the isolate, *A. phagocytophilum* may contain >80 copies of msp2 variants. An existing real-time PCR multiplex assay targets the msp2 gene of *A. phagocytophilum* and the 23S gene of *Borrelia burgdorferi* (Courtney JW et al., 2004). In this study, we validate the *A. phagocytophilum* msp2 primer/probe set as a singleplex real-time PCR assay for testing clinical specimens in the CDC Rickettsial Diagnostic Laboratory. A second set of primers were designed to flank the original amplicon (77-bp) extending the product to provide a template (213-bp) suitable for DNA sequencing. Primer/probe concentrations were optimized and analytical specificity was tested with exclusivity panels of environmental DNAs (33) and bacterial near neighbor DNAs (41). Sensitivity of the assays was determined by spiking known concentrations of *A. phagocytophilum* into PBS and blood. The assays have a limit of detection of 10 copies of the msp2 gene. Assay verification was performed by testing a blind panel of DNA extracted from blood with high, medium, and low concentrations of the organism. Results from the blinded panel correlated with expected values. Assay reproducibility (inter-assay and intra-assay) was also evaluated, generating consistent results. In conclusion, two sensitive and specific real-time PCR assays have been validated and can be used as effective diagnostic tools for the detection of *A. phagocytophilum* in clinical blood specimens.

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SEROLOGICAL SURVEILLANCE OF RICKETTSIAL DISEASE IN NORTHEASTERN AND CENTRAL CAMBODIA

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Rickettsiosis are arthropod-borne diseases caused by intracellular bacteria of the genera *Rickettsia* and *Orientia* and are classified into 3 groups: spotted fever group (SFG), typhus group (TG) and scrub typhus group (STG). Rickettsial diseases generally present as a febrile illness that can range from moderate to severe symptoms. Limited data has been generated on the prevalence of rickettsial diseases in Cambodia. To investigate further, we have conducted febrile syndromic surveillance in the Northeastern provinces of Cambodia near the Lao PDR border and a second region within central Cambodia from July 2010-April 2011. Subjects who reported to local clinics with an acute fever greater than 37.5°C were enrolled. Data thus far includes a total of 390 enrolled patients who tested negative for influenza, dengue and malaria were checked for the presence of specific antibodies against SFG (*R. rickettsii*), TG (*R. typhi*), and STG (*O. tsutsugamushi*) by ELISA. Convalescent sera positive for antibodies specific to one of the three rickettsial groups were detected in 116 of 390 (29.7%) samples and positive in 77 (19.7%), 23 (5.9%) and 48 (12.3%) of cases for SFG, TG and STG, respectively. Titrations were performed on positive convalescent specimens and corresponding acute specimens. Recent infections, defined by a fourfold rise in antibody titers, or seroconversion were determined in 13 (11.2%) of SFG, 3 (3.9%) of TG and 4 (8.3%) of STG rickettsiosis. These results suggest that rickettsial infections may be a common etiology of fever for this area. Continued monitoring of this population and molecular characterization of these specimens will provide further insight to the epidemiology of rickettsiosis in this area.

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RICKETTSIA SPECIES DETECTED IN *DERMACENTOR VARIABILIS* FROM NORTH CAROLINA

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We screened *Dermacentor variabilis* (American dog tick) for the genus *Rickettsia* under the hypothesis that less <10% of the ticks would harbor rickettsiae. To test our hypothesis, we extracted genomic DNA from 532 *Dermacentor* variabilis that were collected from several NC counties over years 2009 and 2010. *Rickettsia* species were identified by PCR amplification of 23S-5S intergenic spacer fragments combined with reverse line blot (RLB) hybridization with species-specific probes. Thus far, amplification of 100 genomic DNA samples has produced 61 (61%) samples, which showed a band of the correct size on agarose gels, and are considered to contain rickettsiae. PCR-RLB of 33 ticks showed that 96% (32/33) of the samples hybridized with a *Rickettsia* genus-specific probe. With species-specific probes, *R. amblyommii* was found in the majority of the ticks (21/33); other *Rickettsia* species detected included *R. massiliae* (3/33), *R. canadensis* (2/33), *R. montanensis* (2/33), *R.belli* (1/33) and *R.conorii* (4/33). Of the 33 samples, 10 samples showed hybridization with more than 1 species of *Rickettsia* and 3 samples contained unknown *Rickettsia* species. DNA sequencing to confirm the detection of *Rickettsia* and to verify species identifications obtained through RLB hybridization assays is in progress. Based on the ticks examined thus far, the presence of *Rickettsia* in D. variabilis is more common than we hypothesized. Our analysis of D. variabilis is ongoing and should provide informative results about the prevalence and species of *Rickettsia* harbored by this tick species.

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STUDY OF SEROPREVALENCE AND VECTORS OF *RICKETTSIA* IN THE COLOMBIAN CARIBBEAN

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The first clinical signs and symptoms of spotted fever caused by *Rickettsia* are nonspecific, so can be easily confused with other febrile infections. The medical diagnosis is even more difficult because there are no diagnostic tests during the acute phase of the disease. Thus, clinical suspicion based on epidemiology, ecology and distribution of vectors, followed by subsequent clinical manifestations of the disease becomes the best strategy for a timely diagnosis. Conduct studies to establish risk areas is necessary to prevent deaths by rickettsiosis, therefore the objective of this work was to determine the seroprevalence in human populations and natural infection with *Rickettsia* in ticks in the department of Sucre, located in the Colombian Caribbean. A blood sample was taken from people, in the rural area of five municipalities of this area, who signed an informed consent and underwent an interview to establish risk factors. An indirect immunofluorescence test was performed to detect anti-*Rickettsia* IgG antibodies. The independence between the seroprevalence and risk factors was tested by a Chi-square test at a 5% level of significance. Additionally, ticks were captured from animals and drag sampling on vegetation, which were taxonomically identified and processed in order

to detect *Rickettsia* DNA by PCR. 320 human samples were taken, where 20 of them were seropositive (6.2%). Seropositive individuals were mainly elderly men ($p<0.05$), who were engaged in agriculture. 10,217 ticks were captured, D. nitens (7922), larvae of *Amblyomma* sp. (909), *R. sanguineus* (882), *R. (B.) microplus* (372), *A. cajennense* (96), *A. dissimile* (23), *H. leporispalustris* (6), *A. ovale* (5) and *A. auricularium* (2). *Rickettsia felis* DNA was detected in *R. sanguineus* and *D. nitens*, with minimum infection rates of 7,4 and 1,9 respectively. This study provides evidence of the presence of bacteria from the genus *Rickettsia* in ticks, and its circulation in human population from rural areas of the department of Sucre.

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Q FEVER AND RICKETTSIOSIS IN U.S. MARINES DEPLOYED TO AFGHANISTAN

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Many dangers face military personnel when deployed in combat zones, but one of the most formidable opponents is infectious diseases. Operation Iraqi Freedom and Operation Enduring Freedom have brought more than 100,000 US troops to Central Asia exposing them to a multitude of pathogens that they may not have been exposed to otherwise. Several of these include the causative agents of the operationally relevant arthropod borne diseases Q fever (*Coxiella burnetii*) scrub typhus (*Orientia tsutsugamushi),* murine typhus (*Rickettsia typhi*), and spotted fever group rickettsioses (*Rickettsia sp.)*. Since 2003, more than 150 cases of Q fever have been confirmed among troops deployed to Iraq and Afghanistan, though the symptoms of infection often mimic a multitude of other infections and can often be misdiagnosed. To assess the risk of these pathogens among military personnel in Afghanistan we initiated a survey of close to 1000 Marines deployed to Afghanistan for at least one continuous year. The DOD Serum Repository provided serum from pre-deployment and post-deployment blood draws, paired for each Marine. Analysis of these sera revealed sero-conversion of approximately 6% for Q fever, and 0.5% for spotted fever, but no sero-conversions for typhus group or scrub typhus. This study leads to a greater understanding of the infectious disease risk in Central Asia and provides valuable information for diagnosis and treatment of acute febrile illness in the area.

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SCRUB TYPHUS IN AFRICA

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Since a report in 1951 evidence has been slowly increasing that scrub typhus exists in sub-Saharan Africa. The previous evidence included two cases (Cameroon and Congo) and evidence of O. tsutsugamushi-specific antibodies in humans (Ruanda-Urundi). With the recent reports of scrub typhus in Dubai and Chile, outside the previously accepted scrub typhus endemic triangle area of Asia, northern Australia and the western Pacific, we decided to investigate whether scrub typhus exists in Africa. Utilizing orientia-specific ELISA-IgG assays and two recombinant protein Western Blot assays (Kp r56 and Kp r47) we assessed human sera collected from abattoir workers in Djibouti and fever patients in Kenya for presence of antibodies-specific to Orientia spp. Antibody prevalence among fever patients in Kenya was 5% (67/1401) and abattoir workers was 4% (2/49). In addition one of the two abattoir workers positive for antibodies against Orientia had a baseline serum sample at the beginning of the study that was non-reactive to orientia-specific ELISA and WB antigens. These results add to the evidence of the presence of scrub typhus in Africa. However, it is unknown whether the scrub typhus in Africa is due to infection with O. tsutsugamushi, O. chuto, both, or another Orientia sp. Also unknown is what the vector(s) is/are. Thus, much more research needs to be performed to characterize African scrub typhus.

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IDENTIFICATION OFA FULL LENGTH TRANSCRIPT ENCODINGA PUTATIVE RELISH-TYPE NF-KB PROTEIN, DVRELISH, IN *DERMACENTOR VARIABILIS*

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As a vector for *Rickettsia rickettsii*, the causative agent of Rocky Mountain spotted fever, *Dermacentor variabilis* are known to upregulate antimicrobial peptides (AMP) in response to rickettsial infection. The relationship between AMP production and vector competence is unknown and the mechanism required for the regulation of AMPs has yet to be characterized in *D. variabilis*. In the model arthropod *Drosophila melanogaster*, the Rel/NF-kB transcription factors Dorsal, Dif, and Relish control AMP regulation. Upon immune challenge in *D. melanogaster*, the Rel/NF-kB proteins localize to the nucleus to regulate the transcription of immune responsive genes. The overall goal of the project is to identify and functionally characterize Rel/NF-kB proteins in *D. variabilis* and examine the importance of these proteins in the regulation of immune responsive genes in *Rickettsia*-infected *D. variabilis*. The broad hypothesis is that differential regulation of Rel/NF-kB proteins occurs in a *Rickettsia*-specific manner, and that this response drives vector competence. Using traditional PCR, we have isolated a 3,183 base pair transcript encoding a 873 amino acid Relish-type NF-kB transcription factor in *D. variabilis*, containing a canonical Rel-homology domain, immunoglobulin/plexin/transcription factor fold domain, nuclear localization sequence and inhibitory ankyrin repeat domains. In *D. variabilis,* after capillary feeding challenge with $2.5x10^8$/ml intracellular *R. rickettsii* in Vero host cells for 1 hour, transcript encoding DvRelish increases significantly as compared to ticks fed only Vero host cells. In contrast, expression of DvRelish transcript decreases significantly in ticks capillary fed for 3 hours with the same number of *Rickettsia*. Further projects will explore the temporal changes in DvRelish transcription and protein expression in response to increasing concentrations of *R. rickettsii* compared to non-pathogenic *Rickettsia montanensis*. These studies will increase our understanding of the molecular regulation of the immunological response to rickettsial infection in ticks, the mechanisms of which may define vector competence.

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A STUDY OF RICKETTSIAL AND ORIENTIA INFECTION AMONG ABATTOIR WORKERS AND THE DETECTION OF *RICKETTSIA* IN TICKS, DJIBOUTI

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Little is known about rickettsial infection in Djibouti located in the Horn of Africa. A study was conducted to examine the exposure to vector-borne and zoonotic pathogens in a high-risk environment, an abattoir in Djibouti. Human serum samples were collected from the abattoir workers in September 2010 and followed up every 4 weeks during a 20 week long period. Ticks were collected from the freshly-slaughtered cattle. 49 baseline and 11 follow-up human serum samples were tested for IgG antibodies against whole-cell antigens of SFGR, TGR and orientiae by enzyme-linked immunosorbent assays (ELISAs). Eight (16%), two (4%), and three (6%) were found seropositive for IgG antibodies against SFGR,

TGR and orientiae, respectively. Analysis of paired sera for 11 workers showed one was seroconversion for antibodies against orientiae during the study period, and the positive reactions were confirmed by Western blot assays using recombinant protein Kpr56 and Kpr47b. No seroconversions were observed for antibodies against SFGR or TGR. DNA extracted from 189 tick pools (contain 1-3 ticks) including 6 tick species from 72 cattle imported from Ethiopia were tested for the infection of *Rickettsia* using a *Rickettsia* genus-specific quantitative real-time PCR (qPCR) assay (Rick17b) and the positives were further tested by a qPCR assay specific for *Rickettsia africae* (RafriG). Overall, 32 (17%) pools of ticks from 26 (36%) cattle were infected with *Rickettsia*, of which 25 (47%) were *R. africae*, the causative agent of African tick-bite fever. Three types of *R. africae* variants were identified from *Ambylomma variegatum* ticks (but none from *A. lepidum*) by PCR and sequencing of a fragment of *ompB*. In addition, *R. sp.* S strain was detected in 2 (6%) pools of *Hyalomma marginatum* ticks. Although future studies are needed to determine the extent and impact of rickettsial infections in Djibouti, healthcare providers should be aware of possibility of these infections among their patients.

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RICKETTSIA AFRICAE VARIANTS

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Rickettsia africae causes African tick-bite fever (ATBF) and is one of the main causes of fever and illness in travelers returning from sub-Saharan Africa. *R. africae* belongs to the spotted fever group rickettsiae (SFGR) and is transmitted to humans by ticks of the genus *Amblyomma* found throughout sub-Saharan Africa. An infection rate in these ticks in endemic areas is high, and may reach 100%. Recent studies have shown existence of *R. africae* variants in ticks from several countries in Africa and New Caledonia. The genetic variants have mainly been detected in *Ambylomma variegatum* ticks and are closely related to wild type *R. africae*, but have not been associated with ATBF. The variability observed is mainly due to nucleotide substitution but some may have deletions and/ or insertions leading to premature stop codons in the amino acid sequence. The impact of these mutations to the infectivity of the *R. africae* variants is currently unknown but we hypothesize that it may result in nonfunctional proteins and preclude human infection. The variability observed among *R. africae* genotype underscores the need to undertake comparative assessment of variants associated with clinical ATBF and those circulating within the tick population to understand the role that these variants play in the epidemiology of ATBF.

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AN ONLINE TOOL FOR MAPPING INSECTICIDE RESISTANCE IN MAJOR *ANOPHELES* VECTORS OF HUMAN MALARIA PARASITES AND A REVIEW OF INSECTICIDE RESISTANCE STATUS FOR THE AFROTROPICAL REGION

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Rapid evolution of insecticide resistance in malaria vectors threatens to erode gains made in malaria control unless action is urgently taken. Upto-date insecticide resistance data are urgently needed for inform vector control policy and programmes. A new online geospatial mapping tool, IR Mapper, is introduced herein. A systematic search of peer-reviewed literature was performed and *Anopheles* phenotypic and resistance mechanisms data extracted to Microsoft Excel 2010 database. IR Mapper (www.irmapper.com) was built on ArcGIS for JavaScript. Additional integrated functionalities allowing triangulation of entomological monitoring data with epidemiological data include *Plasmodium falciparum* and *P. vivax* endemicity layers, entomological inoculation rate layers, and an "Add Own Data" functionality enabling users to add and temporarily view their own data on the application, and print user-generated maps. IR Mapper contains 8, 596 data points from 1, 587 georeferenced localities in 55 countries for data collected between 1954-2013. Susceptibility reports were available for 53 countries; 43 reported resistance confirmed to at least one insecticide. One or more types of resistance mechanisms were detected in 37 countries. In the Afrotropical region pyrethroids and DDT were commonly tested in susceptibility assays than carbamates or organophosphates. Between 2001 and 2012, there was an increase in cases of confirmed resistance of *An. gambiae s.l.* to pyrethroids (from 41 to 87%) and DDT (from 64 to 91%). Resistance mechanism assays in *An. gambiae* mainly focused on target site mutations with very few reports on elevated metabolic enzyme assays. Mapper is a dynamic tool that collates resistance information from various sources and in various formats into a single platform. Mapper is useful for investigating temporal and spatial trends in *Anopheles* insecticide resistance, informing rational deployment of vector control interventions based on the evidence, as well as for pointing out gaps in resistance monitoring for appropriate action.

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INSECTICIDAL PROPERTIES OF LOCAL PLANTS USED AGAINST *ANOPHELES GAMBIAE*, MALARIA VECTOR IN BURKINA FASO, WEST AFRICA

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Malaria remains a serious public health problem killing mostly in Africa. It is estimated to 207 millions of cases and 627000 deaths the burden of malaria in 2012. Malaria control is still heavily dependent of insecticides for vector control but the emergence of resistance to insecticides in Malaria vector population can jeopardize this control effort. The main objective of this study was to evaluate, larvicidal and adulticidal properties of essential oil of local plants, namely, *Ocimum canum, Hyptis suaveolens, Hyptis spicigera* and *Lantana camara* extracts on *Anopheles gambiae* and their inhibiting activity of acytylcholinesterase (AChE). Young branches with leaves were collected from local plants and the extraction of essential oil was processed. Adults of 3-5 old days and third-fourth stage larvae were used for bioassay tests based on WHO protocols. Different concentrations have been used for each plant essential oil and results have been assessed as mortality and Knock down rate of adults after 1 hour exposure. Larvae mortality after 24 hours and the lethal concentration were calculated for each essential oil. The inhibition effect of essential oils on acetylcholinesterase activity was assessed using Ellman's spectrophotometric method. All tested essential oils exhibited adulticidal and larvicidal activities. The LD50 and LD90 lethal doses value observed were 0.82% and 1.55% respectively on adults for L. camara. On larvae, the LC50 and LC90 values of this oil were 61 and 125 ppm respectively. The high inhibitory activity on acetylcholinesterase was observed with O. americanum and H. suaveolens essential oil with 50% Inhibitory concentration (IC50) of 0.21 and 0.55 µg/ml respectively. Our results highlighted that essential oils of these plants have a potential as insecticides for malaria vector control and can be considered as an interesting source of natural and ecofriendly substances for vector control.

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FIELD EFFICACY OF VECTOBAC GR AS A MOSQUITO LARVICIDE FOR THE CONTROL OF ANOPHELINE AND CULICINE MOSQUITOES IN NATURAL HABITATS IN BENIN, WEST AFRICA

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The efficacy of Vectobac GR (potency 200 ITU/mg), a new formulation of bacterial larvicide Bacillus thuringiensis var. israelensis Strain AM65-52, was evaluated against *Anopheles gambiae* and *Culex quinquefasciatus* in simulated field and natural habitats in Benin. In simulated field conditions, Vectobac GR formulation was tested at 3 dosages (0.6, 0.9, 1.2 g granules/m² against *An. gambiae* and 1, 1.5, 2 g granules/m² against Cx. quinquefasciatus) according to manufacturer's product label recommendations. The dosage giving optimum efficacy under simulated field conditions were evaluated in the field. The efficacy of Vectobac GR in terms of emergence inhibition in simulated field conditions and of reduction of larval and pupal densities in rice fields and urban cesspits was measured following WHO guidelines for testing and evaluation of mosquito larvicides. Vectobac GR caused emergence inhibition of ≥80% until 21 [20-22] days for *An. gambiae* at 1.2 g/m² dose and 28 [27-29] days for *Cx. quinquefasciatus* at 2 g/m² in simulated field habitats. The efficacy of Vectobac GR in natural habitats was for 2 to 3 days against larvae and up to 10 days against pupae. In conclusion, treatment with Vectobac GR caused complete control of immature mosquito within 2-3 days but did not show prolonged residual action. Larviciding can be an option for malaria and filariasis vector control particularly in managing pyrethroid-resistance in African malaria vectors. Since use of larvicides among several African countries is being emphasized through Economic Community of West Africa States, their epidemiological impact should be carefully investigated.

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EFFECTS OF COMBINATIONS OF A SUNLIGHT-ACTIVATABLE PORPHYRIN AND NEEM TREE PARTS AGAINST LARVAE OF *ANOPHELES GAMBIAE* S.L., POTENTIAL COMPLEMENTING TOOLS FOR INTEGRATED STRATEGIES OF INSECTICIDE RESISTANCE MANAGEMENT

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Meso-tri(N-methyl-pyridyl),mono(N-dodecyl-pyrydyl)porphine is a porphyrin known as C12. Loaded on ground cat food pellets (CF), the C12 has rapid killing effect on larvae of *Anopheles gambiae* s.l. The present study aimed at screening combinations of C12 and neem portions with the ability of both rapid killing and delayed-acting effects (additive effect) and subsequent residual efficacy (synergistic effect) on larvae of the malaria vectors using outdoor tray experiments. C12-neem combinations were obtained by incubating finely ground neem leaves (NL), bark (NB), fruit (NF) and 10% NeemAzal-loaded cat food (CFNA) in 500-µM C12 solutions. Batches of 60 larvae of wild or laboratory colonies (2nd - 4th instars) were exposed outdoors with 20 - 30 mg of C12-neem products in water samples from typical water bodies. Larvae treated with CF and C12-CF free of neem served for negative and positive controls respectively. Three consecutive experiments were run allowing to determine respectively larval mortality efficacy after i) a short time of 48 h post-treatment, ii) a prolonged-exposure up to 9 days post-treatment and iii) residual efficacy as larval mortality and subsequent inhibition of pupation up to 9 days post-treatment. Alike the C12-CF, C12-neem combinations particularly C12-NF and C12-NL yielded rapid killing efficacy of ~100 % larval mortality after 48 h post-treatment in spring water. This effect decreased in turbid water types. However, a prolonged-exposure allowed to achieve a delayed-effect of 70 - 100% mortalities in turbid water type after 4 - 5 days post-treatment. Upon a prolonged-exposure time of 9 days in spring water, C12-NF and C12-CFNA yielded residual effects of 70 - 100% larval mortalities. However, C12-CF yielded such residual activity for only 21% mortality. C12-NF possesses at least an additive efficacy on larvae of *An. gambiae* s.l. in this preliminary study. Further investigations should help to reinforce efforts towards the development and validation of such larvicidal tools in the context of integrated management of insecticide resistance.

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EVALUATION OF THE FACTORS AFFECTING THE FUNCTIONALITY OF INDOOR RESIDUAL SPRAYING AND LONG LASTING INSECTICIDE NETS PROGRAMS USED FOR MALARIA CONTROL IN WESTERN KENYA

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Indoor Residual Spraying and long-lasting insecticidal nets have been extensively used for malaria prevention and control in Kenya. However there are concerns that certain factors greatly affect the efficacy of these programs. We evaluated the persistence of ICON and deltamethrin on the sprayed mud walls and the bio efficacy of Long Lasting Insecticide nets. Wall bioassays were performed on artificial mud walls and filter papers sprayed with ICON and Deltamethrin using mosquitoes collected from seven sites in western Kenya and Kisumu strain as a control. Net cone bioassays was performed on nets collected from the fields using wild mosquitoes from Emutete and Bungoma and Kisumu susceptible strain was used as a control. Chemical analysis of the netting material was done using gas chromatography. Kisumu strain was susceptible to the insecticides with 100% mortality. ICON persisted on the mud wall for Six months where as Deltamethrin Persisted on the Mud walls for four months. Sprayed artificial walls showed lower mortality rates compared to sprayed filter papers. ICON had high mortality rates on the mosquitoes compared to Deltamethrin. Mosquitoes from Chulaimbo, Ahero, Emakakha and Kisian showed susceptibility to both deltamethrin and ICON with the mortality rates ranging between 80% - 85% but mosquitoes from Bungoma and Emutete had lower mortality rates to both ICON and Deltamethrin with mortality rates ranging between 69%-74%. Wild mosquitoes showed low mortality rates to LLINs nets collected from the field (60%-75%), compared to the control strain (100%). Net chemical analysis results indicated that there was no difference in the net chemical content in the nets between 6months - 2yrs old, with the net chemical content ranging between 0.06 wt% - 0.13 wt%. In conclusion, insecticide resistance, persistent of the insecticide on the sprayed surfaces, bioavailability of the insecticide and the physical condition of long-lasting insecticide nets affect the efficacy of indoor residual spraying and Long Lasting Insecticide nets, therefore these factors should be considered in malaria control programs.

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NEAR-INFRARED CAN DETECT PRESENCE OF *WOLBACHIA* IN FEMALE AND MALE *AEDES AEGYPTI*

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Vector control strategy utilizing endosymbiotic bacterium; *Wolbachia* has been reported successful in Australia. The ability of *Wolbachia pipientis* to shorten the lifespan of mosquitoes and block dengue transmission simultaneously makes it a very effective vector control intervention. The success of such an intervention is determined by its ability to rapidly spread in a given mosquito population. Currently, *Wolbachia* infected mosquitoes can only be differentiated from non-infected population by use of Polymerase Chain Reaction (PCR) assay. However, PCR is costly and time consuming. In this study, we applied the Near-infrared spectroscopy to detect the presence of two pathogenic strains of *Wolbachia pipientis*; wMel and wMelPop in male and female *Aedes aegypti* reared in the laboratory. Using cross validation technique, the accuracy of differentiating infected from non-infected females of the wMel and wMelpop *Wolbachia* was 86.5% and 85%, respectively. wMel and wMelP*op* infected males were predicted with an accuracy of 86% and 96% respectively. Furthermore, independent female sets of wMel and wMelPop were predicted with an accuracy of 84% and 97%, respectively whereas independent male sets were predicted with an overall accuracy of 90%. Where processing of large field samples are required to evaluate the efficacy of strategies involving *Wolbachia*, NIRS could be a complementary diagnostic tool as thousands of samples can be scanned daily. However, we recommend a further assessment of the accuracy of this tool to detect *Wolbachia* infected wild mosquitoes relative to PCR.

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OVERDOMINANCE OF KDR MUTATION IN *ANOPHELES GAMBIAE* ON A BEHAVIORAL TRAIT UNDER INSECTICIDE SELECTIVE PRESSURE

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The anthropophilic *Anopheles gambiae* malaria mosquito females need a blood meal to mature eggs. Faced to the new physical and chemical barrier (i.e. the pyrethroid insecticide treated bed net), *Anopheles* mosquitoes exhibit evolutionary responses such as the Kdr mutation that provides physiological resistance to the pyrethroid insecticides family. Nevertheless, the impact of physiological resistance specifically Kdr mutation on the short-range of host seeking behaviour, when the host is under treated net remains poorly studied. In the present study, we investigated the ability of *An. gambiae* s.s. of the three genotypes of the Kdr mutation to find a hole in a piece of net (either untreated or insecticide treated) to reach a bait in a wind tunnel. Homozygous resistant mosquitoes were the less efficient to pass through an untreated holed net indicating for the first time a cost for the Kdr mutation in *An. gambiae*. This reduced performance was likely to be due to a reduced host-seeking activity as revealed by video-tracks analysis of the mosquitoes. When faced to insecticide treated net, heterozygous mosquitoes were the most efficient evidencing overdominance of the Kdr mutation under pyrethroid selective pressure. Our findings brought valuable new insights in the study of heterosis with an evidence of overdominance of a single gene through behavioral traits. These striking results raise a lot of questions the evolution of such insecticide resistance mutations in natural conditions.

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ENTOMOLOGICAL MONITORING OF INDOOR RESIDUAL SPRAYING INTERVENTION IN LAKE VICTORIA BASIN REGIONS, TANZANIA

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In response to the success of indoor residual spraying (IRS) intervention in Kagera region, which resulted to the decline of entomological indices. The Government of United Republic of Tanzania through financial support of the US President's Malaria Initiatives decided to scale-up the IRS programme in all regions of the Lake Victoria basin, Tanzania. Entomological monitoring activities were carried out at seven representative sentinel districts in Mwanza, Mara, Geita and Kagera regions where IRS intervention have been implemented from July 2011 - August 2012. Mosquitoes were collected using CDC light traps, Pyrethrum spray catch (PSC) and pit trap (PT) method to determine malaria vector species, abundance and sporozoites rates. Vector species were identified by PCR and sporozoite rates were determined by ELISA technique. To determine residual effect of insecticide on various sprayed surfaces, eight houses were selected in each sentinel site. Two to five days old 20 laboratory susceptible female *Anopheles gambiae* s.s. (Kisumu strain) were used. A total of 1713 anopheline mosquitoes were collected. Out of these, CDC light traps collected 879(513%), PT 755(44.5%) and PSC 79(4.7%). A total of 637 anopheline mosquitoes were identified to species. Predominant malaria species were An. *arabiensis* accounted 52.4%, *An. funestus* 24.8%, *An. gambiae* s.s 17.9% and *An. parensis* 4.9%. A total of 972 mosquitoes were tested for sporozoites. The prevalence of sporozoite rates was 1.01% for *P. falciparum*. Bioassays results indicate that wood and cement surfaces have long retention rate. In conclusion, mosquito abundance indicates that high numbers of mosquitoes were collected indoors using CDC light traps. Generally, anopheline mosquitoes were collected in high number in the period of May and June in all sentinel sites. The IRS intervention may have contributed to the decline of malaria in the area.

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PYRETHROID KNOCKDOWN RESISTANCE (KDR) HAPLOTYPES IN *ANOPHELES GAMBIAE* POPULATIONS FROM MALARIA SURVEILLANCE SITES IN THE GAMBIA

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Knockdown resistance (kdr) is a target-site resistance mechanism implicated for DDT and pyrethroid insecticides resulting from point mutations at the domain II of the voltage-gated sodium channel gene of *Anopheles gambiae* s.s. Indications of DDT and pyrethroid resistance among *An. gambiae* s.l. in The Gambia were reported in a recent bioassay study. The aim of the present study was to investigate the underlying target-site resistance mutation and associated kdr haplotypes in the same mosquito population. A combination of allele-specific polymerase chain reaction (AS-PCR), sequencing and a high resolution melting (HRM) assay modified by use of primers further away from kdr point mutation on the sodium channel gene was employed to describe the kdr genotypes in over 1000 Gambian *An. gambiae* specimens from four sites analysed in previous bioassays. Five haplotypes of kdr mutations to pyrethroids describing, L1014S, kdr east and L1014F, kdr west mechanisms

were identified with a total frequency of 15% mainly in *Anopheles* arabiensis. With the modified HRM assay, four unique melt profiles could be distinguished for kdr mutant genotypes representing six mutant haplotypes from polymorphisms at positions 1013 (T/C) and 1014 (T/A). Four kdr wild-type haplotypes resulting from 1013 (T/C), 1014 (T/A), 1033 (G/T), 1042 (C/G) and 1059 (A/T) substitutions could also be identified by different clusters of melting profiles and sequenced. The contribution of these different haplotypes to vector survival and impact on vector control remains undetermined. These results provide evidence of different pyrethroid kdr haplotypes in The Gambia and present HRM as a useful assay for rapid scanning of kdr-based insecticide resistance in a mosquito population.

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ELECTROPHYSIOLOGICAL RESPONSES OF DIFFERENT *ANOPHELES GAMBIAE* RESISTANT STRAINS TO INSECTICIDE AND HOST ODORS

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Major mean of malaria vector control are based on use of insecticides. Their efficiency is threatened by widespread resistance mechanisms. In addition to the physiological resistance mechanisms already well studied, the issue of the behavioral modulation as cause or consequence of the resistance is largely overlooked. Nevertheless there are evidences that insecticide-based control tools alter mosquito behavior before any contact, suggesting that the mosquitoes can detect the presence of the insecticide. In the present study, we tested this hypothesis by investigating the olfactory responses of different resistant strains of *Anopheles gambiae* to host odors and volatile insecticide chemicals. We used *An. gambiae* females aged of 5-8 days old from different strains sharing the same genetic background but insecticide resistance mutations *Kdr* and *Ace1*^{R}. We run electroantenograph (EAG) experiments with different genotypes for these loci (SS, RS, RR) and compared the electrical responses of the antennae. We investigated the responses to the following odours: Octenol, human sweat odour, permethrin, deltamethrin, bendiocarb, propoxur. We described the dose-response relationship to all of these chemicals (except human sweat odour) and run a comparative analysis of the responses of each genotype for the two insecticide resistance loci. Genotypes responded differentially to the odour suggesting that resistance mutations impact olfactory sensitivity of mosquitoes to their environment. Moreover, sensitive strain, Kisumu, responded to permethrin, evidencing that mosquitoes are able to smell insecticide molecule.

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DETECTION OF G119S ACE-1R MUTATION IN FIELD-COLLECTED MOSQUITOES USING ALLELE SPECIFIC LOOP MEDIATED ISOTHERMAL AMPLIFICATION METHOD (AS-LAMP)

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Malaria vectors have developed resistance to the four families of insecticides available for public health purpose. The resistance to organochlorines, pyrethroids, organophosphates and carbamates has increased along with the increasing of the *1014F kdr* and the *G119S ace-1*^{R} mutations frequency in Burkina Faso. The spread of *kdr* and *ace-1*^{R} mutations in malaria vectors is the main constraint for effective insecticide based control of malaria. There is an urgent need to improve resistance management using existing insecticides and new tools to assess resistance level on time for quick decision-making. With the aim of developing a fast and reliable tool to detect *ace-1*^{R} mutation, we report an allele specific LAMP (AS-LAMP) method to detect the *ace-1*^{R} allele. We designed specific primers with the mutation on the 5' end of the inner primers (BIP and FIP) and additional mismatch nucleotides to increase the specificity and distinguishing between the resistant and wild type of *ace-1*^{R} alleles. Plasmids inserted with targeted DNA sequence as well as genomic DNA were used as DNA template to set up the detection method. The reaction was processed in a real time turbidimeter at 63C for 75 min and detected by the turbidity values as well as by naked eye. The results have been confirmed by direct sequencing and the specificity and sensitivity were compared to RT-PCR using more than 100 mosquitoes. The primers designed for LAMP can distinguish between the wild type allele (*ace-1*^{S}) and the mutated type allele (*ace-1*^{R}). The detection time for the wild type homozygous is 50 min and 64 min for the heterozygous using the wild type specific primers. Using these primers, there is no amplification for the resistant type (*ace-1*^{R}) until 75 min. For the *ace-1*^{R} resistant type, the detection time is 51 min for the resistant homozygous and 55 min for the heterozygous, there is no amplification for the wild type (*ace-1*^{S}) until 75 min when using the resistant type primers. The gel electrophoresis of the LAMP products confirmed that the amplification is primer-DNA specific, the primers can only amplify their target specific DNA. Comparing the AS-LAMP to RT-PCR for *ace-1*^{R} detection, the sensitivity and specificity of the of methods were globally similar This fast and reliable detection method which can be performed with just a water bath at 63°C and yields a result detectable by the naked eye, can be used for resistance monitoring at the site of transmission for quick decision-making.

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LABORATORY SELECTION FOR PYRETHRIOD RESISTANCE IN FIELD *ANOPHELES SINENSIS*, THE MAJOR ASIAN MALARIA VECTOR

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Anopheles sinensis (Diptera: Culicidae) is one of the major malaria vectors in China and other Asian countries. Increased attention has been shifted to this species due to its wide distribution, high abundance, and modest susceptibility to malaria parasites. The rapid spread of insecticide resistance becomes the major obstacle for insecticide-based strategy of vector control, which has placed current national efforts in malaria elimination at risk. Since we already have colonized the insecticide-susceptible in *An. sinensis*, the establishing of insecticide-resistant population is essential for the genetic approaches to further understand the molecular mechanism of insecticide resistance and to enhance resistance surveillance in this species. In 2013, the natural *An. sinensis* mosquitoes (F0) were collected from regions, where pyrethriod resistance had been demonstrated in central China, and identified by the morphological characters on both adults and eggs to avoid the species contamination. The adults (F1) 3 days post emergence were treated by standard WHO tube bioassay with 0.05% deltamethrin, and the survivors 24 h post exposure were left for blood-feeding, followed by the forced mating method to overcome the low-mating rate. Eggs from each generation were reared through to adults and selected for resistance to deltamethrin in the same way. After the continuous pyrethriod selection, the survival rate in F3 generation significantly increased to 99.16% and 99.73% in male and female populations, respectively. Two types of kdr mutations (L1014F: TTG ◊ TTT; L1014C: TTG to TGT) at codon position 1014 of the para-type sodium channel gene were detected during the selection process, and the kdr mutation frequency reached to 100% in F2 and F3 generations, with a predominant kdr mutation L1014F. As a conclusion, we have selected and colonized the deltamethrin-resistant population from field *An. sinensis*, which would provide the fundamental material for genetic studies in this important malaria vector.

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UNDERSTANDING THE VARIATION OF INSECTICIDE RESISTANCE IN *ANOPHELES GAMBIAE* S.S FROM CÔTE D'IVOIRE: TOWARD ALTERNATIVE OPERATIONAL STRATEGIES FOR MALARIA ELIMINATION

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Malaria control is heavily dependent on the use of insecticides that target adult mosquito vectors via insecticide treated nets (ITNs) or indoor residual spraying (IRS). In Côte d'Ivoire, where IRS is not implemented, ITNs and more recently LLINs remain the main control measure. We used different approaches based on literature review, field collections, Geographical Information System (GIS) and IR mapper tool to investigate the report the spatial and temporal variation of resistance to WHO-approved insecticide for LLINs and IRS. Overall, mosquitoes collection covered the period from 1993 to 2013, with 60 % of records from 1993-2003 and 40 % between 2004 and 2013. Data were stratified according to the four ecological zones of the country with pyrethroids (OP) and organochlorines (OC) largely tested compared to carbamates and organophosphates ($X2 = 10.8$, $p<0.05$). Organophosphate (52.2%) and carbamates (47.8%) were tested in 23 sites mainly, with no fenitrothion and pirimiphos methyl resistance detected in ecological zone 2, but multiple insecticide resistance detected in only four sites mainly located in zone 1. Within OP and OC, permethrin, deltamethrin and DDT were largely tested between both decades. Temporal trend based on three clusters gathering three yearly mean mortality data per insecticide (exception for DDT in cluster 1, no available data for 1993) showed non-significant increase of susceptibility to deltamethrin in cluster 2 ($X2= 3.879$, $p= 0.144$). We then checked the trend between clusters for each insecticide across zones. Overall, no significant difference was observed in ecological zone 1 between cluster 1 and 2 these insecticides ($P> 0.05$). Mortality to DDT has significant increase in in zone 3 ($X2= 49.94$, $p= 0$), while non-significant variation was detected in 4 ($X2= 1.07$ $p= 0.300$). Data of this study provide valuable information to National malaria control programme for vector control intervention. Deltamethrin could be used for vector control in several areas of ecological zones 3 and 4. In ecological zone 2 where no resistance to fenitrothion and pirimiphos methyl have been detected for most investigated sites, OP could be a potential alternative for IRS. Finally, in ecological zone 1, where multiple insecticide resistance was detected in four sites, new strategies and tools for vector control are needed for vectors control.

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SUSCEPTIBILITY TO PYRETHROIDS OF *CULEX* SPP AND *ANOPHELES* SPP FROM TWO DIFFERENT TYPES OF ENVIRONMENTS IN A CONTEXT OF MASSIVE USE OF INSECTICIDES IN AGRICULTURE FROM TIASSALE, SOUTHERN COTE D'IVOIRE

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The use of insecticides in agriculture and public health has led the emergence of resistance in mosquitoes. Previous studies in the locality of Tiassalekro have shown that the malaria vectors, *Anopheles*, from rice field are resistant to various insecticides. Given that *Culex* mosquitoes also breed in the same rice field and subject to the same selection pressure, it would be interesting to look at their resistance profile, and compare this to *Anopheles*. The current study was undertaken to determine the resistance status of larvae and adult of both *Culex* and *Anopheles* from rice field. The investigation was extended to neighboring houses in order to find out whether the resistance level was the same in the two environments. *Culex* and *Anopheles* larvae were collected in rice fields and reared until adult stage. Blood fed *Culex* and *Anopheles* were collected in sleeping rooms, reared until oviposition. Larvae from eggs were reared to adult stage. Late third instar larvae were exposed to a range of deltamethrin concentrations to determine the LC_{50}. Beside this, *Culex* and *Anopheles* adult females 2-5 days old were exposed to 0.05% deltamethrin treated papers. Physico-chemical parameters of the breeding site in the two environments were determined. *Culex* larvae from houses (LC_{50} = 0,014ppm) were more resistant than *Culex* from rice field (LC50 = 0,0001 ppm) contrary for *Anopheles* larvae from rice field (LC_{50} = 4,35ppm) which appeared more resistant than those from houses (LC50= 2,32 ppm). Mortality was not significantly different between adult *Culex* from the two environments (47%). By cons, *Anopheles* from houses (9,78%) were more resistant than those from rice field (37,11%). Furthermore, according to WHO criteria, *Culex* and *Anopheles* from rice field were both resistant. The same trends were seen with *Culex* and *Anopheles* from houses. Parameters such as conductivity, salinity, redox and pH are significantly different in the two environments contrary for dissolve oxygen and temperature ($p>0.05$). In conclusion, adult mosquitoes are resistant to deltamethrin according WHO interpretation. However, the metabolism of resistance in adult and larvae stages are likely not similar. Molecular analysis wil determine if existing any genetic similarity between *Culex* and *Anopheles* collected in the houses and those collected in the rice fields.

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TARGET-SITE RESISTANCE MECHANISMS *KDR* AND *ACE-1* PRESENT UNIQUE CHALLENGES TO REGIONAL MALARIA ELIMINATION IN THE AMERICAS

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In the region of the Americas, malaria transmission intensity tends to be low and/or focal, and as such, the Americas are seen as a region where malaria elimination may potentially be achieved. However, a variety of anopheline vector species displaying a wide range of breeding, bloodfeeding, and resting behaviors present a challenge for vector control strategies in the Americas. Strategies that successfully control malaria vectors elsewhere are not as well-characterized in the Americas, and little research has been conducted on how insecticide resistance might further compromise efforts for malaria control, and ultimately, elimination. For example, *Anopheles albimanus* are highly resistant to multiple classes of insecticides in coastal Peru, including an area where *Plasmodium falciparum* was recently re-introduced. Our data suggest that voltage-gated sodium channel target site resistance (*kdr*) has been present in *albimanus* throughout Mesoamerica for at least 20 years. To date, the *kdr* polymorphisms we have detected in *An. albimanus* throughout the region are L1014C (associated with permethrin, deltamethrin and beta-cypermethrin resistance in other anophelines) and L1014F (associated with resistance to DDT and a broad range of pyrethroids in other anophelines). We have also identified a novel mutation on the *ace-1* gene and our data suggest that *ace-1* gene duplication and balancing selection are allowing resistant phenotypes to persist at high levels with minimal fitness costs. Even in the absence of routine insecticide application for vector control, resistance selection pressure throughout coastal Mesoamerica and South America is likely maintained through the use of agricultural pesticides, as *An. albimanus* habitats are often associated with lowland crop cultivation. The high frequency of alleles associated with resistance to pyrethroids, DDT, carbamates, and organophosphates in these populations is worrisome and could pose significant vector control challenges as interventions are scaled up as part of regional malaria elimination strategies.

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WIDESPREAD EMERGENCE OF PYRETHROID RESISTANCE AND PRESENCE OF KNOCK DOWN RESISTANCE (KDR) MUTATIONS IN INDIAN *AEDES AEGYPTI* POPULATIONS

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Aedes aegypti, primary vector of yellow fever in Americas, is the primary vector for dengue and chikungunya in India. Resistance to DDT, temephos, permethrin and deltamethrin has been reported in this species in America, Brazil, China, Thailand, Indonesia, Vietnam and many other tropical and subtropical countries; however there is scanty information about insecticide resistance of this species in Indian populations. We collected immature of *Ae. aegypti* from different geographical regions of India; Haryana, Bangalore, Chennai, West Bengal, Bhopal and Khandwa. Larvae/pupae were allowed to emerge into adults. WHO standard insecticide susceptibility tests for DDT (4%), permethrin (0.75%) and deltamethrin (0.05%) were carried out on 2-3 days old adult females. We found varying susceptibility for different insecticides in different populations. Very high level of resistance against DDT was observed in Kolkata population (12.07% mortality) moderate resistance in Bangalore, Chennai and Haryana (55.13% - 66.16% mortality) and incipient resistance in Bhopal and Khandwa populations (91.11% mortality). Deltamethrin resistance was found to be moderate level in Bangalore, Chennai, (48.48% - 80.83% mortality) and low level in Bhopal, Kolkata and Haryana (82.48% - 83.58% mortality). Permethrin was still effective in most of the population showing moderate level of resistance Kolkata population (74.29 % mortality) and low level in Bangalore, Chennai, Haryana and Bhopal populations (92.31% - 98.37% mortality). Two populations (Haryana and Kolkata) tested for Malathion were found susceptible (100% mortality). Here we also studied a knock down resistance (kdr) mutation reported worldwide F1534C and found it few populations. This mutation was absent in Bangalore, Chennai, Khandwa and Bhopal populations. In Haryana population the frequency for mutant allele is very low 0.095 but the high degree of resistance in Kolkata population can be correlated to the high frequency of mutant allele 0.48. Resistance against pyrethroids is alarming and has negative effect on the success of pyrethroid based personal protection methods. Understanding of resistance mechanism is helpful for effective vector control strategies.

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INSECTICIDE SUSCEPTIBILITY OF *ANOPHELES GAMBIAE* S.S MOSQUITOES IN IBADAN, SOUTHWEST NIGERIA

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The emergence of insecticide resistance in *Anopheles* mosquitoes has great implications for malaria control in Nigeria. This study aimed to determine the dynamics of insecticide susceptibility levels and frequency of knockdown resistance (kdr) mutations (L1014F) in wild *An. gambiae* s.s. (Diptera: Culicidae) from Ojoo and Bodija areas of Ibadan, South-west, Nigeria. Insecticide susceptibility to pyrethroids, organophosphates carbamates and organochlorines was assessed using WHO bioassays. A subset of the mosquitoes exposed to pyrethroids and DDT was used for species and molecular form identification and their kdr genotyping was determined using the Taqman assay. The mosquitoes were resistant to pyrethroids and DDT but completely susceptible to organophosphates and carbamates. All samples were identified as *An. gambiae* s.s. Bodja sample comprised of the S form (91.4%) and the M form (8.1%) while one M/S hybrid was recorded. All the mosquitoes screened in Ojoo belonged to the S form. The 1014F kdr mutation was detected at a frequency of 24.52% and 5.8% in Bodija and Ojoo respectively. No correlation was observed between kdr genotypes and resistance phenotypes. The results indicate that metabolic resistance probably plays an important role in the resistance observed and calls for a need to implement insecticide resistance management strategies.

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EFFICACY OF OLYSET DUO® (A PYRIPROXYFEN AND PERMETHRIN BI-TREATED NET) AGAINST PYRETHROID RESISTANT *ANOPHELES GAMBIAE* IN BENIN, WEST AFRICA

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Pyrethroid resistance threatens to undermine the efficacy of longlasting insecticidal nets (LNs) in Africa and without prompt action, the fragile gains in malaria control could be reversed. Alternative compounds which can replace or complement pyrethroids on LNs are urgently needed. Pyriproxyfen (PPF), an insect growth regulator, reduces the fecundity and fertility of adult female mosquitoes. Mixing pyrethroids and PPF on LNs could provide personal protection through the pyrethroid and reduce vector abundance through the sterilising effects of PPF. Experimental hut studies were performed in Benin, West Africa to assess the efficacy of Olyset Duo, a new LN incorporating permethrin (pyrethroid) and pyriproxyfen against pyrethroid resistant *Anopheles gambiae* which are difficult to control with current LNs. Comparison was made with LNs treated with permethrin alone (Olyset Net) and pyriproxyfen alone (PPF LN). Laboratory bioassays were performed with resistant and susceptible strains of An gambiae to substantiate the hut studies. Mortality of wild pyrethroid resistant An gambiae in the experimental huts was significantly higher with Olyset Duo (40-50%) than with Olyset Net (20-30%, $P<0.05$). Olyset Duo also provided significantly higher levels of personal protection than Olyset Net. The reproductive rate of bloodfed pyrethroid resistant mosquitoes surviving in huts with the pyriproxyfen treated LNs was significantly reduced relative to the control (71-100% reduction with Olyset Duo and 91-100% with PPF LN). Cone bioassay and tunnel test results were consistent with the experimental hut studies and showed complete sterilisation and life shortenning effects in mosquitoes surviving exposure to Olyset Duo and PPF LN. The mixture net (Olyset Duo) demonstrated superior performance to the pyrethroid net (Olyset Net) in terms of mortality and personal protection; and also sterilised and reduced the lifespan of surviving mosquitoes. Combining PPF with pyrethroids on bednets shows potential for improved malaria vector control and management of pyrethroid resistance.

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HOW LONG DO BED NETS LAST? PHYSICAL DURABILITY AND INSECTICIDE ACTIVITY OF LONG-LASTING INSECTICIDAL NETS AFTER THREE YEARS IN USE, CRUZEIRO DO SUL, BRAZIL

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As regional malaria control efforts in the Americas begin to consider the possibility of elimination, the role of vector control tools such as long-lasting insecticidal nets (LLINs) must be well-defined. With the number of malaria cases reported in the Americas yearly, LLINs have become

an integral part of malaria control in the Brazilian Amazon region. The monitoring of LLIN durability is crucial to guide vector control programs and refine distribution and replacement strategies. In 2007, public health authorities in Brazil distributed 7,000 PermaNet 2.0 LLINs in Cruzeiro do Sul, Acre State covering all sleeping spaces in selected areas of the municipality. We evaluated the physical integrity and insecticide content of LLINs distributed in three high-transmission areas of Cruzeiro do Sul municipality after nets have been in use for 3 years. Physical integrity was evaluated by counting and measuring the holes on each of the five panels of the net (4 lateral sides and roof). Cone bioassays were used to assess the bioefficacy of the LLINs and a standard colorimetric assay was used to estimate the amount of deltamethrin available on the surface. Of the 27 evaluated LLINs, the lateral panels showed the greatest amount of physical damage, although most holes (61.3%) were <1.5 cm in diameter. Bioassay results indicated that only two LLINs achieved mosquito mortality >80%. The colorimetric test showed that 59.3% (16/27) of the nets had insecticide on the surface ≥0.36 µg/net, which is considered the minimal efficacy threshold. In summary, most LLINs contained holes. However, most were small, so the LLINs likely still provided some degree of physical protection against host-seeking mosquitoes. More importantly, bioefficacy was severely impaired. The colorimetric data also showed depletion of insecticide levels on the surface of the nets. These data suggested that the lifespan of LLINs in this part of Brazil should be further evaluated at time periods <3 years to help refine the planning of LLIN distribution and replacement strategies.

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ACCURACY OF GOOGLE EARTH FOR HOUSEHOLD ENUMERATION AND PLANNING OF AN INDOOR RESIDUAL SPRAYING CAMPAIGN FOR MALARIA IN CHIBOMBO DISTRICT, ZAMBIA

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Satellite imagery is increasingly used for public health research and operations. Indoor residual spraying (IRS) for malaria requires extensive planning including enumeration of structures and procurement of insecticides. Using free satellite imagery from Google Earth may be more efficient and cost effective than ground based processes for planning and monitoring IRS campaigns. In 2014, a 5 x15 km area was selected and all structures were enumerated using Google Earth imagery; location, number of rooms in the house, and wall material based on the roof (tin, thatch or asbestos) was recorded. These were compared to IRS data from 2011 captured by the mSPRAY tool which collects GPS coordinates of households sprayed, the number of rooms in the house and the wall surface type. One hundred structures from the mSPRAY data and 25 visited by field staff were analyzed to compare Google Earth enumeration to ground processes. Google Earth was able to identify household locations within 10-meter accuracy. The number of rooms determined based on Google Earth showed strong agreement to data captured at the household with a mean difference of 0.26 (95% confidence intervals =-0.01, 0.52, p=0.06, 100% agreement to ground visits). Among thatch roof structures, agreement was 100%; tin or asbestos roof structures, however, frequently have unfinished walls meaning they are porous instead of non-porous. This may vary regionally; ground visits will help determine the proportion with finished or unfinished walls. Previously, ground enumerations required 25-30 trained technicians to visit each household in a designated area and conduct an extensive questionnaire for 30 days resulting in an estimated 7,200 structures enumerated (~ 900 person days). In comparison, a single trained GIS technician was able to enumerate the same structures in only 6 days (6 person days) using Google Earth. Google Earth and satellite-based enumerations may be useful and more cost effective in both planning for and conducting an IRS campaign to ensure high IRS coverage.

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CROSS RESISTANCE TO ALTERNATIVE MOLECULES AFTER LONG-TERM USE OF PERMETHRIN AND TEMEPHOS IN *AEDES AEGYPTI* FROM YUCATAN, MEXICO

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Aedes aegypti chemical control campaigns have applied exclusively two insecticides, permethrin and temefos, during the last 12 years in Mexico. Recent reports of insecticide resistance threaten the effectiveness of such mosquito control strategy. An updated list of insecticides for vector control re-introduced the use of organophosphates, carbamates and other pyrethroids type I and II. Therefore, it is very important to track the susceptibility status to these molecules, as well as cross resistance patterns. We performed bottle bioassays using seven adulticides, including permethrin, bifenthrin, transfluthrin and deltamethrin; and the organophosphates chlorpyrifos and malathion, and one carbamate, bendiocarb. We also ran WHO larval assays using temefos. We calculated the lethal concentrations that killed 50% (LC_{50}) of two Yucatan mosquito collections - Hunucma and Vergel – that differ in permethrin resistance profiles. LC_{50} for each insecticide were used to calculate the resistant ratios (RR) relative to the New Orleans susceptible strain. We found that the Vergel collection is highly resistant to permethrin (RR = 98 fold) and this phenotype also confers resistance to other pyrethroids type I and II (RR= 92-117 fold); however, it remained susceptible to organophosphates and carbamate adulticides (RR = 2 - 7 fold). Hunucma had moderate levels of resistance to pyrethroids (RR= 3 -17 fold) and was also susceptible to organophosphates and carbamates (RR = 3 -5 fold). Vergel and Hunucma were resistant to temefos by 21 and 17 fold relative to New Orleans, respectively. Temefos resistance phenotype in larvae seems to be unrelated with adult resistance against molecules that share the same mode of action, organophosphates and carbamates. These results indicate that a rotation scheme for insecticides could be suitable in these locations.

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USE OF A SEMI-FIELD SYSTEM TO EVALUATE THE EFFICACY OF TOPICAL REPELLENTS UNDER USER CONDITIONS PROVIDES A DISEASE EXPOSURE FREE TECHNIQUE COMPARABLE WITH FIELD DATA

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Before topical repellents can be employed as interventions against arthropod bites, their efficacy must be established. Currently, laboratory or field tests, using human volunteers, are the main methods used for assessing the efficacy of topical repellents. However, laboratory tests are not representative of real life conditions under which repellents are used and field testing potentially exposes human volunteers to potential disease. There is, therefore, a need to develop methods to test efficacy of repellents under real life conditions while minimizing volunteer exposure to disease. A lotion-based, 15% N, N-Diethyl-3-methylbenzamide (DEET) repellent and 15% DEET in ethanol were compared to a placebo lotion in a 200 sq m (10 m × 20 m) semi-field system (SFS) against laboratory-reared *Anopheles arabiensis* mosquitoes, and in full field settings against wild malaria vectors and nuisance-biting mosquitoes. The average percentage protection against biting mosquitoes over four hours under SFS and field setting was determined. A Poisson regression model was

then used to determine relative risk of being bitten when wearing either of these repellents compared to the placebo. Average percentage protection of the lotion-based 15% DEET repellent after four hours of mosquito collection was 84.02% (95% CI 78.58-89.46) in the semi-field experiments and 87.77% (95% CI 83.02-92.05) in the field experiments. Average percentage protection of 15% DEET in ethanol after four hours was 67.08% (CI 51.11-83.05) in the semi-field system and 86.43% (81.19-91.67) in the field. In conclusion, semi-field evaluation results were comparable to full-field evaluations, indicating that such systems could be satisfactorily used in measuring efficacy of topically applied mosquito repellents, thereby avoiding risks of exposure to mosquito-borne pathogens, associated with field testing.

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ROLE OF *ANOPHELES BALABACENSIS* IN TRANSMISSION OF SIMIAN MALARIA IN KUDAT DIVISION, SABAH, MALAYSIA

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Simian malaria, *Plasmodium knowlesi* is currently a cause of human malaria leading to fatal infections. High proportion of knowlesi malaria cases were reported in Kudat Division. A study was conducted in Kudat division, Sabah, Malaysia from June 2013 to February 2014 to determine the vectors. A total of 1192 anopheline mosquitoes belonging to 13 species were collected from two localities in Pulau Banggi and one in Kudat using human landing catch (HLC). The mosquitoes were dissected, and the mid gut and salivary glands were examined for oocysts and sporozoites. From the study, *Anopheles balabacensis* was the predominant anopheline, consisting 90.74% of the total collection. They bite early in the night which accounts in 61.17% came to bite before 2200 hours. There are 27 *An. balabacensis* positive for malaria parasites, of which 6 was positive for sporozoites, 10 for oocysts and 11 had both. From the ongoing molecular work, mono-infection of *Plasmodium inui* was detected in both midgut and salivary glands while mixed infection of *P. inui+P. knowlesi* and *P.inui+P. cynomolgi* were detected in salivary glands only.

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ECOLOGICAL NICHE MODEL OF *ANOPHELES DARLINGI* AND *AN. ALBIMANUS* (DIPTERA:CULICIDAE) IN COLOMBIA

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The potential distribution of two primary malaria vectors in Colombia, *Anopheles albimanus* y *Anopheles darlingi* and the environmental factors that influence habitat occupancy were evaluated. Presence records were obtained from published literature from 1997-2011 and field collections performed between 2012 and 2013. Two ecological niche models (ENM) were developed per species using environmental and topographic layers: elevation, land cover and 15 of 19 bioclimatic layers from WorldClim. The models were evaluated using the area under the curve (AUC). The first model included 15 bioclimatic layers and altitude, while, a land cover layer was incorporated in the second model. AUC for the two *An. albimanus* models was 0.94, with altitude being the most influential environmental variable. These results were consistent with the predicted potential distribution for *An. albimanus*, mainly spanning low land areas of the Atlantic and Pacific Coasts. AUC for *An. darlingi* was 0.97, and altitude was also the variable contributing the most to both models. Land cover was the second variable in contribution to the second *An. darlingi* model (24.4%), which is consistent with previous reports of a strong association of this species with forested covers. The potential distribution range was higher for *An. darlingi* than for *An. albimanus*. In addition, *An. darlingi* showed a highest probability of occurrence in the endemic area Urabá-Bajo Cauca-Alto Sinú. This information contributes to future studies on the eco-epidemiology of these species

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PHENOTYPIC STABILITY IN EXTRINSIC INCUBATION OF WEST NILE VIRUS STRAINS IN *CULEX TARSALIS*

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Temperature is a critical factor that regulates the efficiency with which West Nile virus (WNV) is transmitted by its mosquito vectors. The interaction between rates of WNV extrinsic incubation, vector biting and vector survival are key determinants of the virus's range as well as the potential for its amplification to outbreak levels. Previous studies have suggested that extrinsic incubation rates of WNV are higher among recent WNV strains, while others have questioned this assertion. Here, we compare the founding North American NY99 strain with two other representative isolates from WNV's initial incursion into California's southeastern deserts and a recent strain from a hyperendemic area of California's Central Valley. We expand on a previous degree-day model to compare the probabilistic range of extrinsic incubation periods among these strains, and we had expected that our more recent strains would exhibit accelerated extrinsic incubation compared to NY99, in agreement with the previously described emergence of a selectively advantaged strain in North America. However, at the two temperatures studied (22 and 30°C), we found no difference in the extrinsic incubation period in *Culex tarsalis* between NY99 and the recent California isolates. We present our comparative findings among strains and to earlier literature, along with a model-based assessment of the epidemiological implications for the efficiency of WNV transmission.

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BITING CYCLES OF YELLOW FEVER AND MALARIA VECTORS ON UDS NAVRONGO CAMPUS IN THE UPPER EAST REGION OF GHANA

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Yellow fever and malaria are both infectious vector borne diseases with high mortality rates among humans and some animals. While yellow fever has a history of a 10 to 12 years cycle in Ghana with minor ones occurring at three-yearly intervals malaria is found at all times. Behavior such as host seeking, biting activities, resting and disease transmission potentials have considerable epidemiological importance for planning and implementing vector control programmes. However very little information is available on these parameters for both *Aedes* species and *Anopheles* species in the Navrongo area. We studied the comparative biting behavior of *Aedes* and *Anopheles* mosquitoes within the Navrongo campus of UDS. Female adult mosquitoes were collected both indoor and outdoor between the hours of 05.00 and 09.00 GMT, 15.00-17.00 GMT and 18.00-06.00 GMT using human landing collections. All mosquitoes were grouped and identified morphologically using appropriate taxonomic keys. 27 *An. gambiae* complex were identified to species and molecular forms of *An. gambiae* s.s. using PCR-RFLP. Also, the presence of the kdr gene mutation was estimated in the *Anopheles* population. A total of 1072 mosquitoes were collected. Out of this, 61.8 % were *Anopheles*, 22.7 % were *Aedes* and 15.5 % were *Culex*. *An. gambiae* complex was predominant of the *Anopheles* genera constituting 59.6 % whereas *Ae. aegypti* was predominant (85.6 %) of the *Aedes* genera. *Aedes* mosquitoes had a bimodal biting pattern peaking at 05.00 - 07.00 hours GMT in the morning and 17.00 - 19.00 hours GMT in the evening as compared to the *Anopheles* species which had a unimodal biting behaviour peaking at 24.00 - 02.00 hours GMT. All the 27 *An. gambiae* were *An. gambiae*

s. s. M forms The kdr gene mutation frequency in the population was RR 29.6 %, RS 51.9 % and SS 18.5 % (n=27). The information on the vector biology, genetic diversity and feeding behavior as well as resistance of vector mosquitoes are important in planning and implementing effective vector control programmes.

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DEVELOPMENT AND EVALUATION OF ELECTRIC GRID TRAP FOR SAMPLING OF HOST-SEEKING MALARIA VECTORS

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Evidence of malaria vectors changing their behaviors in response to control measures has been documented. These changes include a shift in the biting time and place of biting behavior, where mosquitoes have been observed to bite in the earlier hours of the night before people are under bed nets, or have developed a preference towards outdoor biting to avoid insecticide treated surfaces. These changes could possibly render the current intervention tools such as Insecticide Treated Nets (ITNs) and Indoor Residual Spraying (IRS) inefficient. To be able to study these behavior changes, efficient and safe sampling tools are required to replace the current gold standard human landing catch technique (HLC) which exposes the human subject to mosquito infectious bites. In this work we develop and evaluate an Electric Grid Trap (EGT) to be used in studying the host seeking behaviors of indoor and outdoor biting mosquitoes. This trap was evaluated at the Lupiro village situated in the malaria endemic Kilombero Valley in South-Central Tanzania in comparison to a commercially available bug zapper (CEGT) and the Human Landing Catch technique (HLC) as the reference trap. The study was done in a Latin Square design conducted in experimental huts over a twenty one-night period. The EGT efficiency in collecting indoor and outdoor biting *Anopheles arabiensis* was 21.8% and 59.9% respectively relative to HLC, while for collecting *An. funestus*, EGT was able to perform at 70.3% and 90.5% for indoor and outdoor sampling respectively relative to HLC. The peak biting time estimated by EGT and HLC was the same for indoor sampling of *An. arabiensis* ($z=0.024$, $p=0.98$), but was different for outdoor sampling ($z=-0.35$, $P<0.018$). No difference in the peak biting times for *An. funestus* sampled the two traps indoors ($z=-0.75$, $p=0.45$) or outdoors ($z=-1.72$, $p<0.086$). With improvements, the current prototype of the EGT could be a possible replacement for the HLC and could be used to sample indoor and outdoor biting mosquitoes giving an estimate of their biting behaviors with comparable efficiency to the HLC technique.

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SUSCEPTIBILITY OF WILD AND LABORATORY-REARED *ANOPHELES DARLINGI* TO INFECTIONS WITH *PLASMODIUM VIVAX* IN THE PERUVIAN AMAZON

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Malaria studies focus on quantify human infectious reservoir, to evaluate transmission reducing interventions or to test the gametocytocidal effects of drugs, are crucial for to the critical evaluation of disease control strategies. Mosquito-parasite relationships are still not well understood and it varies as a function of vector species and parasite strains. *Anopheles darlingi,* the main malaria vector in South America, could present different responses to *Plasmodium* infection from females reared under laboratory conditions or wild An. darlingi specimens. The aim of this study was to compare the susceptibility of laboratory-reared versus wild *An. darlingi* to *Plasmodium vivax* using membrane feeding assays (MFA). The study was carried out in Iquitos (Peru) in 2009-2010 and seven patients with positive thick smears for *P. vivax* malaria were enrolled in the study. Sexual and asexual parasites stages were recorded and subsequent mosquito feeding was performed using the MFA technique. To detect number of oocysts, mosquito midgut dissections were performed 8 days postinfection (pi) and counted by microscopy. To determine the proportion of infectious mosquitoes, salivary glands were dissected 14 days pi and performed an ELISA assay to detect VK210 and VK247 *P. vivax* strains. A total of 1333 An. darlingi females, 701 wild and 632 laboratory-reared, were used for this study. Our results showed that there was no statistically significant difference between oocysts means of wild and laboratory-reared An. darlingi ($p=0.105$). Likewise, no statistically difference was identified between sporozoites detection in the two groups ($p=0.137$, $p=0.170$, respectively). ELISA assays showed that VK210 was the most common strain, representing 59% and 63% in wild and laboratory-reared mosquitoes, respectively. In conclusion, the MFA might be a realistic ex-vivo surrogate technique for measuring first insights into malaria transmission, and provides the basis for human-mosquito interaction studies such as transmission-blocking vaccines studies.

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SUSCEPTIBILITY TO *PLASMODIUM VIVAX* AND INSECTICIDE RESISTANCE OF *ANOPHELES CAMPESTRIS* AND *AN. SUBPICTUS* ALONG THAI-CAMBODIAN BORDER

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Changing malaria prevalence from *Plasmodium falciparum* to *P. vivax* along the Thai-Cambodian border has been observed. We have investigated the potential vectors that may contribute to this change in two selected endemic areas, Sa Kaeo and Chantaburi provinces. Two potential vectors, *An. campestris* and *An. subpictus*, were collected by cow bait or human landing catch during the wet and dry seasons of 2012-2013. In Sa Kaeo, 17.8% of 2,355 mosquitoes from 22 species collected were *An. campestris*. In Chantaburi 88% of 433 mosquitoes from 7 species collected were *An. subpictus*. Susceptibility to *P. vivax* for these mosquitoes was studied in laboratory using F1 to F15 progeny and artificial membrane feeding. Both species were found susceptible to *P. vivax*. The parasite developed normally to oocysts in the midguts and sporozoites in the salivary gland. In contrast, development of *P. falciparum* was not observed in either species when compared with An. dirus, the major malaria vector in Asia. Study of insecticide susceptibility indicated that An. campestris (F1 to F3) from Sa Kaeo had potential to resist 0.1% Bendiocarb, 1% Fenitrothion and 4%DDT. An. subpictus (F1-F3) had shown to resist 0.15% Cyfluthrin, 0.05% Lambda-cyhalothrin, 5% Malathion, and 0.05% Deltamethrin. The change in species composition and malaria vector biology on the Thai Cambodian border may contribute to the shift in the *P. falciparum* to *P. vivax* prevalence; therefore, the malaria control program including vector control will have to be planned accordingly.

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SEASONALITY OF MULTIPLE BLOOD FEEDING BEHAVIOR IN ANOPHELINE MOSQUITOES AND IMPLICATIONS FOR MALARIA TRANSMISSION IN NCHELENGE DISTRICT, ZAMBIA

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As part of the Southern Africa International Centers for Excellence in Malaria Research (ICEMR) project, mosquito collections were performed during August-September 2012 and March-May 2013 in Nchelenge District, Luapula Province, Zambia. Located along the environs of Lake Mweru and Kenani Stream, Nchelenge experiences hyperendemic

transmission and has the highest malaria infection rate in children under the age of 5 years despite implementation of indoor residual spraying (IRS) and long-lasting insecticide-treated net (LLIN) distribution. Center for Disease Control light traps (CDC LTs) and pyrethroid spray catches (PSCs) were performed at three villages along Lake Mweru and two villages along Kenani Stream. The collections revealed that during the dry season, *Anopheles funestus* sensu stricto is the dominant vector near both the lake and stream. In contrast, during the wet season, *An. gambiae* sensu stricto is the dominant vector in the lakeside villages, whereas *An. funestus* s.s. is the primary vector with secondary contribution from *An. gambiae* s.s. in the streamside villages. Both vector species are highly anthropophilic and *An. funestus* has a higher *Plasmodium falciparum* sporozoite rate than *An. gambiae*. In the wet and dry seasons, it was found that the *P. falciparum* infection rate of the vector populations was higher near the streamside villages than those of the lakeside villages. The multiple blood feeding rate for *An. funestus* is higher during the dry season, whereas the multiple blood feeding rate for *An. gambiae* is higher in the following wet season. As a result, the entomological inoculation rates (EIRs) for each vector during the dry and wet seasons are underestimated. The results also suggest that there are spatial differences in vector composition and their multiple blood feeding rates, which contribute to differences in human malaria risk during the dry and wet seasons. Overall, the vector data in Nchelenge present unique opportunities to further our understanding of malaria transmission and implications for malaria control in high-risk areas.

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INCONSISTENCY IN THE RELATIVE PERFORMANCE OF HUMAN LANDING CATCHES AND LIGHT TRAPS IN SAMPLING ANOPHELINE POPULATIONS ACROSS DIFFERENT AREAS OF AFRICA

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The need for surveillance of adult host seeking mosquitoes is of foremost importance in determining levels of disease transmission and for appropriate allocation of interventions. The gold standard for estimating mosquito - human contact rate has thus far been obtained based on Human Landing Catch (HLC), where human volunteers catch mosquitoes that land on their exposed body parts. This approach is necessitates exposure to potentially infectious mosquitoes, such a risk it is unethical calling the need for safer and accurate tools. Centers for disease control light traps (LT) have been used widely in malaria endemic setting as an alternative tool to HLC in estimating human biting rate (HBR). Here, multi sites paired mosquito collections of LT against HLC are evaluated for their consistency in the sampling indoor host seeking mosquitoes. Regression models were applied to determine the site specific as well as the overall LT sampling efficiency and their trend across increasing mosquito density for two major malaria vectors across Africa, *Anopheles gambiae* sensu lato and *Anopheles* funestus sensu lato. Generally, LT were able to collect more mosquitoes than HLC, though the ratio of LT:HLC varied between sites and mosquito density. Across sites LT had an overall sampling efficiency of =1.07 [0.76-1.51] in sampling *An. gambiae* s.l. and =1.78 [0.90-3.44] in sampling *An. funestus* s.l.. There was variation in sampling efficiency of LT across mosquito densities and only in a few locations did LT sample proportionally to HLC. More often LT either underestimated human exposure by under-sampling or over-sampling at high mosquito densities, in particular for An. funestus. Such inconsistency necessitates calibration of LT against HLC for each location and across seasons. Also advises against the use of a single calibration factor across all geographical locations since no evidence of a geographical pattern in the sampling efficiency of LT against HLC was demonstrated.

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IDENTIFICATION OF THE FIRST OVIPOSITION ATTRACTANT FOR GRAVID MALARIA MOSQUITOES USING NEWLY DEVELOPED METHODS FOR MEASURING OLFACTORY ATTRACTION IN *ANOPHELES GAMBIAE* S.S..

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Little is known about how *Anopheles gambiae* s.l. find aquatic sites to lay eggs. Some evidence suggests that they use both vision and smell to locate and select breeding sites. Like many nocturnal insects with efficient mechanisms for processing chemical cues, olfaction is suspected to play the major role in this process and has been the subject of many investigations often with contradictory conclusions. Over 2000 gravid mosquitoes were evaluated individually. Every mosquito was given two cups with oviposition media in a cage. We measured the number of eggs laid by individual mosquitoes and determined whether they laid eggs in more than one cup. Thereafter we developed cage experiments that takes into account newly described behaviour. In addition, a semi-field test was developed using modified BG sentinel traps. This test measured olfactory attraction of gravid females to substrates over time and space. Together these two tests enabled the screening and description of an assortment of oviposition substrates. The number of eggs laid by individual mosquitoes varied widely. In addition individual mosquitoes skip-oviposited: laying their eggs in more than one cup on the same night. This is the first report of within-cage skip oviposition in the species and demonstration of how it could lead to an illusion of substrate preference with common egg count bioassays. A new two-tier two-choice cage test that adjusts for large variation and eliminates the risk of artefact preferences as a result of skip oviposition was developed and used to identify the first confirmed attractant (patent pending) for gravid *An. gambiae* s.s. Our study confirms that gravid mosquitoes use olfactory cues to select breeding sites. These findings pave the way for the development of new strategies for controlling malaria vectors; one that will also help target insecticide resistant and outdoor biting populations previously left out by indoor residual spraying and long lasting insecticidal nets.

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ANOPHELES DARLINGI RESTING BEHAVIOR USING INTERCEPTION SCREENS IN THE PERUVIAN AMAZON

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Anopheles darlingi, the main malaria vector in the Neotropics, is highly anthropophilic. However, extrinsic drivers of host seeking and oviposition are poorly understood. Analysis of anopheline flight patterns within villages could enhance malaria risk assessment and control. Data from preliminary studies using a single barrier screen were problematic because several factors may have affected mosquito behavior. The aim was to

intercept vectors seeking hosts, resting sites, and/or oviposition sites. The design was modified using 4 screens for 2 nights/month (6PM-6AM), January-March 2014, in two riverine localities in the Peruvian Amazon, Lupuna and Cahuide. Screens, 2m high x 15m long, were placed such that the distance from the house to the river/forest was 2-7 meters. Screens were checked hourly and resting anophelines recorded for height (>or <1m above ground) and side of screen (next to forest/house/river). For every collection, screen orientation and wind speed and direction were noted. A subsample of mosquitoes was dissected for parity status and PCRs were performed to determine blood meal source. Our results showed that *An. darlingi* was the most abundant species in both localities, with peak densities between 9PM and 2AM on the village side, suggesting a need for additional investigation on behavior during this time. 75% and 78% of the females in Lupuna and Cahuide, respectively, were captured <1m from the ground, and, by visual inspection, 2% of the mosquitoes had blood fed. These data provide important information about mosquito flying behavior and pose new questions about anophelines seeking shelter, mating and/or oviposition sites.

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INVESTIGATING THE ROLE OF DIVERSE ANOPHELINES IN TRANSMITTING ARTEMISININ-RESISTANT *PLASMODIUM FALCIPARUM* IN CAMBODIA - A CHALLENGE FOR MALARIA CONTROL AND ELIMINATION

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Cambodia is a hotspot for the rapid evolution of artemisinin (ART)-resistant *Plasmodium falciparum* parasites, which endanger the effectiveness of all ART-based combination therapies (ACTs), and harbors an exceptional level of *P. falciparum* and *Anopheles* diversity. Subpopulations of ART-resistant and -sensitive parasites have recently been identified, but the role of diverse anophelines in transmitting them has not yet been investigated. We approached this question by screening field-collected anophelines from three Cambodian provinces for *P. falciparum* infection and infecting multiple Southeast Asian and other regional anophelines with ART-resistant and -sensitive parasite strains from Cambodia. More than 10,000 *Anopheles* mosquitoes from three provinces (Pursat, Western Cambodia, where ART resistance is established; Preah Vihear, Northern Cambodia, where ART resistance is emerging; and Ratanakiri, Eastern Cambodia, where ART resistance is rare) were screened for *Plasmodium* infection. Contrary to the current dogma that only one or two vectors efficiently transmit malaria in this region, we found 14 distinct anophelines infected with *Plasmodium* (at least 4 infected with *P. falciparum* and at least 10 infected with *P. vivax*) using nested PCR. Molecular analysis of rDNA ITS2 loci identified 27 distinct *Anopheles* species and revealed only a 50% concordance between morphological and molecular identification methods. *P. falciparum* infections of An. dirus A isolates from Western Cambodia and An. minimus, *An. gambiae* s.s., and An. stephensi lines were achieved in the laboratory. Rates of infection of these and diverse Cambodian anophelines by ART-resistant and -susceptible parasite isolates will be presented. The ability of multiple vectors to carry ART-resistant parasite subpopulations presents a startling challenge for the control and elimination of falciparum malaria in this region.

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ANOPHELES FUNESTUS IN MUTASA DISTRICT, ZIMBABWE: THE ROLE OF VECTOR BIONOMICS AND POTENTIAL INSECTICIDE RESISTANCE IN MALARIA TRANSMISSION

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Mutasa District, Zimbabwe is an area marked by seasonal malaria transmission with severe outbreaks occurring during the wet season. A research arm of the International Centers of Excellence in Malaria Research (ICEMR) in Southern Africa began in 2012 to assess malaria burden in the region. This area is characterized as a region of resurgent malaria, having had increasing rates in recent years despite being previously considered as under control. In order to assess the reasons for this transmission dynamic, the ICEMR project seeks to analyze malaria epidemiology, parasite genomics, and entomology, which is of particular importance for this study. Samples were collected during the wet seasons of 2012-2014 using CDC light traps and pyrethroid spray catches, and morphological identification indicates that the predominant malaria vector is *Anopheles funestus* senso lato. Molecular identification of samples from the 2012-2013 collections confirms these results, with samples being *An. funestus* senso stricto or *Anopheles leesoni*, both members of the *An. funestus* species complex. Blood meal analysis indicates that these mosquitoes feed predominantly on human populations. Sporozoite infection rates for 2012-2013 were approximately 5%. Of additional interest is the high level of pyrethroid resistant *An. funestus* as the dominant malaria vector in the region. During collections in December 2013, 80% of mosquitoes were collected in houses that had been recently treated (within 2 months) with pyrethroids used for IRS control methods. Of these samples, 93% were morphologically observed to be blood fed or gravid, suggesting that these mosquitoes had been resting on treated surfaces for many hours. Although the findings are preliminary in nature, they suggest that potentially insecticide-resistant *An. funestus* is the dominant malaria vector in the region and may be contributing to the resurgence of malaria in spite of control efforts. Further collections are scheduled and will help to further elucidate the vector component of this study. With this knowledge it may be possible to determine areas of highest malaria risk and provide information on how to most effectively deploy limited resources to achieve control.

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INVENTORY AND EVALUATION OF CULICIDAE NUISANCE IN URBAN POST-CONFLICTUAL AREA: CASE OF BOUAKÉ CITY (CÔTE D'IVOIRE)

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Culicidae are vectors of many diseases. They are sources of nuisance by their painful bites,spots and noises. In Côte d'Ivoire malaria remains a major public health problem. Contact between man and the vector is based on the epidemiology of malaria. It is directly modulated by human activities and indirectly through environmental modification. After the politico-military conflict of 2002 in Côte d'Ivoire, many factors may have influenced environment including overcrowding of some cities. Such modification of the environment can impact on the mosquito's composition and nuisance. The objective of this study was to inventory and evaluate the culicidae nuisance in previously overpopulated urban post-conflict area. The study was carried in two quarters of Bouaké city. Ahougnansou was overpopulated quarter before the crisis and less populated after the crisis and Sokoura was overpopulated before and after the crisis. The study was based on human landing catch inside and outside the housing, from 18 pm to 6 am. Mosquitoes were

morphologically identified by genus and species. However, the breeding sites have been characterized and the larvas were collected by "dipping" method and identified the adult stage. A total of 1030 mosquitoes were collected on human landing catch. Four genus of culicidae were identified: *Anopheles, Aedes, Culex* and *Mansonia*. The Culicidae fauna inventoried in these areas consists of 9 species (2 *Anopheles*, 4 *Culex*, 1 *Aedes* and 2 *Mansonia*). In Sokoura the culicidae nuisance was mainly due to *Culex* (98.2%). Mansoni, *Aedes* and *Anopheles* were virtually low. The breeding sites encountered in Sokoura are gutters, drainage channels contained solid wastes. However in Ahougnansou it were the irrigated rice and water drums. The culicidae fauna in Ahougnansou is dominated by *Culex* (48.5%) and *Anopheles* (48 %. The *Aedes* and *Mansonia* were poorly represented. The biting rate in Ahougnansou was 30.6 biting per human per night (b/h/n) and 53.1 b/h/n in Sokoura. Mosquito abundance and biting rate varied by site. The culicidae nuisance remains important. This was due to the environment modification, thus creating favorable conditions of mosquitoes breeding and their adaptation to their new environment. In Sokoura the high density of population involved the *Culex* breeding sites. However in Ahougnansou, the high rate of *Anopheles* is due to irrigated rice exposing the population to the malaria risk transmission.

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SHIFTING PATTERNS OF *AEDES AEGYPTI* FINE SCALE SPATIAL CLUSTERING IN IQUITOS, PERU

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Aedes aegypti abundance is spatially heterogeneous, with some areas and larval habitats producing more mosquitoes than others. There is a knowledge gap, however, regarding temporal persistence of *Ae. aegypti* abundance hotspots. We used a longitudinal entomologic dataset from the Amazonian city of Iquitos, Peru, to (1) quantify spatial clustering patterns of adult *Ae. aegypti* and pupae counts per house, (2) determine overlap between clusters, (3) quantify temporal stability of clusters over nine entomologic surveys spaced 4 months apart. Data from 13,662 household entomological surveys performed in Maynas and Tupac Amaru, two Iquitos neighborhoods differing in *Ae. aegypti* abundance and dengue virus transmission, were analyzed using global and local spatial statistics. Evidence of clustering of pupae presence and abundance was observed in 44% and 11% of surveys, respectively, for Maynas and in 55% and 33% of surveys in Tupac Amaru. The estimated overall mean ± SD clustering distance was 16.6 ± 5.0 m for pupae presence and 10.3 ± 7.8 m for pupae abundance. On average, 3.1% of Maynas and 1% of Tupac Amaru households were members of a cluster of high adult abundance. There was no consistent temporal pattern of adult clusters; i.e., the location of clusters in one survey differed from the location of clusters in future or prior surveys. The probability of finding adults clustering beyond the household was 42% (95% CI,57.8-25.8%). Our analyses indicate that *Ae. aegypti* distribution was highly focal and hotspots of high vector abundance were common on every survey date, but temporally unstable over the period of study. Our findings have implications for understanding *Ae. aegypti* distribution and the design of surveillance activities relying on household-level data. In settings like Iquitos, where there is a relatively low percentage of *Ae. aegypti* in permanent water-holding containers, identifying and targeting key premises will be significantly challenged by shifting hotspots of infestation.

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EFFECT OF HOLDING CONDITIONS ON THE DETECTION OF CHIKUNGUNYA AND VENEZUELAN EQUINE ENCEPHALITIS VIRUS IN MOSQUITO POOLS

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Emerging and re-emerging arboviruses continue to be a threat to global public health. With the recent introduction of chikungunya virus (CHIKV) into the Caribbean and its potential spread across the Americas, there will be a need to increase surveillance of mosquito populations for viruses. Due to the tropical climate of many of the affected areas, it will be difficult to maintain a cold chain as the samples travel from collection sites to laboratories for testing. We determined how suboptimal holding temperatures affected the ability to detect viruses in pools of mosquitoes. Adult female *Aedes albopictus* and *Ae. taeniorynchus* were inoculated with CHIKV or Venezuelan equine encephalitis virus (VEEV) suspensions, respectively, and placed at 26°C for 7 days. One infected mosquito was then added to a vial of 24 negative mosquitoes and then held at -70°C, -20°C, 4°C, 22°C, or 35°C for selected time intervals. Mosquito pools were triturated in cell culture media and processed for detection of CHIKV and VEEV. Samples were analyzed for both infectious virus by plaque assay and for viral RNA with real-time RT-PCR. At high temperatures the amount of infectious virus decreased rapidly, but virus in samples held at 4°C or lower remained relatively stable. In contrast, viral RNA was detectable from pools held at all temperatures and holding times by real-time RT-PCR, although Ct values increased as temperatures and holding times increased. These findings suggest that if viral RNA detection is the goal of surveillance efforts, then mosquito pools do not need to kept at 4°C. This enhances the feasibility of field-based arbovirus surveillance programs where maintaining a cold chain may not be a possibility.

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SOCIOECONOMIC AND ECOLOGICAL FACTORS OF *AEDES AEGYPTI* PRODUCTIVITY IN DHAKA, BANGLADESH: IMPLICATIONS FOR DENGUE VECTOR CONTROL

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In the absence of commercially available dengue vaccine, the primary option for formulating dengue prevention strategies is vector control programming. Although numerous dengue vector control efforts have been implemented, none of them has proved to be sustainable.In our research,we have argued that the general failure of vector control practices demands consideration of the interrelationships within the socio-economic system and ecological system.The first hypothesis we tested was that most *Aedes aegypti* pupae are being produced in a few types of household containers, and socio-economic characteristics of households are responsible for possession of container types.The second hypothesis tested in our study was that the risk of exposure to dengue virus (DENV) is directly associated with the magnitude of *Aedes* productivity.Two household (n=1200)level entomological surveys were conducted in the City of Dhaka (2011, 2012).During our second survey in 2012, blood samples from human population were also collected from members of the same households.During the 2011 entomological survey,a total of 3,651 immature *Aedes* was counted in 1,501 containers with water in 826 premises.The first hypothesis was supported by the data,revealing that 82.1 % of total *Aedes* pupae were produced from only 9 types of containers. We applied multiple criteria(i.e., container use,two-step cluster analysis based on surrounding ecological variables of containers,and multivariate nominal regression model of container types)to classify the containers and to evaluate the role of household's socio-economic factors

for possessing the specific containers types. Most pupae were 3 of 10 types of container usage categories. The cluster technique revealed that most pupae were produced from in-house,tap water-filled containers with no vegetation nearby,and under shade.Household income(χ2=11.9, df=4, p=0.01)and purpose of storing water(χ2=16.9, df=4, p=0.003)were most important explanatory variables to affect possession of productive containers types.ELISA and PRNT results of collected blood samples showed high seroprevalence (80%)and 95 new DENV infections among survey population.Interestingly, the correlation of number of pupae from a household and seropositivity led us to infer that household income,the purpose of water storage,and indoor potted plants are the most significant factors of *Aedes* productivity and seroincidence.

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MOSQUITOCIDAL NECTAR DELIVERY: EVALUATION OF CANDIDATE PLANT SPECIES

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A transgenic toxic protein delivered via nectar could be used to kill or weaken mosquito males, which feed on nectar, and females, which require nectar for energy to seek a blood meal. In addition, transgenic nectar proteins could be used to deliver any peptide, such as a hormone, a lectin, or antipathogen peptide, directly to the gut of wild mosquito populations. However, most mosquito-attractive plants listed in the literature are not amenable to genetic transformation. We selected plants with known insect/plant symbioses and published plant transformation protocols for evaluation of mosquito feeding attractiveness and the nectar protein profile. In all tests, cages with 20 (10m/10f) *Culex pipiens* or *Aedes aegypti* were tested and delivered similar results. Mosquitoes survived best in cages with Impatiens plants (*Impatiens walleriana*), followed by passion flower vines (*Passiflora edulis*), tropical milkweed (*Asclepias curassavica*), red trumpet flower vines (*Campsis radicans*) and 10% sucrose control, followed by castor bean (*Ricinus communis*). With red dye added to the nectaries, mosquitoes were seen to ingest dye within a few hours, with the speed and percentage of acquisition following the same order of plant species. When Impatiens with dyed nectaries was placed in a large cage with three other species with undyed nectaries plus an undyed sucrose tube, 80% of the mosquitoes were dyed within one day, implying that Impatiens nectar would be sampled by mosquitoes even in a complex garden setting. Finally, the nectar protein profile was examined and Impatiens nectar was found to contain a 20 kDa protein at 3 mg/ml. Since *Impatiens walleriana* has a facile transformation protocol, currently in use in the authors' laboratory, produces seeds easily, and belongs to a mainly tropical genus containing 800-1000 species, it represents an excellent candidate plant system for the creation of a mosquitocidal nectar plant.

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THE UBIQUITIN PROTEASOME SYSTEM IS REQUIRED FOR VENEZUELAN EQUINE ENCEPHALITIS VIRUS REPLICATION

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Proteins destined for degradation are tagged with multiple copies of ubiquitin. Traditionally, the proteasome was restricted to disposing of misfolded proteins or general recycling in the cell. Recently, the ubiquitin proteasome system (UPS) has been depicted in roles such as signal transduction and intracellular trafficking. Many viruses have been implicated in utilizing or modulating the UPS to enhance viral replication and/or to sustain a persistent infection. The mosquito-borne virus Venezuelan Equine Encephalitis Virus (VEEV) belongs to the Togaviridae family and is considered an important biodefense pathogen and select agent. There are currently no approved vaccines or therapies to treat the disease, therefore it is imperative to identify novel targets for therapeutic development. An initial screen of a limited FDA-approved drug library indicated that Bortezomib (Velcade) inhibited replication of the attenuated TC-83 strain of VEEV in infected cells without apparent toxicity to the cell. We hypothesized that a functional UPS is required for efficient VEEV replication. Proteasomal inhibitors deplete free ubiquitin that may be needed to modify viral proteins for efficient viral budding. Bortezomib, a dipeptidyl boronic acid, specifically and reversibly inhibits the 26S proteasome. We have shown that at non toxic concentrations Bortezomib proved to be a potent inhibitor of VEEV replication in the human astrocytoma cell line U87MGs. Bortezomib inhibited both the virulent Trinidad Donkey strain and TC-83 strain of VEEV. Additional studies with virulent strains of Eastern Equine Encephalitis Virus and Western Equine Encephalitis Virus depicted that Bortezomib is a broad spectrum inhibitor of the New World alphaviruses. Time of addition assays showed that Bortezomib may affect early time points in the viral life cycle thus influencing viral replication. Mass spectrometry analyses indicated that VEEV capsid was ubiquitinated, which was validated by western blot. Subsequent studies revealed that capsid is stabilized in the presence of Bortezomib in treated cells while the nuclear and cytoplasmic distribution of capsid did not undergo any marked changes. Ongoing studies are focused on elucidating the mechanism by which Bortezomib inhibits alphavirus replication. This study will aid future investigations in identifying host proteins as potential broad spectrum therapeutic targets for treating VEEV infections.

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SINDBIS AND BUNYAMWERA VIRUSES CIRCULATE AMONG PATIENTS WITH FEBRILE ILLNESS IN MFANGANO ISLAND OF LAKE VICTORIA WESTERN KENYA

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Bunyamwera and Sindbis viruses are medically important arboviruses in the tropical and subtropical regions of the world, with an estimated 2.5 billion people being at risk. Early detection of any arbovirus infection has great importance for the clinical management of patients, surveillance, and to prevent possible disease outbreak. The aim of this study was to investigate if patients with acute febrile illness without *Plasmodium* detectable by microscopy or RDTs were arbovirus positive. Patients whose ages ranged from 13 to 70 years with a mean age of 29 were used for the study. There was preponderance of females (65%) over males (35%) among the patients studied. Whole blood was sampled (n=105) from consenting febrile patients at Sena Health Centre, Mfangano Island of Lake Victoria, Kenya. Information on body temperature, type of occupation (indoor/outdoor), clinical manifestation, nature of exposure (direct or indirect) to animals and type of treatment received were collected using a briefed close ending questionnaire. Arbovirus specific RNA sequences extracted directly from blood were amplified by reverse transcriptase, multiplex PCR and amplicons resolved by High Resolution Melt (HRM) for virus detection and differentiation. Four patients (3.8%) tested positive for bunyamwera virus while two representing (1.9%) had Sindbis virus infection. Among all the patients studied, 102 (97.1%) received anti-malarial treatment while 3(2.9%) were treated with antibiotics. These findings demonstrate the presence of arboviruses among febrile illness cases in Western Kenya and the possibility of these viruses being the aetiological agents of in the cases exist. In addition, inadvertent use of antimalarial and antibiotics treatments for malaria negative cases should be discouraged. This study underscores the need to differentially diagnose febrile illnesses for arboviruses to guide and direct appropriate treatment and management.

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DEVELOPMENT OF EILAT VIRUS, A HOST-RESTRICTED ALPHAVIRUS, AS A VACCINE VECTOR

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Eilat virus (EILV) is an alphavirus isolated from a pool of mosquitoes collected in Israel. It replicates efficiently in insect cells but is unable to replicate in vertebrate cells. EILV is host-restricted at two points in its replication cycle: 1) attachment/entry, and 2) viral RNA replication. The host-restricted nature of EILV provides an opportunity to develop it as a potential "pseudoinactivated" vaccine vector. Our goal was to develop EILV chimeras as a new platform for alphavirus vaccines. For proof-of-concept, we chimerized EILV with eastern equine encephalitis virus (EEEV), a highly virulent alphavirus that can cause fatal disease outbreaks in both humans and equids with high case-fatality rates. Our central hypothesis was that a chimeric alphavirus containing the non-structural protein genes of EILV and the structural protein genes of EEEV will retain the vertebrate host restriction of EILV and provide safe, effective protection against lethal challenge with EEEV. To test this hypothesis, we generated a chimeric EILV/EEE infectious cDNA using standard cloning techniques, and rescued the virus in insect cells. We then performed immunogenicity and safety experiments in mice. After a single vaccination, EILV/EEE protected mice against lethal challenge with EEEV and produced a higher titer of neutralizing antibodies when compared to a commercial EEE vaccine for horses. Additionally, EILV/EEEV showed no neurovirulence in suckling mice after intracranial inoculation. These results suggest that the chimeric EILV-based alphavirus vaccine platform represents a safe and efficient approach to protect against EEEV and other highly pathogenic alphaviruses in mice.

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DEVELOPMENT OF A CHIKUNGUNYA VIRUS RNA REFERENCE REAGENT FOR STANDARDIZATION OF NUCLEIC ACID TESTS

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Chikungunya virus (CHIKV) is an Alphavirus transmitted by the mosquitoes *Aedes aegypti* and *Aedes* albopictus, the same vectors that transmit Dengue viruses (DENV), a group of four Flaviviruses that often co-circulate with CHIKV in the same geographical area. CHIKV has caused explosive epidemics in Africa, Asia and Indian and Pacific Ocean islands. It recently appeared in the Caribbean islands, posing risk to the rest of the Americas. Some CHIKV human infections are asymptomatic. Most cause a febrile illness similar to that of DENV, characterized by high fever, polyarthralgia, headache, back pain, myalgia, nausea, vomiting, and rash. There are no vaccines or specific treatments for DENV or CHIKV. However, DENV infections require prompt differential diagnosis and not infrequently hospitalization to prevent fatalities. Laboratory diagnosis for these viruses is made by serology, viral isolation or by nucleic acid test (NAT), the most sensitive method. There are no FDA-approved CHIKV diagnostic or blood screening assays. The lack of a reference reagent for CHIKV is a barrier for proper evaluation of NAT assays. This work aims to produce a well-characterized CHIKV RNA Reference Reagent (CHIKV-RR) for use as a standard for evaluation of performance of existing assays, and to facilitate the development of NAT assays that fulfill the requirements for blood screening. The CHIKV-RR candidate was produced by (a) expansion of a CHIKV clinical strain in Vero cells; (b) heat-inactivation (HI) of the viral stock; (c) preliminary in-house titration of the HI CHIKV stock; (d) validation of results in external collaborative studies; (e) formulation of the Center for Biologics Evaluation and Research of the U.S. Food and Drug Administration CHIKV-RR. Preliminary results showed that the CHIKV stock has a concentration of ~10^6 PCR-detectable units/ml. The final formulation of the CHIKV-RR was shipped to collaborators for a second round of testing. External results are expected within 3 months, when it will be subjected to statistical analysis for assignment of a final number of units.

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EFFECT OF THE LYSOSOMOTROPIC COMPOUNDS ON CHIKUNGUNYA VIRUS *IN VITRO*

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Alphaviruses are mosquito-borne viruses that primarily alternate between the arthropod vectors and vertebrate hosts. These viruses utilize the class-II fusion protein-mediated viral membrane fusion in the acidic intracellular compartments during the process of viral entry. The treatment of lysosomotropic compounds has been extensively used to characterize the viral entry of alphaviruses in mammalian cell lines and have been found effective for the inhibition of viral entry. Such characterization has not been performed for the analysis of chikungunya virus (CHIKV) entry in mosquito cell lines. In this study, three lysosomotropic compounds, ammonium chloride, chloroquine and monensin were tested to evaluate the inhibitory effect of viral entry *in vitro*. The entry of CHIKV is assessed by the fluorescent signals from the expression of the green fluorescent protein (GFP) genetically engineered into the viral genome. The inhibition of viral entry was assed at concentrations which maintained the viability of the cells. The effective $dose_{50}$ of each compound was calculated based on the percentage of reduction in the fluorescent signals and the concentrations of the compounds. The results and the possible mechanisms responsible for the entry of CHIKV and alphaviruses into mosquito cells will be discussed.

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INSIGHTS INTO THE SPREAD OF CHIKUNGUNYA IN BANGLADESH FROM A SEROPREVALENCE STUDY

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Chikungunya virus is an emerging infectious disease in Southeast Asia. The first chikungunya outbreak in Bangladesh was reported in 2008 in Chapai Nawabganj, a district in northwest Bangladesh bordering India. Two more outbreaks occurred in 2011 in the same district. It is unclear whether these outbreaks are markers of large-scale transmission throughout the district or isolated events. We conducted a serosurvey in Chapai Nawabganj to better understand chikungunya dispersal and to determine factors associated with exposure. Between March — May 2013, we randomly selected 40 communities and selected all household members to participate in the study until we enrolled 30-50 individuals per community. We collected a blood sample and socio-demographic characteristics and typical travel behavior from all participants. We used IgG ELISAs (NovaLisaTM, Dietzenbach, Germany) to identify past chikungunya infection. We used a multivariate logistic regression model with a random intercept for each community to identify risk factors. Overall 6.7%

(87/1290) of individuals had evidence of chikungunya infection. Half of the communities, located in the north of the district, had no seropositive cases at all. Communities in the south, nearby previous outbreak sites, had up to 56% seropositivity. Individuals < 20 years were 2.5 (95% confidence interval [CI], 1.5-4.2) times more likely to be seropositive than older individuals. Having another seropositive individual in the same household increased the risk of previous infection 1.4 (95% CI, 1.2 - 2.0) times and each additional seropositive individual within the community (but outside the home) increased the risk of past exposure 1.3 (95% CI, 1.2 - 1.3) times. Gender and travel history were not associated with past exposure. Very high proportions of people infected in some communities and no infections in communities in a small geographic area suggests that transmission was very intense in outbreak communities, but the virus failed to transmit beyond outbreak communities. Research into spatial difference in vector ecology may help us understand heterogeneities in outbreak risk across this area.

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DISSECTING E2 PROTEIN DOMAINS INVOLVED IN ALPHAVIRUS CELL TROPISM

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Alphaviruses represent a diverse set of arthropod borne viruses, many of which are important veterinary or human pathogens. One of the most medically relevant alphaviruses, Chikungunya virus (CHIKV), is the cause of an ongoing outbreak of arthritic disease in the Caribbean islands and South America and its spread to North America appears likely. Other alphaviruses such as Venezuelan equine encephalitis virus (VEEV) and Semliki Forest virus (SFV) cause encephalitic disease. The role of viral genetics determining these different disease phenotypes is unclear. E2, the alphavirus receptor binding protein, has been implicated as a critical determinant of tissue tropism and host range. For example, previous reports have demonstrated that domains A and B of E2 contain residues important for host range expansions; however, little research has been performed to implicate which critical domain (e.g., A, B, and C) of this protein confer variations in cell tropism. Therefore, we created chimeric viruses between a normally arthritic virus (CHIKV) and a normally encephalitic virus (VEEV or SFV) to probe the effect of each domain on cell tropism both *in vitro* and *in vivo*. Chimeric CHIKV/VEEV were not viable, likely due to high sequence dissimilarity between the viruses. In contrast, CHIKV/SFV chimeras were rescued successfully and replicated well in several cell lines, albeit to lower levels than parental CHIKV. All chimeric viruses were attenuated as compared to parental CHIKV. When delivered intracranially to type-1 interferon deficient mice, the virus containing domain B and to a lesser extent domain A from SFV caused neuron degeneration and demyelination, similar to parental SFV. The chimera containing domain A from SFV appeared to cause more perivascular cuffing, similar to SFV. Transfer of these two domains from SFV to CHIKV altered tropism to reflect that of SFV. This indicated that the different domains of E2 may be playing an important role in determining cell tropism for alphaviruses. Further study of these domains may provide useful insight into alphavirus pathogenesis.

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ELUCIDATING THE MECHANISM BY WHICH THE INTERFERON-INDUCED EXORIBONUCLEASE, ISG20, RESTRICTS CHIKUNGUNYA VIRUS REPLICATION

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Chikungunya fever is an important emerging arboviral disease characterized by painful and often persistent, flaring polyarthritis and arthralgia affecting more than six million people worldwide. The causative agent, chikungunya virus (CHIKV), is a positive sense, RNA virus from the genus *Alphavirus*, family Togaviridae, whose geographic distribution has recently increased to include Europe, the Caribbean and South America. Currently, there are no approved vaccines or antivirals available for the treatment of CHIKV or related arthritogenic alphaviruses. We have previously demonstrated a crucial role for type I interferon (IFN) in the protection against musculoskeletal CHIKV disease in mice. Using a systems biology approach for identifying novel interferon stimulated genes (ISGs) with potent anti-alphaviral activity, our lab has identified a subset of genes with antiviral activity against CHIKV and its relatives. Among the genes identified with the greatest anti-alphaviral activity was the 20 kDa, nuclear exoribonuclease, ISG20. Using an inducible over-expression system and dominant-negative inhibitor, we have characterized this antiviral activity against CHIKV infection and identified early genomic translation as an early target of ISG20 function. Interestingly, over-expression of ISG20 also controlled the upregulation of a subset of other ISGs, independently of type I IFN production. Ongoing experiments are examining the mechanism, direct or indirect, by which ISG20 abrogates CHIKV genomic translation and the extent of host regulatory networks influenced by ISG20 upregulation. Our results indicate a crucial role for ISG20 in IFN-mediated protection against CHIKV infection and may reveal targets for future therapeutic intervention.

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MODELING THE POTENTIAL FOR A CHIKUNGUNYA OUTBREAK IN THE MIAMI METRO AREA

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Once found only in parts of Africa, southeastern Asia, and the Indian subcontinent, chikungunya has spread globally in the past decade with major outbreaks occurring in parts of Europe, La Réunion Island, and most recently in the Caribbean. The expansion of the disease has been facilitated in part by global travel, increased vector competence due to mutations in the virus, and increased ranges of habitats of the primary vector species, *Aedes aegypti* and *Ae. albopictus*. Expansion of the disease into the Western Hemisphere coupled with recent outbreaks and autochthonous transmission of dengue fever (which is vectored by the same species) in the U.S. raises concern that a chikungunya outbreak in the U.S. is imminent. Southern Florida, including the densely population Miami metropolitan area, is particularly susceptible to an outbreak due to the tropical climate, cohabitation of *Ae. aegypti* and *Ae. albopictus*, and heavy travel between the region and tropical locations worldwide, particularly Central and South America and the Caribbean. To assess the potential for an outbreak in the Miami metro area, we developed a mathematical model parameterized to study chikungunya dynamics in this region. We utilize the model to study the impact that timing, location, and size of an introduction have on the ability of an introduction to lead to an outbreak. Further, we study the influence of variation in viral strains and differential competence between vectors on disease dynamics. We calculate the probability that introductions will lead to outbreaks in the Miami metro and discuss the potential for the disease to spread from Southern Florida to other parts of the U.S. We also highlight the flexibility of the model by proposing its utility in studying the potential for outbreaks of other diseases, such as dengue fever, in the same region.

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HOST-PATHOGEN INTERACTION DYNAMICS OF HUMAN ASTROCYTES INFECTED WITH VENEZUELAN EQUINE ENCEPHALITIS VIRUS

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Venezuelan Equine Encephalitis Virus (VEEV) is an emerging arthropod-borne virus responsible for causing acute encephalitis, and often death, in animal and human hosts. VEEV is a priority pathogen that has previously been weaponized. Due to its continued environmental persistence in the Americas, it represents a significant threat to U.S. public health and economic security. The increased circulation and spread in the Americas of VEEV, and other encephalitic arboviruses such as Eastern Equine Encephalitis Virus (EEEV) and West Nile virus (WNV), underscores the need for research aimed at characterizing the pathogenesis of viral encephalomyelitis as a foundation for the development of novel medical countermeasures. In this study, we have sought to characterize the host-pathogen dynamics of VEEV in the human neuronal cell line U87MG by carrying out RNA sequencing of poly(A)+ mRNAs. We aim to identify critical alterations in the host transcriptome that take place within the first 24 hours following VEEV infection. Triplicate samples were collected at 4, 8, and 16 hours post-infection and RNA-Seq data acquired using an Ion Torrent PGM. Significant differentially expressed genes were part of the following super pathways: immune response IFN alpha/beta signaling, immune response IL-2 activation and signaling, regulation of nuclear SMAD2/3 signaling, and development glucocorticoid receptor signaling. Specifically we observed an increase in interferon regulated genes IFIT1, IFIT2, IFIT3, and OASL following VEEV infection. We also observed an increase in EGR-1 and differential expression of a number of genes that are involved in the EGR-1 pathway including ADAMT21, ATF3, KLF6, MYC, JUN, and PTGS2. Data from these studies will be leveraged towards identifying specific host mRNA transcripts or pathways suitable for therapeutic intervention, as well as provide mechanistic details regarding how alphaviruses manipulate the host transcriptome to facilitate replication.

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

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A majority of the research on dengue (DENV) virus-induced changes in gene expression has focused on the role of the adaptive immune response, which is undeniably important. However, epidemiological data suggest that the host's genetic background may also contribute important susceptibility factors that could exert their effect in a manner independent from the adaptive immune response. Cellular responses with the potential to make a difference between life and death outcomes are ultimately mediated by the actions of proteins encoded in the genome. Thus, an understanding of differential global gene expression at the proteome level is essential to understand how DENV infection can result in dramatically different disease outcomes. Ribosome profiling is a new technique that enables direct measurements of protein expression at the whole cell level. In so doing, it generates all the information needed for a comprehensive understanding of how global gene expression may influence particular disease phenotypes. We have recently completed the first ribosome profiling-based study of DENV-2 infected human cells. Our results indicate that ribosome profiling is a powerful tool to study changes in cellular dynamics upon DENV infection. Specifically, we are able to pinpoint differentially regulated genes and corroborate previously identified putative predictors of disease progression. As a whole, these data sets elucidate differentially regulated genes in the context of the host's genetic background. Furthermore, this approach has the potential to enable discovery of genes not previously associated with particular disease states, and in so doing, lead to the development of improved vaccines, diagnostics and therapeutics.

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POTENTIAL PROTECTIVE ACTIVITY OF DENGUE NANOVACCINE DELIVERY BY BCG/CHITOSAN NANOPARTICLE

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In this study, we report the development and testing of a novel dengue nanovaccine (DNV) composed of UV-inactivated DENV-2 (UVI-DENV-2), strain 16681 and BCG/chitosan nanoperticles (BCG/CS-NPs). We prepared BCG/CS-NPs by emulsion-polymerization method, yielding 299.1 ± 1.67 nm particles in size. The adjuvant properties of BCG/CS-NPs were tested using primary human immature DCs. A significant increase in the activation markers of DCs (CD80, CD86 and HLA-DR) and stimulation of IL-12 production were observed. By these properties, we used BCG/CS-NPs as an adjuvant and vaccine delivery system for the UVI-DENV-2, a vaccine immunogen. The inactivation and antigenicity of UVI-DENV-2 were confirmed by plaque assay and ELISA using specific monoclonal antibodies (4G2, 3H5 and 2H2). To prepare DNV, we loaded UVI-DENV-2 into BCG/CS-NPs which yielded DNV of 372.0 ± 11.21 nm particle in size. We also showed that up to 98.6% of UVI-DENV-2 found on DNV. We tested the immunogenicity of DNV in Swiss albino mice. Mice were vaccinated with three DNV doses, 15 days apart. The results showed that DNV stimulated anti-dengue IgM/IgG antibodies along with dengue-neutralizing antibodies in a dose-dependent manner, peaking 15 days post-dose three when using 10 µg of DNV per dose. We also demonstrated the cell-mediated immunity properties of DNV by flow cytometry. We showed an increase in the frequency of interferon (IFN)γ-producing CD4+T cells and CD8+ T cells after stimulation with 10 µg of DNV. We developed a mouse dengue challenge model to test the protective qualities of the DNV. Mice were vaccinated with three doses (10 µg each) of DNV and challenged with 10^6 PFU of a mouse-adapted DENV-2 (NGC strain) 14-day post dose three. Serum and spleen were collected at various dates post-challenge to measure viremia by RT-PCR. The decreasing of viremia in vaccinated mice indicated a potential use of the DNV in protecting against dengue virus infection.

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COMMUNITY KNOWLEDGE AND ATTITUDES AND HEALTH WORKERS' PRACTICES REGARDING NON-MALARIA FEBRILE ILLNESSES IN EASTERN TANZANIA

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Although malaria has been the leading cause of fever for many years, with improved control regimes malaria transmission, morbidity and mortality have decreased. Recent studies have increasingly demonstrated the importance of non-malaria fevers, what has significantly improved our understanding of etiologies of febrile illnesses. A number of non-malaria febrile illnesses including Rift Valley Fever, dengue fever, Chikungunya

virus infection, leptospirosis, tick-borne relapsing fever, Brucellosis and Q-fever have been reported in Tanzania. This study aimed at assessing the awareness of communities and practices of health workers on non-malaria febrile illnesses. Twelve focus group discussions with members of communities and 14 in-depth interviews with health workers were conducted in Kilosa district, Tanzania. Transcripts were coded into different groups using MaxQDA software and analyzed through thematic content analysis. The study revealed that the awareness of the study participants on non-malaria febrile illnesses was low and many community members believed that most instances of fever are due to malaria. In addition, the majority had unrealistic beliefs about the possible causes of fever. In most cases, non-malaria febrile illnesses were considered following a negative Malaria Rapid Diagnostic Test (mRDT) result or persistent fevers after completion of anti-malaria dosage. Therefore, in the absence of mRDTs, there is over diagnosis of malaria and under diagnosis of non-malaria illnesses. Shortages of diagnostic facilities for febrile illnesses including mRDTs were repeatedly reported as a major barrier to proper diagnosis and treatment of febrile patients. Our results emphasize the need for creating community awareness on other causes of fever apart from malaria. Based on our study, appropriate treatment of febrile patients will require inputs geared towards strengthening of diagnostic facilities, drugs availability and staffing of qualified health workers.

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NEW INSIGHTS INTO THE MOLECULAR EVOLUTION OF DENGUE VIRUS TYPE 4 IN PUERTO RICO OVER TWO DECADES OF EMERGENCE

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Dengue has emerged globally as a human health problem since the 1950s and is now the major arboviral disease infecting hundreds of million people annually. While some cases are asymptomatic, others develop a febrile illness (dengue fever) or even progress to severe dengue which can be fatal. Dengue is caused by any of four related viruses (DENV-1 to -4) that are maintained in endemic transmission in large urban centers of the tropics by *Aedes* mosquitoes. Since the late 1960s, Puerto Rico (PR), a major population center in the Caribbean, has experienced increasingly severe epidemics following the introduction of multiple dengue serotypes. A particularly severe outbreak in 1998 was dominated by a novel DENV-4 strain that evolved in PR, replacing earlier forms and spreading throughout the region. Here we show that this DENV-4 strain is genetically distinct based on unique changes in the NS2A and NS5 genes. Its replacement of earlier forms in Puerto Rico progressed rapidly from 10% of samples in 1994 to 95% in 1997, suggesting that strong natural selection played a role in its fixation. This study confirms that dengue viruses evolve through rapid lineage turnover driven in part by natural selection, important contributors to its ongoing emergence in human populations. *In vitro* experimental infection did not support higher replication rate as the cause of severity in the 1998 outbreak, however similar experiments are now being analyzed from *Aedes aegypti* mosquitoes.

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POOR CORRELATION BETWEEN BLEEDING AND THROMBOCYTOPENIA IN DENGUE

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Thrombocytopenia is a common finding in Dengue. Advice for prophylactic platelet transfusions in some guidelines has led to many un-necessary platelet transfusions. Threshold of platelet transfusions is often set at $20x10^9$/L, an arbitrary figure set without clinical evidence. Hence, it is important to study the relationship of bleeding in Dengue to low platelet count. We studied all the patients with dengue infection who were admitted under and referred to the principal investigator at Asiri Hospitals PLC, Colombo, during a period of 24 months from 1st of June 2011. Dengue infection was confirmed by positive antigen and/or antibody test. DHF was diagnosed in patients who had ultrasonically confirmed fluid leakage. Study included 254 patients with 142(55.9%) males and 112(44.1%) females. Mean age was 28.28 yrs. While 118 of them had DHF, 136 had DF. Bleeding manifestations were seen in 47(18.5%) patients; GI bleeding (n=6, 2.4%), gum bleeding (n=4, 1.6%), PV bleeding (n=31, 12.2%) and occult bleeding (n=6, 2.36%). Bleeding in DHF patients was significantly higher than that of DF patients. (DHF- 36, DF - 11, p=0.000). Bleeding was significantly associated with low platelet count when all the Dengue patients were considered with a mean platelet count of $34.02x10^9$/L (range 80-$5x10^9$/L) in bleeders and $53.66x10^9$/L in those who had no bleeding (p=0.000). When only the patient with platelet counts <80x109/L were analysed, there was a significant difference in the two groups of patients with platelet counts >20 and <$20x10^9$/L (p=0.022). However, when the patients with minor bleeds (gum bleeding) were excluded, there was no significant difference between these two groups (p=0.136). Mortality rate in this study was zero. Of the total, 33(12.99%) had platelet counts <$20x10^9$/L, but only 10 of them had any bleeding. None had prophylactic platelet transfusions. Therapeutic blood transfusions were required in 18(7.1%) and 01 had both platelet and blood transfusions as they were hemodynamically unstable. Mean lowest platelet count of this group was $30.44x10^9$/L and 56% had lowest platelet counts above $20x10^9$/L. This study confirms that the transfusion threshold of $20x10^9$/L in Dengue is arbitrary and relationship with clinically significant bleeding in Dengue and low platelet counts is not straight forward. Therefore, recommendations on prophylactic platelet transfusions need to be reconsidered.

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DENGUE HEMORRHAGIC FEVER VIRUS DETECTION IN *AEDES* MOSQUITOES, IBADAN NIGERIA

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Dengue Hemorrhagic Fever is cause by arthropod-borne virus that is transmitted to humans through mosquitoes bite. The subtropical climate of southern Nigeria is often hot and humid which promotes mosquitoes breeding. The consequences are reports of feverish illnesses most often attributed to malaria or label as fever of unknown origin when treatment is intractable. This study was carried out in Ibadan, southwest Nigeria to Detect Dengue Hemorrhagic Fever Virus in mosquito vectors and as a possible cause of fever of unknown origin in order to shed more light on the ecology of infectious disease in the locality. Five hundred and ten anthropophillic mosquitoes were captured, identified and processed for detection of arboviruses by molecular techniques. Nucleic acid was extracted from mosquitoes pool following homogenization in cell culture medium. Extracted RNA was thereafter transcribed to cDNA with random Hexamers. Thereafter, primers that primarily target alpha and flaviviruses were used for gene amplification. Subsequently, gene specific primers for yellow fever, West Nile, Chikungunya, rift valley and dengue viruses were used to amplify conserved regions of the different gene segments. Amplified products were thereafter separated in agar gel electrophoresis and viewed in UV light with a transilluminator. Out of 17 mosquitoe pools, 7 *Aedes* species pool tested positive for Dengue Haemorrhagic Fever Virus. DENV-1 was found in 6 pools, DENV-2 in 0 pools, DENV-3 in 0 pools and DENV-4 was tested positive in 5 pools. We detected Dengue Haemorrhagic Fever virus in *Aedes* mosquitoes in the study area, this provide recent data on Dengue Haemorrhagic Fever Virus ecology in Nigeria. It is also an indication that arboviruses may contribute to the burden of fever of unknown origin in the sub-region and should be considered for public health interventions.

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DEVELOP ALGORITHMS TO DIFFERENTIATE PRIMARY AND SECONDARY DENGUE INFECTIONS USING A SINGLE SPECIMEN IN ACUTE PHASE

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Dengue is the most important arboviral disease affecting humans. It can be caused by any one of four dengue viral serotypes, DENV1-4. Among symptomatic cases clinical presentation varies widely, ranging from mild febrile illness to severe and fatal disease. Although the pathogenesis is poorly understood, there is good epidemiological evidence indicating that heterotypic secondary infections are associated with development of severe disease. Therefore differentiating clearly between primary and secondary infections is crucial for studies on dengue pathogenesis. However the current methods are not ideal - often only a single specimen is available, and none of the available methods take into account the evolving nature of the immune response over time during the acute illness. The aim of this study was to develop a model to distinguish primary from secondary dengue infections using a single specimen obtained early in the illness course. The results of plaque reduction neutralization tests (PRNT) at 6 months after fever onset, when the acute immunological response had settled, were taken as the gold standard for classifying the recent infection as either primary or secondary. Daily plasma samples obtained during the acute illness were assayed for a) IgG using commercial PanBio Indirect ELISA kits, b) anti-whole dengue virus IgG and IgM in-house Capture ELISAs, and c) anti-dengue E protein IgG in-house indirect ELISA. Using logistic regression models were developed according to the day of illness (DOI), with separate models prepared for DOI 3; DOI 6; both DOI 3 and DOI 6; and for all days from DOI 3 to DOI 6. The models were internally and temporally validated. Preliminary results show that simple univariate logistic models based on the commercial PanBio Indirect ELISA or the anti-whole dengue virus IgG in-house Capture ELISA using DOI 3 samples performed well (AUCs (95% CI) of 0.88 (0.82-0.95) and 0.82 (0.74-0.9) respectively). Performance of the early acute phase models was as good as both convalescent, and dual phase, models.

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PRESENCE OF DENGUE FEVER IN SEMI-URBAN AREAS OF TWO HEALTH DISTRICTS IN BURKINA FASO

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The global incidence of dengue has grown dramatically in recent decades. In Africa, particularly in Burkina Faso, it has been neglected or ignored, the last description of acute cases were back in 1982. All fever cases are systematically considered presumptive malaria cases. During a regular populations survey, unexplained persistent fevers facing anti malaria treatment were observed. We therefore conducted an epidemiological investigation for non-malaria cases. The study was conducted in a panel of febrile children 0-10 years old, with temperature of 37.8°C or higher, living in semi- urban areas of Kaya and Zorgho. Rapid tests from SD Bioline Dengue Duo (AgNS1-IgG/IgM) were added to a panel of malaria rapid test. Samples on filter paper were taken from every patient with a rapid dengue test positive and for every tenth negative patient to perform qTR-PCR analyses. A total of 264 children were included, 153 from Kaya and 111 from Zorgho. Among them, 6.7% (18/264) had positive results in the two districts. In Kaya, 9.8% (15/ 153) positive cases were reported and 2.7% (3/ 111) in Zorgho. From all positive results, 11.1% were NS1 antigen (2/18) and 88.9% IgG/IgM (16/18) antibodies. The qRT-PCR results show the presence of DENV2, further analyses are ongoing to characterize the virus and to define the possible presence of other serotypes. Thirty years after from its last report, dengue presence has been document in febrile and symptomatic children, from semi- urban areas of Burkina Faso. Due to the contextual/local limitations, it was important to explore different ways to obtain the information and to analyze the samples. Therefore, filter paper use was a feasible tool to perform molecular analyses needed to confirm the presence of the virus. These findings reveal the need to address research and actions towards non-malaria diseases in the region. In this case thinking about dengue as one of the causes for febrile diseases. (More data about the viral sequencing and epidemiological description of the cases will be available for presentation at the conference)

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MOLECULAR DETECTION OF DENGUE VIRUS SEROTYPES CO-CIRCULATING WITHIN MOSQUITOES IN PERI-DOMESTIC ECOSYSTEMS IN NIGERIA

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Dengue Virus (DENV) causes devastating health impacts with an estimated 500,000 infections annually. The first DENV-1 isolate in Africa was reported from Nigeria. Reports of importation of DENV from West Africa into Europe have been increasing, yet the burden of the disease in Africa has not been estimated. Surveillance activities are weak or non-existent in many African countries. There is currently no vaccine against DENV, although several research activities are underway for an effective tetravalent vaccine. In this study we provide recent epidemiological data of co-circulating DENV serotypes in mosquitoes involved in peridomestic transmission. This is by far the most elaborate study in Nigeria with comparison between habitats where non-human primates are kept and aboreal habitats without nonhuman habitats where humans visit. The modified CDC light trap was used to catch both *Aedes albopictus* and *Ae. aegypti* from Agodi garden Ibadan; University of Ibadan zoo, botanical garden, botany forest Oyo State. They were sorted and identified morphologically into 26pools of 50mosquitoes each. It was screened after RNA extraction using primers designed to target and amplify the nonstructural proteins region of the genome using a thermalcycler and the amplicon was observed using a trasilluminator. Temperature of diurnal activity was between 26-36oC. The number of positive pools are as follows; DENV-1 (11), DENV-2 (14), DENV-3 (16), DENV-4 (10). DENV was detected in all 26pools and the minimal rate of infection was DENV-1 (22.2%), DENV-2 (44.4%), DENV-3 (77.8%) and DENV-4 (44.4%). *Aedes albopictus* was found to be more abundant all year round compared to *Aedes aegypti*, both competent in transmitting DENV. Co-circulation of four DENV serotypes within peridomestic cycles has been established in Nigeria. There is the need for immunological investigation of host factor interaction within the area, this may enhance vaccine production. Also, sustained surveillance activities in hospitals are encouraged to confirm clinical cases and improve patient care.

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THE ROLE OF DENGUE VIRUS MATURATION IN VECTOR INFECTION

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Dengue, a mosquito-borne flavivirus, affects up to 100 million people a year with over 40% of the global population currently at risk. Infections range from mild febrile illness to severe dengue hemorrhagic fever and dengue shock syndrome, both of which can be lethal. Present primarily in tropical and subtropical regions, dengue's expansion into the U.S. and other areas has become a major public health concern. Due to antibody-dependent enhancement of a secondary infection, a dengue vaccine should contain all serotypes or risk putting vaccinated individuals at higher risk for severe disease. While progress has been made to develop a vaccine with the four established serotypes, the discovery of a fifth

possible serotype presents a significant setback. With no vaccine or specific treatment for dengue, understanding the viral interaction with the mosquito vector is particularly important. Knowledge regarding dengue pathogenesis in the mosquito could lead to novel treatments and preventative measures. Dengue is a positive-stranded RNA virus which contains three functional proteins: capsid protein C, envelope protein E, and membrane protein M. Immature virus within the cell is coated with immature M protein (prM). This protein is cleaved by furin during exocytosis, which results in mature particles coated with E protein. This process is imperfect and can lead to the release of viral particles in various stages of maturation. The infectivity of these particles has been well-documented in mammalian cells, with the addition of prM-antibody rendering immature particles infectious. However, since mosquitoes lack antibody receptors, the mosquito-specific infectivity of immature virus particles remains unclear. We and others have found that completely immature dengue particles are not infectious in mosquito cells *in vitro* yet the *in vivo* infectivity remains to be seen. It is possible that enzymes or other factors may render the immature stages of the virus infective to the mosquito. Recent and ongoing experiments have given us valuable insight into the function of these immature virus particles when it comes to vector infectivity. Results point to exciting new roles for virions in various stages of maturity when taken up by the mosquito vector.

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HIGH PREVALENCE OF FLAVIVIRUS EXPOSURE AMONG PREGNANT WOMEN IN MYSORE, INDIA

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Dengue is a flavivirus infection whose prevalence has been steadily growing in recent years, especially in the developing world. It has also been shown to affect pregnant women by increasing the rate of cesarean section deliveries and risk of pre-eclampsia. This pilot study describes the baseline incidence of dengue in a sample of pregnant women in Mysore, India. While 349 samples (89%) were seropositive for dengue, there was no significant association with demographic factors that were studied. The high numbers of flavivirus infection however, indicate that further research is crucial to better understanding and implementation of preventive measures.

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MOLECULAR CHARACTERIZATION OF DENGUE VIRUSES IN THE PHILIPPINES 2008-2013

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Dengue is the most important arboviral disease in the Philippines with over 166,000 cases from January to November 2013. All four serotypes of dengue are found in the Philippines. However, to our knowledge, there have been no studies to characterize dengue strains at the molecular level in the Philippines. Molecular characterization is important to monitor changes in circulating strains. The aim of this study was to characterize the dengue strains circulating in the Philippines from 2008-2013. To date, 34 serotype 1, 18 serotype 2, and 24 serotype 3 samples have been sequenced and analyzed. Substitution rates were analyzed using BEAST software giving mean rates of 1.99x10-3 substitutions/site/year for serotype 1, 1.3136x10-3 substitutions/site/year for serotype 2, and 8.3243x10-4 for serotype 3. Single Breakpoint Recombination (SBP) and Genetic Algorithm for Recombination Detection (GARD) analysis showed potential breakpoints at 157 in capsid-premembrane gene of serotype 1 in seven samples and 194 in CprM gene and 587 in the Env gene of Serotype 3 in 4 samples. Initial analysis of the recombinant sequences of serotype 3 indicates recombination between genotypes I and IV. The recombination in both dengue serotypes 1 and 3 was first detected in samples from 2010 and serotype 1 recombinants were also detected in samples from 2011. The occurrence of these recombinants may be linked to virulence and improved transmission. These are probable factors in the evolution and dynamics of the dengue virus in the Philippines. No recombination was found in serotype 2 samples. More samples will be sequenced and analyzed with a target completion date of June 30, 2014.

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VIROLOGICAL AND SEROLOGICAL INVESTIGATION OF THE FIRST DENGUE FEVER OUTBREAK IN ETHIOPIA

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Dengue fever is transmitted by the bite of a mosquito infected with one of the four dengue virus serotypes. In recent years, transmission has increased predominantly and has become a major international public health concern. In Ethiopia Dengue Fever has never been reported or laboratory confirmed previously. Unusual increment of febrile cases of unknown etiology was reported from Dire Dawa town, in eastern Ethiopia, in September 2013. Serological and virological investigations were carried to confirm or refute if arbo-viruses is the causative agent of the outbreak. Fifty serum samples were obtained from acute febrile illness patients who visited health facilities and also active case search for patients in convalescent phase of illness. The laboratory investigation of the serum samples included testing for the presence of IgM against arbo-viruses; namely flavi viruses (Yellow fever, Dengue, West Nile and Zika viruses) and Rift Valley Fever, Crimean-Congo Hemorrhagic Fever and Chikungunya viruses by ELISA as well as PCR testing for the presence of viral nucleic acids and nested PCR for sero-typing of dengue viruses. The results of the laboratory investigation showed that 15 of the 50 tested samples were positive for IgM against Dengue infection by ELISA (30%) and 11 were also positive by PCR (22.0%), one case was positive by both techniques giving a total dengue infection positivity rate of 50%. Relatively lower rate of Flavi-virus cross reactive IgM positivity was also observed for yellow fever (22%), Westnile (20%), and Zika (10%) viruses as compared to dengue. None of the samples were positive for IgM against rift valley fever, Crimean-Congo Hemorrhagic Fever and Chikungunya. Except for dengue, none of samples were positive by PCR for the tested viruses. Sero-typing of the virus revealed that the infection was caused by dengue virus sero type 2. It is evident from the laboratory investigation that the acute febrile illness outbreak was caused by Dengue fever virus sero type 2 and this is the first laboratory finding confirming the first dengue outbreak in Ethiopia.

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INTRACELLULAR COMPARTMENTALIZATION OF DENGUE VIRUS DURING ANTIBODY-DEPENDENT INFECTION

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Dengue virus (DENV) continues to put billions at risk of life-threatening disease annually. Infection is enhanced when DENV is opsonized with sub- or non-neutralizing antibodies that augment entry into monocytes and dendritic cells through Fc-gamma receptors (FcγRs), a process termed antibody-dependent enhancement of DENV infection (ADE). It has also been suggested that besides augmenting entry, ADE occurs through other intrinsic factors activated by FcγR-mediated signaling. We recently reported a role for leukocyte immunoglobulin-like receptor B1 (LILRB1) in downregulating activating FcγR-mediated induction of the interferon stimulated genes (ISG). Co-ligation of LILRB1 by antibody-opsonized DENV recruits the phosphatase SHP-1 to dephosphorylate Syk and hence reduce ISG induction. In this work, we test the hypothesis that reduced Syk

signaling would also lead to differences in the compartmentalization of DENV-containing phagosomes, which may influence the outcome of ADE. We observed that higher levels of phosphorylated Syk permitted faster phagocytic trafficking of DENV immune complexes through Rab-5, Rab-7 and LAMP-1 compartments. When Syk activity was inhibited either by LILRB1 co-ligation or by piceatannol, a Syk-selective inhibitor, phagosomes displayed reduced levels of trafficking markers. Further interrogation of proteomic changes in phagosomes isolated under differential levels of Syk phosphorylation provides insights into how phagosomes are trafficked for ADE. Collectively, our data suggests that antibody-dependent infection enables DENV to be trafficked into compartments that may be more congenial for replication.

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A DENGUE HUMAN INFECTION MODEL TO EVALUATE THE PROTECTIVE EFFICACY OF THE LIVE ATTENUATED TETRAVALENT DENGUE CANDIDATE VACCINE TV003

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Dengue virus (DENV) has become the most important arbovirus worldwide with estimates of as many as 400 dengue infections occurring annually. These infections result in approximately 100 million symptomatic cases of which more than 2 million are classified as severe disease and approximately 21,000 result in death. Neutralizing antibody, the accepted immunologic endpoint chosen to move candidate DENV vaccines forward, was not predictive of protection against DENV illness in the field in a recently completed Phase 2b efficacy trial of the lead DENV candidate vaccine CYD. These results have made it more difficult for vaccine manufacturers to determine which candidates should be further evaluated in large efficacy trials in endemic areas where vaccine failure could predispose subjects to more severe disease should they subsequently become infected with DENV. A dengue human infection model (DHIM) would be useful in down-selecting candidate vaccines prior to testing in endemic areas as well as identifying putative correlates of protection. We have developed a DHIM to evaluate the protective efficacy of the live attenuated tetravalent dengue vaccine TV003 developed by the U.S. National Institutes of Health. In Phase I clinical trials, TV003 induced neutralizing antibody to DENV-1, DENV-2, DENV-3, and DENV-4 in 92%, 76%, 97%, and 100% of vaccinees, respectively. It induced seroconversion to all four DENV serotypes in 74% of vaccinated subjects and to three or more serotypes in 98%. Because the seroconversion rate to DENV-2 was lowest, we sought to evaluate protection elicited by the vaccine against a heterotypic strain of DENV-2. We vaccinated 24 flavivirus-naïve subjects with a single dose of TV003; an additional 24 subjects received a placebo. Six months later, all 48 subjects received a challenge dose of 1,000 PFU of rDEN2Δ30, an under-attenuated DENV-2 vaccine candidate. DEN2Δ30 was previously demonstrated to induce viremia in 100% of inoculated subjects with a mean peak virus titer of 2.5 log10 PFU/mL. In addition, 80% of subjects developed a maculopapular rash and 40% developed neutropenia. The study is powered to detect 60% efficacy of TV003 to prevent viremia caused by rDEN2Δ30 (the primary outcome). Secondary outcomes include protection against rash and neutropenia. Preliminary clinical, virological, and efficacy outcomes of the trial will be presented.

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CHARACTERIZATION OF CELLULAR RESPONSES TO A TETRAVALENT LIVE-ATTENUATED DENGUE VACCINE IN NON-HUMAN PRIMATES AND FLAVIVIRUS-NAÏVE HUMAN VOLUNTEERS

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We have developed a live attenuated tetravalent dengue vaccine candidate based on an attenuated dengue 2 virus (DENV-2) and three chimeric viruses containing the pre-membrane and envelope genes of DENV-1, -3 and -4 expressed in the context of the attenuated DENV-2 genome. The cellular responses elicited by this vaccine were analyzed in: 1) non-human primates after a single subcutaneous administration, and 2) flavivirus-naïve human volunteers vaccinated twice (day 0, 90) via the subcutaneous or intradermal routes. Using peptide arrays and intracellular cytokine staining, we demonstrated that the vaccine elicits $CD4^+$ and $CD8^+$ T cell responses targeting the non-structural NS1, NS3 and NS5 proteins of DENV-2, and E proteins of each DENV serotype. Both T cell subsets produced IL-2, IFN-γ, and TNF-α, and expressed the CD107a marker. $CD8^+$ T cell responses in humans were highest on day 90 after the first immunization and were still detectable on day 160 post-secondary immunization. In both species, $CD8^+$ T cells were multifunctional, producing ≥2 cytokines simultaneously, and cross-reactive to the NS proteins of the other serotypes. Overall, these findings highlight the immunogenic profile of our candidate dengue vaccine and support the further evaluation of clinical samples from ongoing phase II clinical trials.

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ORGAN-HOMING TARGETS OF ANTIBODY SECRETING CELLS DURING ACUTE DENGUE VIRAL INFECTION

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B cell responses play important roles during dengue viral infection due to the ability to produce both protective and disease enhanced antibody. Although many studies demonstrated a major expansion of antibody secreting cells (ASC), the precise mechanisms for this massive increase in the frequency of ASC and their mobilization remain unclear. The analysis of cell surface homing molecules on ASC may provide some clues for the organ or tissue distribution of these cells. In this study, the blood samples from dengue infected patients were analyzed by polychromatic flow cytometry for B cell subset distribution based on the expression of CD21, CD27 and CD38 and the expression of cell surface homing molecule on ASC including CCR7 and CD62L (to the regional lymph nodes), CXCR3 and ICOS (to the lung), CCR10 (to the skin), beta7, CCR9 and CD103 (to the gut tissue), CCR2 and beta1 (to the central nervous system) and CXCR4, CD122, CD132 and CD137 (to the bone marrow). The results showed that the frequencies of ASC (CD21-/CD27hi/CD38hi) varied from 16.1% to 87.1% of total B cells depend on the time when samples were collected. At the time of defervescense, ASC had a highest frequency (47.9%) when compared to naïve (32.5%), resting memory (9.3%), activated memory (4.4%) and tissue memory (6.1%) B cells. Frequencies of ASC remained higher than other B cell subsets even 2 days after defervescense date. Although high frequencies of ASC were observed, the results showed no significant difference when compared between patients with dengue fever and dengue hemorrhagic fever. When the expressions of homing markers were determined, the results showed high frequencies of ASC that expressed CD62L (79.4%), CXCR3 (71.9%), CCR10 (99.8%), beta7 (75.7%), CXCR4 (84.13%) and CD132 (99.9%). Almost half of

ASC expressed CCR2 (48.3%) whereas only a minor population expressed CCR7 (29.4%) or CCR9 (22.8%). Low frequencies of ASC expressed beta1 (3.9%) or CD122 (5.7%) whereas ICOS, CD103 and CD137 could not be observed (less than 1%). The results suggested that large proportions of ASC may distributed to various organs and tissues including lymph nodes, lung, skin, gut and bone marrow whereas minor proportions mobilized to the central nervous system. The results obtained in this study provide novel insights on the target distribution of ASC to specific organ and tissue where it plays protective or disease enhanced roles during acute dengue infection.

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HOUSEHOLD LEVEL ECONOMIC BURDEN OF DENGUE VIRUS INFECTION IN PUERTO MALDONADO, PERU

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Dengue virus (DENV) is an arbovirus with global distribution. DENV was reintroduced to Peru in the 1990s and has been reported in Puerto Maldonado (population ~80,000) in the Peruvian southern Amazon Basin since 2000. This region also has the highest human migration rate in the country, much of it from areas where DENV is not endemic. Several studies have reported on the economic burden of dengue disease from societal, health care system or governmental perspectives but few have focused on the financial burden at the household level. We therefore sought to assess the household income diverted to costs incurred when a household member contracts DENV and to compare costs between recent migrants (RM) and longer-term residents (LTR), defined respectively as residency in Puerto Maldonado for less than or greater than 5 years. We administered a standardized questionnaire to persons diagnosed with dengue disease at Hospital Santa Rosa in Puerto Maldonado from December 2012 to March 2013. We compared direct and indirect medical costs and compared between RM and LTR. Demographic data, socioeconomic characteristics and assets were also compared between RM and LTR. Of the 80 persons who completed the survey, 28 (35%) were RM. Each dengue disease episode cost the household an average of US$ 105 (SD=107), representing 24% of their monthly income. Indirect costs were the greatest expense (US$ 56, SD=87), especially lost wages. LTR households had a higher average monthly income than RM ones (p=0.041) and were significantly more affluent based on wealth index (p=0.002). Costs did not differ significantly between RM and LTR households. The study highlights the very significant financial burden incurred by households when a family member suffers dengue disease, especially for RM since their overall monthly income is lower.

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COMPARING FLUORESCENT FOCUS ASSAYS (FFAS) WITH PLAQUE ASSAYS (PAS) IN DENGUE CONFIRMED CASES

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Plaque formation assays and mosquito inoculations are two widely used techniques to quantify dengue viruses. Each of these techniques has its limitations; for example, mosquito inoculations are sensitive but require regular maintenance of a mosquito colony, and the sensitivity of plaque assays (PA) depends on the type of cell line used and the ability of a particular virus to induce a desired cytopathic effect (CPE). The sensitivity of C6/36 *Aedes albopictus* cells to dengue virus (DENV) infection has been well documented; however, DENV causes little apparent CPE in this cell line. Fluorescent focus assays (FFA) are a variation of PA where focused areas of infection are visualized by detecting viral antigen with monoclonal antibody. To quantitate DENV infections in cell culture, we performed serial dilutions of all four DENV serotype seeds, infected Vero 76 and C6/36 cells using the FFA in 8-well chamber slides, and performed PA using BHK-21 clone 15 cells in 24-well plates. We also titrated 8 and 15 samples positive for DENV-2 and DENV-4 by viral isolation, respectively. Seed viruses showed higher titers of DENV-1, DENV-3 and DENV-4 using FFA in 2-3 days in both cells lines compared with 5-7 day in PA. Compared with PA, FFA in C6/36 cells resulted in higher titers of DENV in 14 samples (61%), similar titers in 2 samples (9%), and lower titers in 2 samples (9%). Only 3 samples (13%) were positive by FFA in Vero cells. Although more expensive than PA, FFA does not require the development of CPE in the infected cell monolayer. In conclusion, C6/36 cells are ideal for DENV titration in humans, increasing sensitivity and producing more accurate quantifiable DENV results.

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DESIGNING GOAL-ORIENTED DISEASE SURVEILLANCE NETWORKS: A DENGUE VIRUS CASE STUDY

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With public health facing increasing budget constraints, disease surveillance is at a critical juncture. To design effective surveillance under resource constraints, we propose a four-step process that produces a surveillance system through systematic evaluation and integration of candidate data streams. This methodology quantifies the performance of individual data streams in terms of the specified surveillance objectives, and prioritizes them for incorporation into surveillance systems. We demonstrate the utility of this method by designing a multi-objective Dengue surveillance network for Puerto Rico. Networks designed using this approach were able to effectively monitor regional and local Dengue outbreaks, track serotype dynamics, and provide early warning for large outbreak years. This system was predicted to perform at least as well as the existing system and only utilized 20% of the existing providers.

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LONGITUDINAL ANALYSIS OF SERUM AVIDITY FOLLOWING SECONDARY DENGUE VIRUS TYPE 2 INFECTION IN PATIENTS WITH DIFFERENT DISEASE SEVERITY

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The four dengue virus serotypes (DENV1-4) are responsible for the most prevalent mosquito-borne viral illness in humans. DENV causes a spectrum of disease ranging from self-limiting dengue fever (DF) to severe, life-threatening dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Secondary (2°) DENV infection is associated with more severe disease, which is attributed to cross-reactive antibodies and T cells. However, antibodies and T cells can also mediate protection. The effectiveness of the antibody response is dependent on both the affinity and avidity of the antibody/antigen interaction, where avidity depends on the antibody isotype and the number and affinity of individual binding sites. We sought to determine how antibody avidity developed over time following 2° infection with DENV2 and whether avidity differed according to disease severity. We analyzed sera from 41 2° DENV2-infected subjects (DF, n=15; DHF, n=15; DSS, n=11) from an ongoing hospital-based study of pediatric dengue in Managua. IgG avidity against DENV2 whole virion antigen (DENV2 strain N172) was measured in samples collected during the acute and convalescent phases as well as 3, 6, 12, and 18 months post-illness using a urea avidity enzyme-linked immunosorbent assay

(ELISA). With a stringent cutoff of background absorbance <0.2 OD, a positive control absorbance of >5X background OD, and positive control within one standard deviation from a quality control plate, the avidity was calculated as a ratio between percent IgG bound in 9M urea-treated wells compared with PBS-treated wells. Longitudinal analysis of serum IgG avidity against DENV2, regardless of disease severity, parallelled our previously published kinetics of avidity in 2° DENV3 infections. Our data show a substantial increase in avidity from acute to convalescent phase followed by a decrease in avidity from the convalescent phase to 3-months post-infection, then a plateau. During the acute phase, individuals who would develop more severe disease demonstrated higher serum avidity against DENV2. In contrast, at 18 months post-infection, sera from individuals with more severe disease displayed lower avidity when compared to sera from cases with less severe disease. Taken together, these data show that the kinetics of serum avidity following 2° DENV2 and DENV3 infection are similar. In addition, the data suggest that serum avidity levels may correlate with disease severity.

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QUAD-COLOR B CELL FLUOROSPOT: A NOVEL APPROACH FOR ANALYZING THE SEROTYPE SPECIFICITY AND CROSS-REACTIVITY OF ANTIBODY-SECRETING CELLS IN DENGUE VIRUS-INFECTED INDIVIDUALS

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The 4 dengue virus serotypes (DENV1-4), members of the flavivirus genus, cause the most prevalent mosquito-borne viral illness in humans, with >3 billion people at risk for infection and up to 96 million cases annually worldwide. DENV infection can result in self-limiting dengue fever or more severe life-threatening disease. Severe disease is thought to be associated with the presence of serotype cross-reactive antibodies (Abs) that enhance viral uptake into target cells during a heterologous secondary infection. In contrast, type-specific Abs after a primary infection are important for homotypic protection. However, little is known about the protective role of cross-reactive Abs. To date, studies have analyzed DENV-specific B cells using ELISPOT assays and flow cytometry or by generating monoclonal Abs (MAbs) from memory B cells (MBCs) or plasma cells. We have developed a novel approach to determine the frequency of serotype-specific and serotype cross-reactive MBC responses on a per-cell basis. Using a modified version of an ELISPOT and applying recent advances in fluorescent detection (FluoroSpot), we can evaluate MBC specificity to all 4 DENV serotypes simultaneously in individual cells. The MBCs from cryopreserved peripheral blood mononuclear cells (PBMCs) of DENV-exposed individuals were stimulated *in vitro* for 3-5 days to become Ab-secreting cells and then incubated in ELISPOT plates coated with a human IgG capture Ab for 1-2 days. Subsequently, OptiPrep-purified virions from the four serotypes (Nicaraguan DENV1-4) were added to bind to DENV serotype-specific and cross-reactive Abs secreted by the Ab-secreting B cells. Visualization was achieved using four mouse anti-E Domain III serotype-specific MAbs that were labeled with different fluorophores. Serotype-specific MBCs and MBCs cross-reactive to 2, 3, or 4 DENV serotypes were detected in different individuals, with type-specific and cross-reactive MAb hybridomas used as controls. We are currently applying this method to analyze longitudinal samples (acute, convalescent, and 3, 6, 12, and 18 months post-illness) from primary and secondary DENV infections in a hospital-based dengue study in Nicaragua. This new technique will further the understanding of how MBC specificity evolves over time, how the serotype-specific and cross-reactive responses associate with disease outcome, and how serial infections with heterotypic serotypes drive MBC specificity.

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CO-INFECTION WITH INFLUENZA AND DENGUE VIRUS LEADS TO SEVERE DISEASE VIA MODULATION OF THE HOST RESPONSE

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Both influenza and dengue are major health problems worldwide. In 2009, Nicaragua experienced a large dengue epidemic, even though the dominant dengue virus serotype (DENV3) and virus clade remained the same between 2008 and 2011. In addition, the 2009 dengue epidemic was atypical, with early onset of compensated shock. The only identifiable epidemiological difference in 2009 was the influenza A-H1N1 pandemic, which was shifted from the usual seasonal influenza peak and instead overlapped with the dengue epidemic for 8-10 weeks. We hypothesized that sequential or co-infection of influenza H1N1 and DENV modulated the immune response, leading to the atypical presentation and more severe dengue disease. We then established a mouse model of dual infection with Nicaraguan pandemic H1N1 influenza clinical isolate A/NI/5227/2009 (intranasal) and the virulent DENV2 strain D220 (intravenous). DENV suppresses the interferon response, replicates, and causes disease in humans but not in wild-type mice. Thus, we used C57BL/6 mice lacking the interferon-α/β receptor (*Ifnar*$^{-/-}$) that were susceptible to DENV2 and influenza virus infection. Influenza virus infection followed after two days by DENV2 caused 90% lethality in *Ifnar*$^{-/-}$ at virus doses that caused mild disease during infection with either virus alone. DENV2 infection followed two days later by influenza H1N1 infection caused 50% lethality in *Ifnar*$^{-/-}$ mice. The viral load of DENV2 and influenza virus H1N1 was similar in the lungs and other tissues of *Ifnar*$^{-/-}$ mice infected with one or both viruses; thus, viral load does not appear to explain the decreased survival of *Ifnar*$^{-/-}$ mice sequentially infected with the two viruses. We are currently investigating the inflammatory response via Nanostring nCounter gene expression analysis of mRNA in mice infected with one or both viruses to establish immunological pathways that determine disease outcome. Preliminary results suggest differential expression of inflammatory genes in the lungs of mice infected with both viruses compared to mice infected with only one virus. Our study may inform treatment and vaccine strategies in endemic areas where dengue and influenza viruses co-circulate.

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A NATURALLY OCCURRING SINGLE POINT MUTATION IN THE ENVELOPE PROTEIN CHANGES THE NEUTRALIZATION PROFILE OF DENV2 ASIAN 1 VIRUSES

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The four serotypes of dengue virus (DENV1-4) cause the most medically important arthropod-borne viral disease worldwide. Each serotype is comprised of several genotypes. A DENV2 genotype replacement event in Vietnam, where the previously dominant Asian-American (AA) genotype was entirely replaced by the Asian 1 (A1) genotype, was observed during 2003-6. We investigated the possible role of pre-existing immunity in driving genotype replacement by generating reporter virus particles (RVPs) representing the two genotypes and analyzing their neutralization profiles by patient sera collected in Vietnam prior to (1997-8) and after (2006-7) the replacement event. The Vietnamese 2007 samples neutralized the A1 better than the AA genotype, as did sera from post-secondary DENV2 infections in a Nicaraguan cohort. However, among A1 isolates

that shared the 5 amino acid changes in charge or side chain between the E genes of A1 and AA -- N83K, D203N, T226K, G228E, and H346Y -- one A1 isolate had a different neutralization profile when analyzed using antigenic cartography with non-human primate antisera. Six A1 isolates were poorly neutralized by both homologous and heterologous antisera, but the outlier A1 was strongly cross-neutralized by DENV1 and DENV3 antisera and had a distinct substitution, K160Q. This mutation was observed in strains isolated at the end of the Vietnam DENV2 A1 epidemic, in a clade that persisted for multiple years. To test if this amino acid substitution determined differences in neutralizing antibody titers with human antisera, the A1 RVP with 160Q was mutated to K. Interestingly, the dominant neutralizing phenotype was lost in the Q160K A1 RVP when tested with Nicaraguan cohort antisera from post-secondary DENV2 infections (1.7-fold reduction, $p<0.001$), post-primary DENV3 infections (2.4-fold reduction, $p<0.001$), and post-primary DENV1 infections (1.5-fold reduction, $p<0.05$). We are analyzing changes in neutralization titer to 160K and 160Q variants by a panel of monoclonal antibodies sensitive to virion structure to determine if mutations at position 160, located in a valley on the surface of E Domain I, alter the global structure of the virus. In sum, our findings from independent analyses indicate that a clade of viruses circulating for multiple years in Vietnam had a single point mutation in the E protein (K160Q) that significantly altered polyclonal neutralization, in particular by heterologous antisera.

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DIAGNOSTIC UTILITY OF ATYPICAL LYMPHOCYTES TO IDENTIFY DENGUE AND SEVERE DENGUE

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Dengue is the most prevalent mosquito-borne viral disease of humans worldwide. Dengue virus (DENV) infection can cause a range of outcomes, from asymptomatic infections and undifferentiated fever to dengue fever and severe illness. Prompt and accurate diagnosis of dengue, as well as the identification of severe cases, remains a challenge, in particular in resource-limited settings. Atypical lymphocytes, aka Downey cells or reactive lymphocytes, have been described in several infectious, autoimmune, and idiopathic disorders, including severe dengue. Atypical lymphocytes can be identified morphologically and quantified in blood smears simply using a light microscope. Here, we analyzed the diagnostic value of atypical lymphocytes, quantified as percentage of total lymphocytes, for the identification of 1) confirmed dengue cases and 2) severe dengue cases among suspected dengue cases. A total of 1,522 suspected dengue cases, aged 6 months to 14 years and enrolled in a prospective, hospital-based study in Managua, Nicaragua, from August 2005 to November 2013 were included (964 laboratory-confirmed dengue-positive and 558 dengue-negative cases). Daily measurements of atypical lymphocytes were performed (total: 4,416; range: 1-6 per case). On days 1 and 2 of illness, no difference was observed in the proportion of atypical lymphocytes between dengue-positive and dengue-negative cases. However, from day 3 on, the proportion of atypical lymphocytes was significantly higher in dengue-positive cases ($p<0.01$). On day 5, the best cut-off value for atypical lymphocytes was estimated to be ≥2%, with a sensitivity of 64.6% (95%CI: 61.0-68.8) and a specificity of 62.3% (57.1-67.3) for dengue positivity. We then compared severe dengue cases, defined using the 2009 WHO classification, vs. non-severe cases. A significant difference was not observed between these groups on days 1-3; however, on days 4-7, the proportion of atypical lymphocytes was higher in severe dengue cases ($p<0.01$). At day 5, the best cut-off that differentiated severe from non-severe cases was ≥3% of atypical lymphocytes, with a sensitivity of 66.7% (60.8-72.1) and a specificity of 52.0% (47.3-56.7). Our results suggest that atypical lymphocytes can be used to aid in the diagnosis of dengue and severe dengue. Additional signs, symptoms, and blood parameters could be used in combination with atypical lymphocytes to improve their diagnostic utility.

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LOWER CHOLESTEROL LEVELS ARE ASSOCIATED WITH SEVERE DENGUE OUTCOME

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Dengue virus is a flavivirus of worldwide importance, with approximately 3.97 billion people across 128 countries at risk of infection and up to 96 million dengue cases annually. Previous studies have shown that lipids and lipoproteins may play a role in modifying virus infectivity and the host's immune response to infection. However, the relationship between total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and severe dengue outcome is unclear. We analyzed data from 789 laboratory-confirmed pediatric dengue cases and 447 other febrile illnesses (OFI) in a prospective hospital-based study in Managua, Nicaragua, between August 2005 and January 2013, and used three different classifications of dengue severity: World Health Organization (WHO) 1997, WHO 2009 and standardized intervention categories. To examine the association between total serum cholesterol at first presentation and risk of severe dengue outcome, relative risks and 95% confidence intervals (CIs) were calculated using modified Poisson models with robust standard errors, adjusted for day of illness and other confounders. We found that total cholesterol, HDL-C and LDL-C decreased over the course of illness and differed across disease outcome groups. Greater decreases were observed in LDL-C compared to HDL-C. Multivariate models showed that lower levels of total serum cholesterol were associated with severe dengue outcome compared to OFI and mild dengue outcome. At first presentation, for each 10 mg/dL decrease in total serum cholesterol, the risk of severe dengue outcome increased by 14% (95% CI: 6-22%) using the WHO 1997 classification, 6% (95% CI: 2-11%) using the WHO 2009 classification, and 3% (95% CI: -0.02-9%) using standardized intervention categories. Models using the cut-point of total serum cholesterol <85mg/dL had cross-validated areas under the curve (AUC) ranging from 0.7-0.8. These results indicate the relatively specific ability of cholesterol to discriminate patients with severe dengue outcome from patients with OFI and mild dengue outcome when they first present to the hospital.

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MOLECULAR SURVEILLANCE OF DENGUE VIRUS (SEROTYPES 1 TO 4) AND ITS SEVERAL GENOTYPES IN A MEDIUM SIZE CITY OF SOUTHEASTERN BRAZIL, DURING EIGHT EPIDEMICS YEARS

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Dengue is a viral infection transmitted by the bite of an infected *Aedes* mosquitos. This illness is endemic throughout the tropics and subtropic (100 countries worldwide) with a geographic distribution similar to that of malaria, but more risk in urban and residential areas. The four serotypes

of dengue virus (DENV 1-4) (family Flaviviridae, genus Flavivirus) are antigenically and genetically distinct and their genotypes vary in virulence. Wherefore, the detection and analysis of spatial and temporal transition are essential. In this work we looked the Dengue viruses circulation in Sao Jose do Rio Preto (a 434K habitants city) from 2006 to 2014. São José do Rio Preto show subquente tropical and humid weather, ideal for the proliferation of vector and has annual epidemics and hyperendemicity situations. We used serum samples of suspected DENV patients provided by the Public Health to profile DENV circulation by PCR. We analyzed 2.162 cases from January 2006 to January 2014. We amplified 1372 (63,45%) samples of DENV: 389 (28,35%) were positive for DENV-1, 177 (12,90%) for DENV-2, 494 (36,00%) for DENV-3, 312 (22,74%) DENV-4 and 4 (0,29%) DENV-1/4 coinfection. Studying the movement of DENV from 2006 to 2014 we get to see the introduction, extinction and re introduction of serotypes in the city. Up to now, we got 20 sequences of DENV-1 (inferred E protein) and 5 sequences of DENV-1 (full genome); 26 sequences of DENV-2 (E protein) and 4 sequences of full genome; and 31 sequences of DENV-4 (E protein) and 4 sequences of full genome. The phylogenetic reconstruction of serotypes 1, 2 and 4 show that the samples identified in this study grouped with genotypes that circulating in Brazil (genotypes V, Asian American and American, for types 1, 2 and 4 respectively). We have identified two lineages of DENV 1 circulating in the region ad the others serotypes showed no lineages divergences. Some E protein aa substitutions were detected between the two strains of DENV-1. This data shows that the phylodinamics of dengue circulation can be much more complex than expected even in a medium size city and reinforces the importance of DENV constant surveillance.

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COMPARISON OF DENGUE TRANSMISSION INTENSITY ESTIMATES OBTAINED FROM DIFFERENT DATA TYPES

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With more than 2.5 billion individuals at risk and an estimated 390 million infections per year globally, the vector-borne viral infection dengue continues to be a major public health problem. Prior knowledge of local dengue transmission is essential to design effective vector control and vaccination strategies. Yet estimates of global distribution and transmission intensity remain highly ambiguous. Most of the models estimating dengue transmission risk have utilised notification data which depend heavily on the quality of the surveillance system. Furthermore since the majority of dengue infections are asymptomatic, assessing the true burden of dengue is difficult. We estimated the force of infection (λ) by fitting a catalytic model to age-stratified seroprevalence IgG data from 7 countries using a Metropolis-Hastings Markov Chain Monte Carlo algorithm. We also estimated λ and the probability of detection of sequential infections by fitting a catalytic model to case-notification data from Puerto Rico. We find that the estimates of the force of infection vary substantially depending on the type of data fitted, with seroprevalence data resulting in higher estimates of λ on average, and that reporting rates and sequential infections play a major role in how dengue burden is currently assessed. Although IgG data are unable to differentiate between the dengue serotypes, they can identify past asymptomatic infections, are easy and cheap to obtain and can provide an insight into local dengue dynamics. On the other hand, case notification time series data, although subject to reporting bias, is more readily available. Combining the two data sources allows a more robust estimation of dengue transmission intensity and helps to identify areas where clinical dengue surveillance may require improvement.

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ESTIMATING DENGUE INTER-SEROTYPE INTERACTIONS FROM AGE-STRATIFIED SEROTYPE SPECIFIC DATA

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With an estimate of 390 million infections per year globally, dengue continues to be a major public health problem. While the majority of infections are asymptomatic, the presence of multiple serotypes circulating simultaneously in a population increases the risks of severe infection due to antibody-dependent enhancement (ADE). Although costly compared to IgG ELISAs, plaque reduction neutralisation tests (PRNTs) can determine the infecting serotype of past infections, are cheaper than PCR and are considered the gold standard for routine serotyping. The serotype with the highest titre is regarded as the most recent infecting serotype. However due to the cross-reactivity of anti-dengue antibodies it is difficult to definitively determine primary and secondary infecting serotypes from PRNTs alone. Thus it is essential not only to identify populations previously exposed to dengue and thus at risk of severe dengue upon heterologous infection, but to estimate the degree of immune-mediated inter-serotype interactions. We developed a catalytic model to estimate serotype-specific forces of infection (λ) and the level of interaction between serotypes (susceptibility enhancement-inhibition following a primary infection) from cross-sectional PRNT data. The model was fitted to multiple PRNT datasets from 1985 - 2010 using a Metropolis-Hastings MCMC algorithm. We find that if one serotype has a significantly higher λ than the others, the basic reproduction numbers are driven by the dominant serotype. We can infer the probable order of sequential heterologous infections by comparison of the interaction parameters assuming interaction was dependent solely on the primary or secondary infection strain. All estimates were comparable to previously published values of R0 estimated from incidence data. Our models provide an insight into dengue transmission intensity in different countries at different time points and highlight the complex transmission dynamics of dengue.

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METHOD DEVELOPMENT AND CHARACTERIZATION OF *IN VITRO* RELATIVE POTENCY (IVRP) ASSAY FOR A TETRAVALENT RECOMBINANT SUBUNIT ENVELOPE-BASED DENGUE VACCINE CANDIDATE

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Dengue is a widespread and potentially fatal disease, with 50 - 100 million infections per year, and over 2.5 billion persons at risk globally. Dengue has four serotypes and a potential vaccine should elicit strong and balanced neutralizing responses to each type to reduce the risk of dengue hemorrhagic fever. Dengue envelope glycoprotein has been the focus of subunit-vaccine development as it is the primary target of neutralizing antibodies. We have developed a tetravalent (DEN1, 2, 3, 4) recombinant subunit envelope (E) protein based Dengue vaccine candidate. An *in vitro* relative potency (IVRP) assay has been developed as an alternative to the *in vivo* immunogenicity assay to support product release and characterization. In this method, Dengue antigens are detected by neutralizing monoclonal antibodies (mAbs) specific for E protein of each serotype. The mAbs for IVRP were selected by neutralization activity, binding affinity and kinetics, and stability-indicating attributes. These mAbs were further characterized by other methods including Biacore and Western blot. The IVRP assay uses a sandwich ELISA format, and measures relative potency of the Dengue

antigen sample as compared to a reference standard. The IVRP method is highly specific for each serotype, has a precision of 10-20% RSD, and can be completed in 2-3 days. The relationship between antigenicity measured by the IVRP and immunogenicity measured by the mouse potency assay was evaluated by testing several heat stressed Dengue antigen samples in both methods. The results showed that stressed samples had decreased antigenicity by IVRP and decreased immunogenicity by mouse potency assay relative to untreated control samples. This study also suggests that the IVRP assay is stability-indicating. With good specificity, precision, stability-indicating attributes, and quick turnaround, the IVRP assay is suitable for use as potency release and stability assay for subunit based Dengue vaccine candidates. Furthermore, this method may serve as a good alternative to the variable and time-consuming mouse potency assay for vaccine product release for licensure in the future.

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MOSQUITO SALIVA FROM THE DENGUE VECTOR *AEDES AEGYPTI* MODULATES ENDOTHELIAL CELL PERMEABILITY *IN VITRO*: A NEW MECHANISM FOR VIRAL DISSEMINATION?

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Dengue is the most important mosquito-borne viral disease, affecting >40% of the world's population, especially in tropical and sub-tropical areas where the main mosquito vector, *Aedes aegypti*, circulates. Infection with any of the four dengue virus serotypes (DENV1-4) causes a wide spectrum of illness from dengue fever to severe, potentially fatal disease mainly associated with plasma leakage, hemorrhage and organ failure. The infection initiates when a DENV-infected female *Aedes* mosquito probing for blood injects saliva, together with virus, into the skin of its human host. Mosquito saliva is known to enhance replication and pathogenesis of many arthropod-borne pathogens. In DENV infection, *Ae. aegypti* saliva has been shown to both hinder infection of dendritic cells *in vitro* and increase DENV titers in infected mice. However, the early events after DENV inoculation into the skin and the role of mosquito saliva in regulating virus dissemination is not well understood. Here we examine the ability of salivary gland extract (SGE) from female *Ae. aegypti* to modulate endothelial cell permeability *in vitro*. Briefly, cultures of human pulmonary microvascular endothelial cells (HPMEC) grown on a transwell permeable membrane system as a model of endothelial barrier function *in vitro* were exposed to SGE (1 SGE unit=5 µl) through either the apical (AP) or basolateral (BL) membrane, and endothelial permeability was examined by continuously measuring the trans-endothelial electrical resistance (TEER). A sharp reduction in TEER after treatment with 2 SGE units was detected with both AP and BL treatment (60%, $p<0.001$ and 30%, $p<0.05$ reduction, respectively). The AP effect was greater after the second dose of SGE compared to BL (TEER ratio: 0.35-0.66), while the BL effect persisted for a longer time (up to 48 hours). These results suggest distinct mechanisms for modulating endothelial permeability by SGE. A complex mixture of anti-hemostatic, anti-inflammatory, and immunomodulatory compounds has been identified in saliva of blood-sucking arthropods. Experiments to determine the effect of SGE on the intercellular junctions by confocal microscopy and to identify the mechanism(s) conditioning the increased endothelial permeability *in vitro* are ongoing. These findings may suggest a new biological mechanism associated with modulation of endothelial permeability that may facilitate systemic viral dissemination.

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THE ROLE OF UBIQUITIN-PROTEASOME PATHWAY IN DENGUE VIRUS EGRESS: LEARNING FROM THE *AEDES AEGYPTI* MIDGUT

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The mosquito-borne dengue virus (DENV) is a cause of significant global health burden. In the mosquito vector, the virus first establishes infection in the midgut before systemic spread to the organs to cause persistent infection. In the midgut, however, infectious DENV titers reaches a peak approximately 8 days after an infectious blood meal but decreases thereafter despite continual viral RNA replication. The mechanism that decouples viral RNA replication and infectious particle production in the midgut may constitute a novel innate immune response employed by *Aedes* mosquitoes against DENV. Using RNA inference studies, we show *in vivo* that down-regulation of ubiquitin proteasome pathway (UPP)-related genes, including proteasomal subunits, ß2 and ß5 decouples RNA replication from infectious titer production. Mechanistically, inhibition of proteasomal function prevented virus egress but not DENV assembly by exacerbating endoplasmic reticulum (ER) stress within the unfolded protein response. The translational attenuation due to increased ER stress led to reduced protein levels of Exoc7, an exocyst complex component required for exocytosis. Our study thus identified a mechanism that contributes to the mosquito midgut barrier. This mechanism also appears to be amenable for clinical translation as inhibition of UPP in primary monocytes with the licensed proteasome inhibitor, bortezomib, inhibited DENV titers even at low nanomolar drug concentration. In conclusion, we suggest here that functional UPP is necessary for virus egress through exocytosis and the inhibition of which could be an effective therapeutic strategy.

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ALARM SIGNALS FOR DENGUE OUTBREAKS: A MULTI-CENTER STUDY IN ASIA AND LATIN AMERICA

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Dengue outbreaks threaten both endemic countries and those with low or no stable transmission. Outbreaks are detected late, and response is often inadequate; early detection of outbreaks is challenging, since no established set of indicators exist for defining an outbreak. Candidate alert signals have been defined, but a systematic literature review found no satisfactory analyses or validations of these indicators and our expert meetings in 2012 and 2013 found that no country uses dengue outbreak alerts routinely for an early response. Within the context of a WHO/TDR-led Research Work Package, part of the European Union funded IDAMS research consortium (International Research Consortium on Dengue Risk Assessment, Management and Surveillance; www.idams.eu), a model dengue outbreak contingency plan based on the available evidence collected through country case studies and literature reviews, is being developed. The aim is to test the validity of alarm signals for dengue outbreaks through a retrospective study in 5 countries (Brazil, Mexico, Dominican Republic, Vietnam and Malaysia), including a large number of districts and 5-10 years of observation. The following candidate alerts are being explored for their ability to predict dengue outbreaks with a defined probability: increased number of dengue cases compared to previous years; entomological indices; levels of sero-positivity in blood samples;

changes in predominant serotype, age shift; climate data (temperature, rainfall and relative humidity). For each covariate, the Shewart Method is used to quantify correlations between outbreak 'alarms' with known dengue outbreaks. Information about the ongoing analytical process and the results to date will be presented and discussed.

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USE OF LOCAL OCEANOGRAPHIC INFORMATION TO PREDICT DENGUE IN ECUADOR

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Dengue fever, a mosquito-borne viral disease, has increased in distribution, prevalence and severity throughout the tropics and subtropics. In this study, we explore the use of local oceanographic variables to predict dengue transmission in the southern coastal of Ecuador. We hypothesize that local oceanographic conditions, such as invasion of warmer water masses from the Panamá Bay and strengthening of the cold Humboldt Current, interact with the El Niño-Southern Oscillation (ENSO) to influence local climate and dengue transmission. We developed a statistical mixed effects model to evaluate the influence of local oceanographic variables (weekly sea surface temperature (SST) and sea subsurface temperature in front of Puerto Bolívar and other coastal localities), ENSO, and local climate on the dengue standardized morbidity ratio from the city of Machala, El Oro Province (weekly SMR, 2003-2013). We found that SST at Puerto Bolivar, El Oro Province, was positively associated with dengue (3-month lag). We also found that the Coastal El Niño index, which was developed in this study using the SST of El Niño 1+2 region, was positively associated with dengue (6-month lag). These findings indicate that local oceanographic variables provide some predictive lead for forecasting dengue outbreaks, and consequently, the potential to be integrated into operative tools of epidemic management, such as climate-driven early warning systems.

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CLINICAL AND HEMATOLOGICAL PARAMETERS THAT DIFFERENTIATE DENGUE VERSUS OTHER FEBRILE ILLNESSES IN VENEZUELA

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Dengue is the most important vector-borne viral disease worldwide. Dengue can be asymptomatic or present a wide range of manifestations from mild dengue fever to more severe disease. To date, there are no vaccines or antiviral treatments for dengue. At the early stage of the disease, general signs and symptoms of dengue can be confused with other febrile illness (OFI) and a late dengue diagnosis can be fatal. Dengue in Maracay, Venezuela, is hyperendemic with co-circulation of the 4 serotypes. In this setting, a longitudinal observational study was set up in 2010 to identify clinical and laboratory parameters that could differentiate patients with dengue versus OFI. Patients presenting with fever and dengue clinical criteria were recruited from 3 designated health centers. Dengue infection was confirmed by IgM ELISA and/or RT-PCR. Patients were followed daily with clinical examination and sequential blood sampling at determined intervals up to 30 days. Severe cases were treated in a tertiary hospital and followed daily until discharge. Hematological parameters and serum levels of selected biochemical markers were determined in acute phase blood samples. Between August 2010 and August 2013, 254 individuals met the inclusion criteria of which 44% were positive for dengue, 31% of positive patients developed alarm signs, while only 6% developed severe dengue. All four serotypes were detected in patients, with DENV-3 predominating. Using logistic regression and mixed effect models clinical, hematological and biochemical parameters where analyzed comparing the first 3 days with days 4-7 after fever onset. Rash, hemorrhagic manifestations including a positive tourniquet test, a concomitant decrease in platelets (~10,000 platelets/day) and white blood cell count and the absence of sore throat were associated with dengue infection in the first 3 days of the disease. A higher proportion of patients with hemorrhagic manifestations were present in days 4-7 after fever onset. A final model of determinants will be presented.

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GLOBAL ECONOMIC COST OF DENGUE CASES TREATED IN THE MEDICAL SYSTEM

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As the most important vector-borne viral disease, dengue is a serious and growing global public health problem, with 2.5 billion (b) people at risk. To estimate the global cost of symptomatic dengue cases treated in the professional health-care system, we reviewed the literature for numbers and cost of dengue cases by country. Using Bhatt et al. (2013) and Brady et al. (2012), we identified 134 countries with active dengue transmission. Shepard et al (2010 and 2013), supplemented by recent field data from India, Mexico, and Philippines, provided the number of dengue cases in the Americas and Southeast Asia. We adjusted the WHO reported dengue cases for South Asia using an expansion factor estimated for India (282). For the rest of the world, we multiplied the number of apparent dengue infections from Bhatt et al. (2013) by 16.25%--the ratio of cases in medical settings to total cases projected in Southeast Asia by Bhatt et al. (2.92 million (m)/17.96 m). To estimate the economic cost of dengue illness (both direct and indirect), we reviewed the literature for 5 studies reporting overall economic cost of dengue complemented by recent field studies from 3 countries. Collectively, these provided the per capita cost of dengue illness for 58 countries. We estimated the log of this per capita cost as a function of log of GDP per capita and log of dengue incidence per 100,000 population ($p<.0001$, $R2=0.66$) and calculated estimated values for the remaining 76 countries. Bootstrapping the selection of 58 countries used with replacement generated a range of estimates. Multiplying the cost per capita times the population gave the aggregate cost of dengue by country, region, and globally. Preliminary results show a total of 30 m locally transmitted symptomatic dengue cases annually treated in the medical system in 134 countries with transmission. The estimated annual global aggregate cost of dengue illness is $8.65 b (range: $3.98-$8.80 b) or $1.58 (range: $0.73-$1.61) per capita. Of this, $3.77 b (44%) was incurred in 8 countries in South Asia; $1.95 b (23%) in 28 countries in East Asia and the Pacific, $2.47 b (29%) in 44 countries in Latin America and the Caribbean; $0.31 b (4%) in 42 countries in Sub-Saharan Africa, $0.14 b (2%) in 6 countries in the Middle East and North Africa, $5.6 m (0.06%) in 4 countries in Europe and Central Asia; and $3.4 m (0.04%) in North America. If control strategies could reduce dengue appreciably, billions of dollars would be saved globally.

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VERTICAL TRANSMISSION OF YELLOW FEVER TO INFANTS VIA BREASTFEEDING

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Before the development of a vaccine in 1937, Yellow Fever was one of the most feared and lethal diseases in human history. The live, attenuated Yellow Fever vaccine provides lifelong immunity and is safe for those with a developed immune system. Breastfeeding mothers, however, should not receive the vaccine as it may cause Yellow Fever in infants. Six cases of Yellow Fever transmitted via breastfeeding have been reported. These cases occurred when Yellow Fever vaccine was administered to breastfeeding mothers with infants from 10 to 15 days old. Serum and cerebrospinal fluid were positive for the Yellow Fever virus, and nucleotide sequences confirmed identical viral profiles between vaccine and cases. Such cases suggest acute central nervous system infection with Yellow Fever through breastfeeding. Further research is needed to evaluate more fully the risk of vertical transmission of such viruses via this route. Although vertical transmission of Dengue Fever and West Nile has been documented previously, little information exists regarding transmission of Yellow Fever via breast milk.

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CHARACTERIZATION OF A MUTATION IN THE YELLOW FEVER VIRUS E LINKER REGION IN *AEDES AEGYPTI* MOSQUITOES

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The linker region of the flavivirus envelope (E) protein connects the C-terminus of the domain I and the N-terminus of the domain III and was first hypothesized to accommodate the mechanical forces resulting from structural rearrangement that occur during viral membrane fusion. Available evidence further demonstrated the importance of the linker region in viral particle assembly and secretion of progeny virions. These conclusions were derived from *in vitro* models since all the mutations in the linker region of DENV-2 E protein are lethal and prevent production of infectious virions, thereby precluding *in vivo* studies. We evaluated the phenotypic effect of a methionine to isoleucine mutation generated at position 299 in the yellow fever virus (YFV) E gene that occurred during serial passage of the wild-type Asibi strain to derive the attenuated 17D vaccine virus. Using the YFV reverse genetics system and *Aedes aegypti*, we characterized the infection, replication and dissemination phenotypes of the mutant and parental viruses in mosquitoes. Mosquitoes were orally infected with virus produced from various YFV infectious clones: Asibi, 17D, Asibi E M299I, and a 17D/Asibi M-E chimera with and without the M299I mutation and sampled at 7, 10, and 14 days post infection to evaluate replication kinetics and assess infection and dissemination phenotypes. The phenotypes of the 17D strain, the Asibi strain and the 17D/Asibi M-E chimera were similar to the phenotypes observed in our previous reports. The results of the characterization of the M299I mutation will be discussed.

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MERCADEO VIRUS: A NOVEL INSECT-SPECIFIC-FLAVIVIRUS

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The genus *Flavivirus* (*Flaviridae, Flavivirus*) is a heterogeneous group of viruses; some of them are important arthropod-borne-virus for both human and veterinary medicine. Recently an explosion of insect-specific-flavivirus has been documented**.** Although, many flaviviruses have been isolated from humans, field-collected-mosquitoes and birds in Panama, insect-specific flavivirus has never been detected in this Central American country. During 2011, mosquitoes were collected in Darien province close to the border of Colombia, where an outbreak of alphaviral encephalitis in both human and equines was reported in 2010. A number of virus isolates were obtained in C6/36 mosquito cell culture from specific pools of *Culex Melanoconion spp.* and *Cx. Culex spp*. Structural and antigenic characterization placed these viruses in the flavivirus group and their complete genome sequence was obtained using Illummina next generation sequencing. Phylogenetic analysis based on the ORF of two isolates shows that our isolates grouped within the insect-specific clade of flaviviruses, closely related to cell fusing agent and Nakiwogo virus that were isolate in Uganda. Based on the location of the mosquito collection site, we named this virus strains as "Mercadeo virus". Here, we report the discovery and characterization of an insect-specific flavivirus in Panama. Our results highlight the importance of combined viral isolation, structural, antigenic and genetic characterization for virus discovery and taxonomic designation.

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HEAT SHOCK COGNATE PROTEIN 70 ISOFORM D IS REQUIRED FOR CLATHRIN-DEPENDENT ENDOCYTOSIS OF JAPANESE ENCEPHALITIS VIRUS IN C6/36 CELLS

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Japanese encephalitis virus (JEV) generally enters host cells via receptor-mediated clathrin-dependent endocytosis that requires the involvement of the 70 kDa heat shock protein (Hsp70). Hsc70 is one member of the Hsp70 family and is mainly constitutive; thus, it may be expressed in the absence of stress. In C6/36 cells, Hsc70 is up-regulated in response to JEV infection. Since Hsc70 shows no relationship with viruses attaching to the cell surface, it probably does not serve as the receptor although it has been reported to exist on the cell face. In contrast, Hsc70 is evidently involved in virus penetration and the resultant acidification of intracellular vesicles, suggesting that it is highly involved in clathrin-mediated endocytosis. The effects of Hsc70 occur particularly at a late stage of viral entry into host cells. Furthermore, we found that Hsc70 is composed of at least three isoforms, with isoform D being the one that helps JEV penetrate C6/36 cells via clathrin-mediated endocytosis. This study provides relevant evidence that sheds light on the regulatory mechanisms of JEV infection in host cells.

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ANALYSIS OF SEROLOGICAL SURVEYS FOR YELLOW FEVER IN AFRICA

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Yellow fever can be a devastating disease that occurs both in large epidemics and in sporadic endemic cases across large parts of Africa and Latin America, with over 90% of the global burden being attributed to Africa. However, assessing the extent of yellow fever virus circulation in the endemic countries in Africa is very difficult due to under recognition of both asymptomatic infections and mild cases with non-specific febrile illness that occur in the majority of persons, and under reporting of severe cases who develop jaundice or haemorrhage . We reviewed the published and unpublished available literature for reliable serological surveys for yellow fever in Africa and identified six suitable studies published since 1980 from five African countries including Nigeria, Cameroon, the Central African Republic, the Democratic Republic of the Congo and the Congo. We analysed the age-dependent sero-prevalence of YF neutralizing antibodies to estimate the annual risk of infection to a sero-naive person that would give rise to the observed seroprevalence profiles and obtained estimates between 0.25% and 2.2% in the five countries; these estimates are similar to those previously established from Nigeria during the 1970s. Using this annual risk of infection, we estimate that there are a total of 180,000 (95% CI 54,000 to 440,000) severe cases annually in these five countries, around 52% of which occur in Nigeria and 43% in the DRC. These case estimates indicate a disease burden several orders of magnitude higher than official case notifications to WHO. This illustrates the challenges of achieving highly sensitive syndromic disease surveillance systems and highlights the utility of systematically collected serological data to assess the true extent of virus circulation. Well-designed serological studies are an indispensable component in evaluating the true extent of the yellow fever disease burden. Indeed, the WHO and partners are currently conducting serological studies in YF endemic countries to assess transmission intensity and inform control strategies.

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INFERRING THE YELLOW FEVER FORCE OF INFECTION FROM THE OBSERVED AGE DISTRIBUTION OF CONFIRMED CASES

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Yellow fever is an arboviral disease, transmitted between humans and non-human primates and mosquitoes of various *Aedes* spp. in Africa. Disease severity shows a broad spectrum with high rates of fatality amongst the more severe cases, but symptoms tend to be non-specific, particularly in milder cases leading to huge underreporting and highly uncertain disease burden. As yellow fever is a sterilising infection, the age distribution of observed cases can give clues to the yellow fever transmission intensity. Using data on confirmed human cases of yellow fever reported to the Yellow Fever Surveillance Database covering 20 countries in western and central Africa in conjunction with population demographics and age-dependent vaccination coverage we estimate the force of infection that could give rise to the observed age distribution of cases, using a number of different assumptions regarding the age-dependence of the exposure. Point estimates of the force of infection are low, but comparison with estimates derived from other data suggest upper confidence bounds obtained might put useful bounds on the extent of transmission. Results are sensitive to the age distribution of the population and vaccination coverage and strongly depend on the age-dependence of exposure assumed, with models assuming age-independent force of infection fitting better in western Africa, but a higher exposure in adults than children fitting better in central Africa. In western Africa, the annual risk of infection to a susceptible person was estimated to be below 2.3%, while the annual infection risk in central Africa was estimated to be below 0.5% for children and 1.2% for adults. The fact that in different areas different assumptions about the age-dependence of the infection risk gave the best fit to the data could be interpreted as indiscriminate exposure in villages in western Africa, where the transmission intensity is thought to be highest, but more limited occupational exposure through activities such as wood clearing in central Africa with a lower overall transmission intensity.

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ROLE OF ENDOGENOUS FLAVIVIRAL GENETIC ELEMENTS IN A VECTOR MOSQUITO

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Remnants of ancestral flaviviral infections can be detected throughout the genomes of *Aedes albopictus* and *Ae. aegypti*, both major disease vectors throughout the world. Although flaviviral genetic elements in *Ae. albopictus* are riddled with stop codons, NCBI BLAST analysis of complete flaviviral polyproteins against the *Ae. aegypti* genome identified two genes (AAEL007866 and AAEL017001) that corresponded to flavivirus NS1 and NS5 proteins. We call these proteins *Aedes aegypti* NS1 (AeNS1) and NS5 (AeNS5). AeNS1 and AeNS5 are most related to the insect only flavivirus Kamiti River virus (KRV). Genetic alignment against wild-type NS1 and NS5 proteins revealed that AeNS1 and AeNS5 are not complete proteins. AeNS1 is missing a conserved tip of unknown function, and AeNS5 retains only the RNA dependent RNA polymerase finger domain, which is thought to interact with RNA templates. The MTase domain, catalytic site, and the priming loop are missing. We hypothesized that AeNS1 and AeNS5 are dominant negative proteins that were co-opted by *Ae. aegypti* to interfere with flavivirus replication. Accordingly, overexpression of AeNS5 inhibited KRV replication. Interestingly, AeNS5 overexpression had no effect on DENV2 replication. We hypothesize that this is due to the large evolutionary gap between insect only and pathogenic flaviviruses. These studies highlight the role of viruses in the evolution of eukaryotic organisms, and implicate AeNS5 as an evolution-guided template for the design of dominant negative proteins that target major viral pathogens.

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IDENTIFICATION OF THE FIRST CASE OF IMPORTED ZIKA FEVER TO THE UK: A NOVEL SAMPLE TYPE FOR DIAGNOSTIC PURPOSES AND SUPPORT FOR A POTENTIAL NON-VECTOR-BORNE ROUTE OF TRANSMISSION

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Zika virus (ZIKV) is a flavivirus related to Dengue, is transmitted by the *Aedes* mosquito and normally causes a self-limiting illness characterised by fever, rash, headache, conjunctival suffusion, myalgia and joint pains. It was discovered in 1947 at Zika Forest, near to Entebbe, Uganda. More recently it has been found outside Africa in outbreaks such as those of French Polynesia and the Cook Islands. We report the first case of imported Zika Fever to the UK, highlight novel samples for testing and a theoretical mode of non-vector-borne transmission. A couple travelled to the Cook Islands during what was thought to be a Dengue outbreak in February 2014. Within 6 days of exposure, both 'Patient 1' and his wife, 'Patient 2', had developed fatigue, followed 48 hours later by fever, headache, aching joints and a widespread maculopapular rash. Both reported symptoms resolving by day 5 of rash onset. In 2011, Foy et al described likely sexual transmission of ZIKV. A scientist from Colorado, travelling back from Senegal whilst incubating the virus, reportedly passed it to his wife, in whom clinical and serological evidence supported the diagnosis. On day 1 of rash onset, Patient 1 had serum sent to the Rare

and Imported Pathogens Laboratory at Porton Down and a panel of serological tests based on stated travel was performed. Dengue testing revealed positive IgM, but negative IgG and PCR. This pattern has been reported previously due to cross-reactivity between the flaviviruses. The sample was then tested by PCR for ZIKV and found to be positive. Further samples were taken on day 28 after rash onset. Blood and urine from both patients were tested in parallel, along with a semen sample from Patient 1. The semen was the only sample found to be positive for ZIKV by PCR. The reasons for persistence in the semen are not yet clear, but this case is significant not only as the first case of Zika Fever imported to the UK, but also as support for the possibility of sexual transmission, though not in this case, and highlights an additional sample type for the confirmation of infection in the future.

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IXODES SCAPULARIS SALIVA ENHANCES *POWASSAN* VIRUS TRANSMISSION TO THE HOST, INFLUENCING ITS DISSEMINATION AND THE COURSE OF DISEASE

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Powassan virus (POWV) is transmitted to humans by infected ticks, and successful transmission of POWV can occur within one hour of *Ixodes scapularis* attachment. Successful tick feeding is facilitated by a collection of pharmacologically active factors/proteins in tick saliva which are secreted into the feeding pool that the tick creates on the vertebrate host. The skin serves as the interface of host-virus-tick interactions; therefore, we sought to understand how tick saliva immunomodulates the tick-host interface and whether it facilitates POWV transmission and dissemination. To achieve this objective, groups of naïve BALB/c mice were infected intradermally in the hind footpad with either POWV alone or with POWV plus *Ixodes scapularis* salivary gland extract (SGE). A third group consisted of mice injected with media only. Mice from each group were sacrificed at 1, 3, 5, 7, and 8 days post infection (dpi). Organs were harvested from each sacrificed mouse and viral titers were determined by detecting POWV using quantitative real-time PCR. Starting at 3 dpi, mice infected with POWV+SGE had viremias that were at least 0.5Log greater than the viremias detected in the mice receiving POWV only. At 3 and 5 dpi the viremias of the POWV+SGE group were statistically significantly higher than the POWV only group. A similar pattern of higher viral loads in the POWV+SGE group was detected in the popliteal lymph nodes; however, there was no significant difference between groups on any of the days. POWV was not detected in the brains of mice until 5 dpi. From 5 dpi until experiment completion, the POWV+SGE infected mice had higher titers than the POWV only group, with 5 dpi being statistically significant. The onset of clinical symptoms occurred one day earlier (4 dpi) in the POWV+SGE group. Overall, this data indicates that *I. scapularis* SGE enhances POWV transmission to the host and influences the course of disease. To further examine the effect that *I. scapularis* saliva in the presence of POWV has on the host's immune response, we fed POWV-infected and uninfected *I. scapularis* nymphs on naïve mice for 3 or 6 hours. 5-um sections were taken near the mouthparts of each feeding tick and infiltrating immune cells and POWV-infected cells at the tick bite site were identified. The location, timing, identity, and quantity of immune cells determined via histology enabled us to define the quality and kinetics of the host response to tick-borne POWV infection.

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OUTBREAK OF YELLOW FEVER VIRUS DISEASE IN UGANDA IN 2010-2011: WERE OTHER INFECTIONS INVOLVED IN THE OUTBREAK?

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An outbreak of yellow fever in northern Uganda was confirmed on 23 December 2010 after samples tested at the US Centers for Disease Control and Prevention (CDC) were positive for Yellow Fever Virus (YFV). Uganda is endemic for YFV however nearly 40 years had passed since the previous recorded transmission of YF in Uganda. The first documented outbreak in the country was in 1939-41. Single cases of yellow fever disease were documented in 1952, 1959, 1964 and 1971. Cases of illness of unknown cause had been reported since October 2010 from twelve districts in northern Uganda. These cases mainly concerned males aged 20 to 34 years mostly presenting with severe frontal headache, fever, lethargy, abdominal pain, diarrhoea and vomiting, as well as with hemorrhagic signs. Blood and tissues samples from patients were sent to the U.S. CDC Special Pathogens laboratories in Atlanta, Georgia. Yellow fever virus was identified as the culprit. Then the CDC laboratory in Ft. Collins, Colorado, confirmed the finding. Overall, 181 cases met the YF suspected case definition; there were 45 deaths (case fatality rate 24.9%). Molecular sequencing revealed 92% homology to the YF virus strain Couma (Ethiopia), East African genotype. Suspected YF cases had fever (100%) and unexplained bleeding (97.8%), but jaundice was rare (11.6%). Over 200 blood samples were tested by RT-PCR and by IgM ELISA, including many samples from people who met the suspected case definition. However, only a total of 13 cases were laboratory confirmed from five districts. It was during a dry spell and the limited number of mosquitoes collected were not positive for YFV. Although this is acknowledged as the largest YF outbreak ever reported in Uganda. It remains unclear if all of the cases of disease were caused by YFV given that only a small number of suspected cases were laboratory confirmed to be YFV infections; and they were randomly scattered in five districts. There could have been other flaviviruses which probably were not tested for.

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MOSQUITO SALIVA-MEDIATED ENHANCEMENT OF YELLOW FEVER VIRUS INFECTION

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Mosquito-borne viruses like those in the genus Flavivirus must interact with both the mosquito and mammalian host in order to complete its life cycle and, to this end, have evolved complex strategies to replicate and disseminate within these different environments. Dengue and West Nile viruses take advantage of the complex mix of proteins in mosquito saliva in order to enhance infectivity in the mammalian host following the bite of an infected mosquito. Our recent work indicates that a non-catalytic serine protease is responsible for mosquito saliva-mediated enhancement of infectivity of these viruses and can be abrogated by the addition of a serine protease inhibitor or specific knockdown of the protein. We now report that in a murine model of the closely related yellow fever virus (YFV), early infection is enhanced in the presence of mosquito salivary gland extract (SGE) by as much as 5,000-fold both at the subcutaneous site of infection and in visceral tissues. However, despite this early enhancement, mice infected in the presence or absence of SGE, develop similar peak virus load and manifestation of clinical disease. Surprisingly, animals infected in the presence of SGE were more likely to survive infection, suggesting the development of a more effective or less damaging adaptive

immune response. We are currently examining the specific role that SGE-enhancement of infection plays in the development of cell-mediated and humoral immunity toward YFV.

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YELLOW FEVER RE-EMERGENCE AFTER FIFTY YEARS, ETHIOPIA, 2013

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In Ethiopia from 1960-1962 there was an outbreak of 100,000 cases of yellow fever (YF), which caused 30,000 deaths. Since 1962, however, no cases of yellow fever had been reported in Ethiopia. In May 2013 we received reports of a suspected outbreak of yellow fever from South Omo Zone situated within the Rift Valley in southern Ethiopia. We investigated to confirm the outbreak, identify risk factors and institute control measures. We used a clinical case definition to identify suspected YF cases and conducted a medical record review at all hospitals and health facilities in the Zone. Laboratory confirmed cases were confirmed by IgM serology at the Institut Pasteur in Dakar. We also conducted an age-matched case control study that included 30 suspected cases and 60 controls, who were neighbours of suspected cases without symptoms of disease. We identified 6 laboratory confirmed and 124 suspected YF cases from 28 November 2012 to 6 June 2013 from four rural districts of South Omo Zone. There were 53 deaths (case fatality rate of 41%). The overall attack rate was 30/100,000, and the highest attack rate (45/100,000) was from South Ari District. The median age of the 130 cases was 27 years, and 73 (56%) were males. Significant risk factors associated with illness included attending a mass gathering (OR=4.0; 95% CI=1.3-6.2) and presence of a person with YF in the village (OR=3.7; 95% CI=1.5-9.4). Working in a forest was a non-significant risk factor (OR=0.92; 95%CI=0.4-2.4). We confirmed the first outbreak of yellow fever in Ethiopia in 50 years. The outbreak was contained with a mass vaccination campaign of more than 500,000 people. We recommended heightened surveillance for hemorrhagic illness throughout Ethiopia.

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TAU ABNORMALITIES, INFLAMMATION AND AXONAL DAMAGE IN MURINE CEREBRAL MALARIA

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Cerebral malaria (CM) is a potentially fatal complication of disease associated with *Plasmodium falciparum* infection. Despite complete clearance of the parasite with anti-malarial treatment, approximately 20% of CM survivors develop long-term neurological deficits; however, the mechanisms that mediate this are not well understood. Neuronal injury has been linked to neurocognitive impairment in several neurodegenerative diseases and may contribute to the deficits seen in CM. In this regard, damage to neuronal axons has been observed in both human and murine experimental CM (ECM). Furthermore, improper regulation of tau, an axonal protein important for microtubule stability and cytoskeletal organization, has been demonstrated in mouse and human disease. We hypothesized that the neuronal injury observed in ECM results, in part, from abnormalities in tau. Improper regulation of tau results in an increase in its phosphorylated levels. We quantified protein levels of two forms of phosphorylated tau known to be pathological in Alzheimer's disease (Ser396/404; Ser202) in several brain regions of mice with ECM and compared our findings with uninfected mice and mice infected with a less neurotropic malarial strain. In the same regions, we also quantified the level of SMI 32, a marker of axonal damage. Phosphorylated tau and SMI 32 were elevated throughout the brains of mice with neurological disease. Treatment of ECM mice with the immunotherapeutic PHF-1 antibody, which clears phosphorylated tau in mouse models of Alzheimer's disease, prevented axonal damage in certain brain regions, suggesting that this protein is contributing to the neuronal injury in ECM. Abnormal tau regulation has previously been linked to dysregulated inflammation, a common feature of CM. We hypothesized that the aberrant tau phosphorylation in ECM is associated with the hyper-inflammation which typically occurs. mRNA levels of several inflammatory cytokines in the brains of our infected mice were found to be consistently elevated during neurological disease. The increases in these cytokines may contribute to the atypical tau regulation in ECM. Our goal is to further establish abnormal tau as a hallmark of CM. This protein may prove to be a viable target to ameliorate both the neuronal damage and subsequent neurocognitive impairment which occur during disease.

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CRYOPRESERVATION OF *PLASMODIUM VIVAX* SPOROZOITES

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Plasmodium vivax represents a great challenge to malaria control because of the ability to relapse of dormant form known as hypnozoite in the liver. Research efforts to understand hypnozoite biology are limited due to the availability of *P. vivax* sporozoites. Mahidol Vivax Research Unit (MVRU) is one of a few centers in the world that has facilities to produce the sporozoites by feeding mosquitoes on blood from *P. vivax*-infected patients. The ability to cryopreserve sporozoites is therefore essential to support liver-stage malaria research community. Hydroxyethyl starch 6% (HESTAR), a currently used cryoprotectant in cryopreservation of *P. vivax* sporozoites at MVRU, retains sporozoite viability up to 30% after thawing. In this study, the protective effect of different combinations of cryoprotectants on sporozoite viability and infectivity was investigated in order to improve the cryopreservation protocol. Sporozoites were harvested from infected mosquitoes and cryopreserved at slow freezing rate of -1°C/min to final temperature of -80°C before plunging in liquid nitrogen. Frozen sporozoites were thawed in a 37°C water bath for a few seconds and viable sporozoites were counted under hemocytometer. Addition of 10% DMSO and 250 mM sucrose into HESTAR solution increased number of viable sporozoites after thawing. The combination of PBS, 0.5% BSA, 250 mM sucrose, and 10% DMSO demonstrated highest sporozoite recovery. However, reduction of sporozoite infectivity was observed in all cryoprotectants tested.

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FOLIC ACID METABOLISM IS DISRUPTED IN A NON HUMAN PRIMATE MALARIA MODEL

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Plasmodium falciparum is a leading cause of severe malaria. Due to the challenge of studying *P. falciparum* pathophysiology in human populations, non-human primate (NHP) models have been developed. As part of an effort by the Malaria Host-Pathogen Interaction Center (MaHPIC), we hypothesized that high-resolution metabolomics (HRM) would further our understanding of the biochemical changes ensuing in the early phase of *P. coatneyi* infection in *Macaca mulatta*. As part of a larger intervention study, we collected serum samples from 4 healthy animals at 2 time points: 15 days before experimental infection (baseline) and 5 days after challenge (early infection). All animals developed malaria after intravenous injection of $4x10^4$ *P. coatneyi* infected erythrocytes/kg obtained from an infected donor monkey. At the early infection blood collection, parasitemias were low and animals did not require antimalarial treatment. Samples underwent randomization followed by HRM. We used xMSanalyzer for data extraction and obtained >20,000 metabolites (unique *m/z* with retention time). Paired analyses for differences due to infection were performed using the Linear Models for Microarray package (LIMMA, R

programming) with adjustment for multiple testing by the Benjamini-Hochberg false discovery rate (FDR) method. 33 metabolites altered during early *P. coatneyi* infection at FDR<0.2 were tested for pathway enrichment using Mummichog. Results showed 2 metabolites in the folic acid pathway (p<0.08): methylene-tetrahydrofolate (THF) and methyl-THF being less abundant in the early infection period. Pathway enrichment analysis with the addition of the top 100 correlated metabolites (33+100 with absolute Spearman correlations between 0.83-0.91 at FDR<0.2) confirmed the enrichment of the folic acid pathway (p<0.005) and pathways related to the metabolism of N-glycans, glycosphingolipids, amino sugars, squalene and pyrimidine (p-values <0.005). This first report of the metabolomic analysis of NHP malaria exposed alterations in the folic acid metabolism and other biochemical pathways. These will serve as a reference for future NHP and human malaria metabolomics studies.

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THE EXPRESSION OF *PLASMODIUM FALCIPARUM* INVASION LIGANDS AND THEIR RECEPTORS IN CLINICAL ISOLATES FROM TIMIKA, PAPUA, INDONESIA

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Plasmodium falciparum invasion is a complex process involving several parasite ligands and their receptors expressed on the red blood cell surface. We reported various receptors used by the parasite ligands during their invasion based on their sensitivity to neuraminidase (N), trypsin (T) or chymotrypsin (C). Sixty nine isolates were subjected to various enzyme treatments including 50 mU/ml neuraminidase, 1 mg/ml trypsin, or 1 mg/ml chymotrypsin. Eight invasion profiles were found in this study showing receptor sensitivity or resistance to those enzymes. Most field isolates in Timika invaded red blood cells through type A receptor that was resistant to all enzyme treatments (NrTrCr; 28,99%) and type B that was sensitive to neuraminidase and trypsin, but resistant to chymotrypsin (NsTsCr; 21,74%). The expression of two invasion ligands; *P. falciparum* Duffy binding ligand (PfDBL) and *P. falciparum* reticulocyte homolog (PfRh) were quantified from the schizonts stage of each isolate. We employed quantitative real-time reverse-transcription polymerase chain reaction (QRT-RT-PCR) to detect the expression of PfDBL family including EBA-140, EBA-175 and EBL-181 and PfRh genes such as Rh-1, Rh-2a, Rh-2b. We demonstrated that EBA-140, Rh-1 and EBA-175 were the major invasion ligands expressed in *P. falciparum* of Timikan isolates. The current study strengthens the support to include these invasion proteins into the malaria vaccine platform. The presence of red cell polymorphisms including the Southeast Asian Ovalocytosis (SAO), Gerbich negativity, and variant hemoglobin (HbE) were not found to affect parasite invasion.

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EFFECTS OF LOW MOLECULAR WEIGHT HEPARIN AND ANTIMALARIAL DRUGS ON ROSETTE FORMATION AND CYTOADHERENCE OF *PLASMODIUM FALCIPARUM*

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The main pathophysiology of severe malaria is due to the mature stage of *Plasmodium falciparum*–infected red cells sequestered in the capillary vessels of the brain and other vital organs known as sequestration. This sequestration is due to cytoadherence, the ligands-receptors interactions and rosette formation which are defined as the adhesion of two or more uninfected red cells to the infected cells. Many studies have shown that the heparin sulfate, which is negatively charged through sulfate groups, was used in the adjunctive treatment of malaria. However, there is no report on the effects of low molecular weight heparin (Sevuparin) on the cytoadherence of *P. falciparum* isolated from patients. We therefore investigated the effects of low molecular weight heparin on rosette formation and cytoadherence of *Plasmodium falciparum i*solates in Thailand. *P. falciparum*-infected blood samples at trophozoite stage was incubated with Sevuparin at 37°C for 30 minutes and an hour before performing the rosetting and cytoadherence assay, respectively. The results shown that Sevuparin (62.5-1000 µg/mL) inhibit rosette formation of *P.falciparum* (N=43) *in vitro*, (P<0.01) in dose dependent manner. The concentration of Sevuparin at 400 µg/mL significantly inhibited cytoadherence (N=24) (P=0.02). This study provides the novel knowledge for understanding the pathophysiology of malaria and improving treatment of malaria patients. The low molecular weight heparin with less anticoagulation activities might be used as adjunctive treatment in severe malaria patients.

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THE IMPACT OF DYSREGULATED ANGIOGENESIS ON BIRTH OUTCOMES IN EXPERIMENTAL PLACENTAL MALARIA

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Each year ~125 million pregnant women are at risk for malaria infection. Placental malaria (PM) due to *Plasmodium falciparum* infection has a profound impact on maternal and child health, including preterm birth and low birth weight (LBW), and results in an estimated 100 000 infant deaths per year. Previous work by our lab showed that PM-mediated LBW outcomes in both human and murine studies are associated with dysregulated angiogenesis including modifying the angiopoietin (Ang)-Tie2, endoglin, and VEGF pathways. Coordinated expression of these angiogenic factors is required for normal placental development during pregnancy. Furthermore, recent studies have implicated complement activation (e.g. C5a) in mediating these changes. Although we uncovered a role for angiogenesis and complement in the pathobiology of PM, the temporal regulation of these factors has not been precisely mapped. Thus, the objective of this study is to compare the kinetics and levels of factors that regulate vasculogenesis and angiogenesis including VEGF, soluble VEGF Receptor-1 (sFlt-1), endoglin, Placental Growth Factor, Ang-1, and Ang-2, required for the normal placental vascular development and gestation. Using the established BALB/c mouse model of PM, we will measure maternal, fetal, and placental levels of the various angiogenic factors throughout gestation by qRT-PCR and ELISA. Further, to establish a causal role for Ang-1 in mediating PM outcomes, we will use heterozygous and conditional Ang-1 knockout mice to determine the impact of Ang-1 deficiency on angiogenesis and fetal growth in an experimental PM model. Finally we will investigate the therapeutic utility of angiogenic factors such as recombinant Ang-1 and determine if adverse birth outcomes in PM can be reversed by interventions to restore normal angiogenesis. Overall, these studies will improve our understanding of the specific roles of the angiogenic factors in normal and malaria-complicated pregnancies and may help identify novel interventions to prevent poor birth outcomes associated with PM.

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DC8-EXPRESSING *PLASMODIUM FALCIPARUM* - INFECTED ERYTHROCYTES BIND EPCR UNDER PHYSIOLOGICAL SHEAR STRESS BUT DO NOT INHIBIT ACTIVATED PROTEIN C GENERATION BY EPCR

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Plasmodium falciparum-infected erythrocytes (IRBCs) expressing the domain cassettes (DC) 8 and 13 of the cytoadherent ligand PfEMP1 bind under static conditions to the endothelial protein C receptor (EPCR). It has been proposed that by interfering with EPCR functions, IRBC adhesion could promote localized coagulation and/or vascular permeabilitycontributing to the pathogenesis of cerebral malaria. Whether EPCR binding occurs under physiological shear stress or has any functional consequences has not been addressed. In this study, we examined the adhesion of the parasite clone IT4var19 expressing DC8 to primary human lung (HLMEC) and dermal (HDMEC) microvascular endothelial cells in a flow chamber assay. In addition, we used CIDRα1.1-coated beads, representing the EPCR-binding domain of DC8, to examine the functional consequence of EPCR binding on HLMEC barrier dysfunction in a transwell assay and activated protein C (APC) generation with an amidolytic APC generation assay. Our results showed that the low expression of EPCR on HLMEC mediated IT4var19 IRBC binding at 1 dyne/cm2. IRBC adhesion was inhibited by the mAb RCR-252 and EPCR mRNA knockdown by 35 and 50% respectively. In comparison, adhesion of IT4var19 to HDMEC, which was 5-6-fold higher than to HLMEC, was completely independent of EPCR. CIDRα1.1-coated beads had no direct effect on endothelial permeability, but inhibited thrombin-induced endothelial barrier dysfunction by 55% in an EPCR-dependent manner. No effect was found for the beads or IT4var19 IRBC on endothelial EPCR-dependent APC generation. Overall, the findings suggest that while EPCR contributes to IT4var19 adhesion to HLMEC under shear stress, its role may vary according to the microvasculature. CIDRα1.1 inhibition of thrombin activity could occur through an EPCR-dependent switch of thrombin-PAR-1 signaling from proinflammatory to cytoprotective signaling as suggested for APC mutants that engage EPCR but do not have proteolytic activity. Additional studies with clinical parasite isolates will further define the clinical relevance of PfEMP1-EPCR interactions.

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FUNCTIONAL MICROENGINEERED MODEL OF THE HUMAN SPLENON-ON-A-CHIP

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We have developed a newfangled microfluidic device that mimics the hydrodynamic behavior and filtering functions of the splenon, the minimal functional unit of the red pulp of the spleen able to maintain its filtering functions. Unlike any other previous microfluidic devices, the human splenon-on-a-chip incorporates for the first time two compartments with different flow velocities (according to the physiological flow division) and two physical barriers representing the reticular mesh and the interendothelial slits (IES) where cells first slow down increasing the hematocrit and then traverse the IES in a unidirectional matter. To validate the use of this platform, several experiments were carried out with different types of red blood cells (RBCs). As a proof of concept, we described that old RBCs showed less deformability than freshly drawn RBCs when traversing the microconstrictions. Posterior analysis allowed studying the passage and deformability of infected RBCs through the IES using peripheral blood of BALB/c mice experimentally infected with the *Plasmodium yoelii* 17X-GFP strain. Results showed that infected reticulocytes are significantly more deformable than non-infected reticulocytes, in agreement with the higher deformability of reticulocytes parasitized by *P. vivax*, a human malarial parasite with reticulocyte tropism. These results suggest that the device is able to reproduce physiological conditions and to distinguish different types of RBCs by means of deformation/mechanical properties. Presently, we are determining if there is hemolysis during the pass of blood through the device, if there is pitting in the absence of macrophages and the rheological properties of malarial infected RBCs.

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ORGAN-SPECIFIC TISSUE FACTOR ACTIVITY IS SIGNIFICANTLY ALTERED IN EXPERIMENTAL MALARIA

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Sequestration of *Plasmodium falciparum*-infected erythrocytes in the brain or the maternal blood space of the placenta results in two severe clinical manifestations of this disease, cerebral (CM) and placental malaria (PM), respectively. Hyperinflammation consisting of increased expression of Tumor Necrosis Factor-α (TNF) and activation of macrophages in affected organs are associated with CM and PM. It is well established in other models of disease that inflammatory damage leads to systemic activation of coagulation and severe thrombosis. Recent evidence showing the existence of a procoagulant state in patients suffering from CM or PM indicates the inflammation-coagulation cycle may play a significant role in malaria pathogenesis and may provide useful diagnostic and therapeutic targets. However, the extent to which coagulation is responsible for the pathogenesis of these diseases is incompletely understood. Thus, nonpregnant C57BL/6J mice were infected with 10^6 *P. berghei* ANKA, a virulent murine malaria species capable of inducing CM, or 10^6 *P. chabaudi* AS, a non-CM strain, then serially sacrificed between days 3 and 6 post-infection and assayed for TF procoagulant activity in brain, liver, and lung tissues. TF activity was significantly higher in brain (>1400-fold; P<0.01) of mice that succumbed to CM 5 and 6 days post-infection relative to uncomplicated malaria cases. In mice sacrificed on days 3 and 4 post-infection, all three organs exhibited a trend toward reduced TF activity, most notably in the brain (50-fold decrease). Preliminary data show reductions in TF activity at day 4 of *P. chabaudi* AS infection in the brain (>360-fold, P = 0.057), lung (>1200-fold, P = 0.016), and liver (>4400-fold, P = 0.057) compared to uninfected controls. Increased TF activity in the brains of mice that succumb to CM, but not those with uncomplicated *P. berghei* ANKA infection, suggests a significant role for coagulation in the pathogenesis of experimental CM. Ongoing experiments seek to further elucidate critical cellular sources of TF with the use of TF-floxed/cre mice. In addition, the importance of TNF in malaria-associated TF activity is being assessed with TNF and TNF receptor (TNFR) null mutant mice. Finally, to determine the effect of TF-driven coagulation on inducing placental pathology during PM, placentae from TF-, TNF-, and TNFR-modified *P. chabaudi* AS-infected mice are also being assessed.

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MALARIA-RELATED ANEMIA IN COLOMBIA

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Anemia and other hematological changes associated with malaria infections vary with the level of malaria endemicity, demographic variables and malaria immunity, among other factors. Information on cofactors associated with malaria-related anemia in Latin America is scarce. This

study aimed at characterizing erythrocytes indices in patients living in endemic areas of Colombia with unstable malaria transmission. A total of 929 malaria patients were enrolled between 2011 and 2013 in three areas of Colombia: Tierralta, Tumaco and Quibdó. Demographic and epidemiological information related to malaria was obtained at the time of diagnosis, and automated complete blood cell counts were performed on samples collected from all participants. Multiple linear regression analyses were then carried out to determine the relationships between erythrocytes indices and independent variables. *Plasmodium falciparum* was found to be the most prevalent species in Tumaco (84%) and Quibdó (70%), whereas *P. vivax* was predominant in Tierralta (92%). Variable degrees of non-severe anemia (Hb 7.1- 10.9 g/dL) were present in 21% of the patients, with similar distribution in the three study sites. Severe anemia (Hb <7 g/dL) was almost absent (0.3%). Afro-descendants from Quibdó presented lower hemoglobin levels than other ethnic groups regardless of the parasite species. Only in Tumaco, patients with *P. vivax* displayed lower hemoglobin levels than those found in *P. falciparum*-infected patients. In Tierralta, hemoglobin levels, hematocrit and RBC counts were negatively associated with days of illness. Moreover, in Tierralta and Quibdó, a direct relationship was found between the number of previous malaria episodes and hemoglobin levels. Both *Plasmodium* species appear to have similar potential to induce malarial anemia, but distinct cofactors at each endemic setting in Colombia seem to modify this clinical manifestation. Early diagnosis and prompt treatment are likely preventing more frequent and serious anemia in Colombia, and the target age in these low transmission settings has shifted towards adolescents-young adults. Moreover, previous malaria history appears to induce protection against anemia development.

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HEME INDUCED ACTIVATION OF TOLL-LIKE RECEPTOR 4 IS ASSOCIATED WITH REDUCED HEMATOPOIETIC STEM CELL VIABILITY AND APOPTOSIS IN SEVERE MALARIA

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Plasmodium falciparum infections are responsible for malaria associated mortalities ranging between 700,000 and 900,000 cases annually. Approximately 30% of the deaths occur after treatments indicating an urgent need for adjunctive therapies. Endothelial progenitor cells ($CD34^+$/$VEGRF^{2+}$) play an important role in the repair and regeneration of vascular endothelium as well as in neovascularization during cerebrovascular diseases, yet they are depleted by an unknown mechanism in severe malaria. Hematopoietic stem cells (HSC) are $CD34^+$ precursors to EPC and are decreased in the peripheral blood of individuals with Cerebral Malaria, a severe form of malaria associated with significantly high mortalities. We have shown that overproduction of free heme induces up-regulation of toll-like receptors (TLR) 4 and 9, as well as inflammation and apoptosis in both human and mouse brain vascular endothelial cells. However, the role of free heme in the depletion of HSC in severe malaria is poorly understood**.** We hypothesize that HSC depletion is a consequence of heme-induced TLR-mediated mechanism(s). We determined the effects of free heme on viability/apoptosis and TLR expression in HSC. Cell viability was assessed by MTT in HSC exposed to heme, apoptosis indices were examined by caspase-3 mRNA expression and TUNEL respectively. The effect of heme exposure on HSC expression of TLRs 1 - 10 was functionally assessed in the presence and absence of TLR specific blockers. Heme significantly decreased cell viability and induced cell apoptosis in both HBVEC and HSC compared to controls ($p<0.05$). Additionally, heme-induced TLR4 was up-regulated in HSC. Our results indicate that 1) free heme contributes significantly to the depletion of HSC and vascular endothelial cells by inducing apoptosis. 2) Free heme could be utilized as a potential biomarker for fatal malaria as it correlates with severity of malaria. 3) Among TLRs, TLR4 played a significant role in the heme-induced pathogenesis of malaria. In conclusion, *Plasmodium* infection activates TLR4 and subsequently induces apoptosis in HSC resulting in decreased numbers of circulating EPC and limited vascular repair in severe malaria pathogenesis.

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PREVALENCE AND DIVERSITY OF HAEMOPARASITES IN BIRDS AT THE RESERVA NACIONAL ALLPAHUAYO MISHANA (RNAM), IQUITOS, PERU, 2013

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Birds, like most wild animals, are frequently found to be affected by an enormous range of parasites, both endoparasites and ectoparasites, which represent a potential health risk for both humans and wild animals as well as a threat to the conservation of biodiversity. There is no information from Peruvian Amazon about the existence of these blood parasites, despite having the second richest ornithological diversity worldwide. The lack of research on wild birds in the Peruvian Amazon motivated us to investigate the presence of 3 hemoparasites that are genetically closely related (*Plasmodium, Hemoproteus*, and *Leucocytozoon*) in the Allpahuayo-Mishana National Reserve (AMNR). This is the first study in the Peruvian Amazon focus on identifying the presence of blood parasites in wild birds, and will provide invaluable information needed to assess the risk of emerging diseases that can affect human populations. For this purpose, a series of 10 parallel mist nets were installed in the study area from March to April 2013. A 75ml of blood sample was taken from each bird that was captured to perform a blood smear and a nested PCR amplification of Cytochrome b. A total of 203 birds belonging to 22 families and 62 species were captured. Our preliminary microscopy results revealed that 64 birds belonging to 28 species (31% of the total), were positive for *Plasmodium/Haemoproteus*. Casicus cela was the specie with the highest prevalence of parasites and mixed infections with Microfilaria and *Plasmodium/Haemoproteus*.

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GENOME-WIDE ANALYSIS OF CHANGES IN BEWO CELLS INDUCED BY CS2 *PLASMODIUM FALCIPARUM*-INFECTED ERYTHROCYTES, SCHIZOGONIC PRODUCTS AND CYTOKINES

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Placental malaria (PM) changes the microenvironment at the intervillous space of the placenta which is essential for fetus growth by exposing the syncytiotrophoblast (ST) to *Plsmodium falciparum* infected erythrocytes (IE), *P. falciparum* schizogonic products (SP) and inflammatory cytokines leading to low birth weight babies. Changes of ST functions occurs during PM, however it is not known what contribute to these changes. We analyzed gene expression changes in the BeWo cell line produced by the binding of IE, IE and parasite SP, and cytokines produced by THP-1 monocytes co-cultured with IE. BeWo cells were syncytialized for 72 hours using 10 μM forskolin in triplicate cultures and exposed to intact CS2 IE, intact CS2 IE plus SP, and conditioned medium (CM) produced by THP-1cells upon exposure to IE and co-cultured for 48 hrs. Gene expression changes were then analyzed by the microarray. Functional annotation of differentially expressed genes (fold change ≥1.5 and adjusted P value ≤0.01) was determined using GO-term context with DAVID version 6.7v and altered pathways were identified using Pathway Miner (BioRag). Conditioned medium containing inflammatory cytokines and chemokines, but not IE alone or IE with SP, significantly altered genes associated with

biological processes and pathways in BeWo cells that are important for fetus and placental growth. The categories of genes enriched (P ≤0.05) among down-regulated genes in BeWo cells treated with CM included, genes linked with vasculogenesis, blood vessel formation and negative regulation of vasoconstriction. Growth pathways significantly altered (P ≤0.05) included insulin, insulin like growth 1, mammalian target of ramphamycin, transforming growth factor beta 1, platelet derived growth factor, epidermal growth factor signaling and prostaglandin synthesis and regulation. Cytokines and chemokine dysregulate pathways and biological processes in BeWo cells that are involved in placental vascular development and remodeling. These results may explain why placental insufficiency occurs as a result of malaria infection.

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HEMATOPOIETIC STEM/PROGENITOR CELL SOURCES TO GENERATE RETICULOCYTES FOR *PLASMODIUM VIVAX*

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The adaptation of *Plasmodium vivax* to *in vitro* culture remains an important challenge. The preference of *P. vivax* for immature erythrocytes (reticulocytes) constitutes one of the main hurdles. Three different sources of hematopoietic stem/progenitor cells (HSPC) i.e; umbilical cord blood (UCB), bone marrow (BM) and adult peripheral blood (PB) were expanded for 5 days and differentiate for 14 days to obtain reticulocytes. The HSPC population could be efficiently expanded and produce reticulocytes that are equally permissive to *P. vivax*. Interestingly the permissiveness of HSPC-derived reticulocytes was higher than the reticulocyte-enriched blood. Our findings might suggest that *P. vivax* has a preference for immature reticulocytes opening new perspective in the research on *P. vivax* invasion mechanism.

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TACI IS REQUIRED TO CONTROL PARASITEMIA LEVELS POST PY NL INFECTIONS

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The TACI (transmembrane activator and calcium-modulator and cyclophilin ligand) receptor binds BAFF (B-cell activator factor) and APRIL (a proliferation-inducing ligand), two cytokines critical for B-cell survival and plasma cell generation. To assess the role of TACI in malaria, TACI/KO and the wild type (WT) C57BL/6 mice were infected (i.p) with 1 million *Plasmodium yoelii* (Py) NL parasites and parasitemia was assessed. Results showed that the level of parasitemia was significantly elevated in TACI/KO mice (55.2% at day 28) compared to wild type WT mice (12.4% at day 16), and the parasitemia clearance was substantially delayed in the TACI/KO (28 days) relative to WT control (21 days). Subsequently, anti-Py NL antibodies and BAFF and APRIL concentrations were measured. In addition, to determine the role of B cells in protection, adoptive B-cell transfers were also done. Measurement of serum BAFF and APRIL levels revealed that in TACI/KO mice, the highest levels of BAFF (33.2 ng/ml) and the lowest levels of APRIL (119 ng/ml) were detected at peak parasitemia. Additionally, less anti- Py NL IgG-antibodies were produced by the TACI/KO mice up to Day 22 post infection when compared to WT mice, but the differences in antibody levels vanished between TACI/KO and WT mice at day 28 post infection. Both, WT and TACI/KO mice were immune to a second Py NL challenge with peak parasitemia less than 1% and parasitemia clearance by day 10 following the secondary infection. Importantly, adoptive transfer of "immune" B-cells conferred protection in both WT and TACI/KO mice against Py NL challenge. Conclusion: TACI plays a key role in the control of Py NL infections.

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PHENOTYPIC CHARACTERIZATION OF THE *PLASMODIUM FALCIPARUM* NOT1 (PF3D7_1103800) GENE KNOCKOUT AND HEAT SHOCK ANALYSIS OF THE PIGGYBAC MUTANT LIBRARY

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Ongoing research in our lab uses genetic approaches to identify new potential targets for therapeutic treatments. The objective of this work is to characterize PF3D7_1103800 and identify other genetic factors in the *piggyBac* mutant library affected by heat shock. The Not1 gene, PF3D7_1103800, is a part of a larger gene regulatory system in eukaryotes known as the CCR4-NOT complex, which is involved in mRNA transcription and degradation. Our research on the asexual stages of the major malaria parasite *Plasmodium falciparum* uses the phenotypic analysis of genetic mutants. The primary phenotypic screens are growth attenuation and changes in virulence factors. My dissertation focuses on the screening for genes involved with stress responses to increased temperature. In the process of a whole genome random transposon mutagenesis of *P. falciparum,* a knockout of *Not1* was isolated. Characterization of protein extracts from mutant and wild type parasites were analyzed by immunoblots with antibodies to EBA-175 and GAP45. The IFA localization of these invasion-related proteins in the *Not1* mutant was compared with wild type patterns. NOT1 and other *piggyBac* mutants were analyzed for altered sensitivity to febrile temperatures during culture through growth analysis with a flow cytometer. The immunoblots display altered expression at mid to late trophozoite stages in the *Not1* mutant when compared to the wild type. The IFAs for the *Not1* mutant reveal patterns similar to that of the *Caf1* mutant, which is also a part of the CCR4-NOT complex and found to be important in parasite egress. Of the more than 40 parasites tested, 2 were significantly increased in growth and 18 were significantly decreased compared to the wild type, including the Not1 knockout. Validation of the *Not1* mutant phenotype should result in delayed growth and invasion of the parasite. Furthermore, exposure of the *piggyBac* mutant library to increases in heat may reveal genetic factors involved with the intraerythrocytic cycle that lead to new drug targets.

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IRON SUPPLEMENTATION AFTER SEVERE MALARIA IS ASSOCIATED WITH DECREASED HEME OXYGENASE-1 LEVELS ONE MONTH AFTER ILLNESS

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Iron treatment in malaria endemic areas can increase the risk of malaria and death. One mechanism by which iron supplementation increases the risk and severity of malaria may be by increasing oxidative stress. To assess changes in oxidative stress after treatment with iron in children with severe malaria, we assessed baseline and 4-week levels of heme oxygenase-1 (HO-1), an enzyme that is upregulated under oxidative stress, in Ugandan children with cerebral malaria (CM) (n= 51), severe malaria anemia (SMA) (n=39) and community children (CC) (n=52). We hypothesized that iron treatment would increase oxidative stress, as measured by HO-1. Upon enrollment, children who were iron deficient (zinc protoporphyrin (ZPP) >=80) either received daily iron therapy immediately (n=62) or 4 weeks later (n=54). All children with SMA and CM and 26 CC had a ZPP>=80, and were randomized to immediate vs. delayed treatment. 26 CC had a ZPP<80 and did not receive iron. Children with CM and SMA had similar baseline HO-1 concentrations, and did not differ in the immediate vs. delayed subgroups. Baseline HO-1 concentrations were lower in CC than in children with CM or SMA (P<0.0001). At 1 month, HO-1 concentrations in children in the CM and SMA delayed iron groups were increased compared to the immediate iron groups (both P<0.05) and the

iron sufficient group (both P<0.001), but HO-1 levels in the iron deficient community control group did not differ between immediate and delayed groups. There was no correlation of HO-1 levels at 1 month in children with CM or SMA who received either immediate or delayed iron treatment with readmission (P>0.05). Contrary to our hypothesis, iron treatment after malaria was associated with significantly lower HO-1 concentrations at 4 weeks. This suggests that iron treatment reduces oxidative stress in iron deficient children after malaria. Future research will explore potential mechanisms for this process.

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SEQUESTRATION MECHANISMS OF *PLASMODIUM VIVAX*-INFECTED ERYTHROCYTES

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We have previously demonstrated that *Plasmodium vivax*-infected erythrocytes (Pv-iEs) cytoadhere to endothelial cell receptors and form rosettes, a typical feature observed for *P. falciparum*. Rosette is a cytoadhesion phenotype where infected red blood cells can adhere to non-infected red blood cells and in falciparum malaria rosettes are normally associate to poor clinical outcomes and severity and might improve parasite infectivity. Nevertheless, it has recently shown that Pv-iEs harvested from vivax patients form rosettes, however correlation with severity and infectivity was not observed. These observations prompt us to investigate the biological role of rosetting formation of Pv-iEs. Thus, using a panel of 59 *P. vivax* Brazilian isolates, we showed that rosettes are mostly observed at late stages, they are formed mostly by normocytes and parasitemia levels correlate with rosetting rates. Nonetheless, we noticed that autologous plasma improved significantly rosette formation, suggesting a key role of plasma components. Indeed, we found positive correlation between IgM levels and an inverse association of IL6 and IL10 with rosetting rates. Moreover, antibodies towards to VIR proteins disrupt rosettes, which are less likely to be phagocyted *in vitro*. Thus, we hypothesize that rosetting probably acts as a mechanism of immune evasion.

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AN ADJUSTABLE GAS-MIXING DEVICE TO INCREASE FEASIBILITY OF *IN VITRO* CULTURE OF *PLASMODIUM FALCIPARUM* PARASITES IN THE FIELD

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A challenge to conducting high-impact and reproducible studies of the mechanisms of *Plasmodium falciparum* drug resistance, invasion, virulence, and immunity is the lack of robust and sustainable *in vitro* culture in the field. While the technology exists and is routinely utilized in developed countries, various factors–from cost, to supply, to quality–make it hard to implement in malaria endemic countries. Here, we design and rigorously evaluate an adjustable gas-mixing device for the *in vitro* culture of *P. falciparum* parasites in the field to circumvent this challenge. The device accurately replicates the gas concentrations needed to culture laboratory isolates, short-term adapted field isolates, cryopreserved previously nonadapted isolates, as well as to adapt *ex vivo* isolates to *in vitro* culture in the field. We also show an advantage over existing alternatives both in cost and in supply. Furthermore, the adjustable nature of the device makes it an ideal tool for many applications in which varied gas concentrations could be critical to culture success. This adjustable gas-mixing device will dramatically improve the feasibility of *in vitro* culture of *Plasmodium falciparum* parasites in malaria endemic countries given its numerous advantages.

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MOLECULAR MARKERS OF RADIATION INDUCED ATTENUATION IN INTRAHEPATIC *PLASMODIUM FALCIPARUM* PARASITES

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Radiation attenuated sporozoite (RAS) vaccination has proved to be a promising approach for malaria vaccine development. While the host requirements for sterile immunity induced by RAS vaccination have been studied, the molecular events that render attenuation to sporozoites in response to radiation remain poorly understood. We performed genome-wide transcriptional profiling of untreated verses radiation-treated intrahepatic parasites on days 3 and 6 post-invasion of human HepG2 hepatic cells by *Plasmodium falciparum* sporozoites of the 3D7 strain to identify parasite gene targets of growth attenuation and enhanced immune protection induced by radiation. Using this approach, we found that 180 parasite genes had significantly altered transcriptional expression in response to radiation on day 3 and 6 post-cultivation in hepatic cells. Among these novel biomarkers, we identified a signature of eight candidate parasite genes that associated with functionally diverse pathways that may regulate radiation induced cell cycle arrest of the parasite within the hepatocyte. In addition, a repertoire of 14 genes associated with protein translation is transcriptionally overexpressed within the parasite by radiation. We hypothesize that this radiation induced enhanced translational activity may be a contributing factor for the sterilizing immunity observed by RAS vaccination by increasing the pool of parasite antigens available for immune presentation either as processed epitopes on the hepatocyte surface or as released antigens from apoptotic infected hepatocytes . These results have significantly increased the repertoire of novel targets for 1) generating genetically attenuated parasite vaccine candidates 2) subunit vaccines against the hepatic stage cycle and 3) biomarkers of safety to define proper attenuation.

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COMPARATIVE GROWTH ANALYSIS OF MALARIA PARASITE, *PLASMODIUM FALCIPARUM*, USING ^{3}H-HYPOXANTHINE INCORPORATION AND SYBR GREEN FLUORESCENCE

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Differential growth of *Plasmodium falciparum* in red blood cells (RBC) is an essential feature of malaria parasite biology. High growth rate results in increased parasite biomass which is correlated with disease severity in patients. 'Growth' is generally ascertained microscopically or through a surrogate reporting system such as tritium labeled hypoxanthine (^{3}H- Hx). The use of tritium has been the 'gold standard' for measuring parasite growth as it effectively mirrors visually determined parasitemias. This method of labeling requires the parasites to be starved of the essential nucleic acid precursor, hypoxanthine (Hx) for 48 hours in order to facilitate uptake of the isotope. SYBR green, a relatively inexpensive fluorescent dye, has been shown to give similar results to ^{3}H-Hx-incorporation in drug assays, providing an alternative method for ascertaining parasite growth. However, a side-by-side comparison of SYBR green and ^{3}H-Hx-incorporation has yet to be completed in the absence of drug. Here

we provide a head to head comparison of the SYBR green and ^{3}H-Hx-incorporation assays in a set of genetically unique progeny to ascertain if these two methods can be used interchangeably in determining parasite growth. Our data shows a correlation between the data sets indicating that the relative growth rate as measured by both assays is comparable. In addition we use the SYBR green assay to measure differences in parasite growth in Hx starved and unstarved conditions. Preliminary data indicates decreased parasite growth in Hx starved conditions. More interesting is the observation that parasites show variable responses to Hx starvation suggesting a possible genetic mechanism may be involved.

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ASSESSMENT OF KNOWLEDGE AND ADHERENCE TO THE NATIONAL GUIDELINES FOR MALARIA CASE MANAGEMENT IN PREGNANCY AMONG HEALTHCARE PROVIDERS AND DRUG DISPENSERS IN RURAL, WESTERN KENYA

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Although prompt and effective treatment is a cornerstone of malaria control, information on healthcare provider adherence to malaria treatment guidelines in pregnancy is lacking. Incorrect or sub-optimal treatment can cause adverse consequences to the mother and fetus. We conducted a cross-sectional study from September to November 2013, in all health facilities and randomly selected drug outlets in Siaya County, western Kenya, to assess healthcare provider adherence to and knowledge of national guidelines for treatment of uncomplicated malaria in pregnancy to inform the Kenyan Ministry of Health and relevant stake-holders on knowledge gaps to be targeted. In health facilities, standardized questionnaires were used to interview all healthcare providers and all women of childbearing age who had been assessed for fever. Mystery clients posing as 1st trimester pregnant women or as relatives of women in 3rd trimester collected information from drug outlets. Information on drugs was recorded from prescriptions or after reviewing drugs in patient's possession. None of the drug outlet dispensers, versus 56% of health facility providers, knew the appropriate treatment for 1st trimester patients, while 39% and 87%, respectively, knew the appropriate treatment for 2nd/3rd trimester. Prescription of the correct drug for pregnancy trimester at the correct dosage, was observed in 66% of cases in health facilities and 50% in drug outlets. Prescribing was more often correct in 2nd/3rd trimester than in 1st (65% vs. 32%, p=0.004, and 38% vs. 0%, p<0.001, at health facilities and drug outlets, respectively). Sulfadoxine-pyrimethamine, which is no longer recommended for treatment of acute malaria, was prescribed and available in 4% of cases in health facilities and 23% of simulations in drug outlets (p<0.001). Exposure to artemether-lumefantrine in 1st trimester, which is contraindicated due to its unknown safety, occurred in 13% and 51% of cases in health facilities and drug outlets respectively (p=0.04); none were a result of quinine stock-out. This study highlights knowledge inadequacies and incorrect prescribing practices in the treatment of malaria in pregnancy. These should be addressed through comprehensive trainings and adequate supervision by the Kenya Ministry of Health to improve the quality of patient care and maximize therapeutic outcomes.

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RANDOMIZED TRIAL TO COMPARE THE SAFETY, TOLERABILITY AND EFFICACY OF BI-MONTHLY INTERMITTENT PREVENTIVE TREATMENT WITH EITHER MEFLOQUINE-ARTESUNATE OR SULFADOXINE-PYRIMETHAMINE PLUS AMODIAQUINE, WITH THE STANDARD REGIMEN OF DAILY PROGUANIL, FOR THE PREVENTION OF MALARIA AND RELATED COMPLICATIONS IN PATIENTS WITH SICKLE-CELL DISEASE IN NIGERIA

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Effective prophylaxis should be provided for children with sickle-cell disease(SCD) in malaria endemic areas. The aim of this study was to find out if intermittent treatment with mefloquine-artesunate (MQ+AS) could be used in this way. Patients with SCD were randomly assigned one of three intervention groups. Patients in group 1 received a fixed-dose combination of Mefloquine-Artesunate at each bimonthly clinic visit, those in group 2 sulfadoxine-pyrimethamine plus amodiaquine (SP+3AQ) bimonthly whilst patients in group 3 received daily proguanil. Patients were given diaries and are asked to return to the clinic every two months and whenever they were unwell. Three days after each clinic visit, patients or their carer were interviewed to ask about compliance and adverse events. The primary outcome of the trial was tolerability, secondary outcomes were adherence to the regimen, incidence of malaria and the number of outpatients presentations and hospitalizations over 12 months. 270 SCD patients were followed-up from September 2011 to May 2013, 90 in each study group. Adherence to the bimonthly regimens was excellent, (87% received 6 doses of MQ+AS and 84% completed all courses of the drug; 88% received 6 doses of SP+3AQ and 86% completed all courses). In contrast, adherence to the daily proguanil regimen was poor (10% took tablets less than three days in four). Incidence of mild adverse events during three days after each clinic visit, was highest in the MQ+AS group with about 20% of children reporting side effects, the most common of which were nausea, vomiting and abdominal pain, but these symptoms did not affect adherence. Incidence of malaria was low in all groups. The number of malaria cases was 17 (proguanil), 14(SP+3AQ) and 9(MQ+AS), rate ratios compared to proguanil were 0.84 (95%CI 0.4,1.9) for SP+3AQ and 0.55 (95%CI 0.2,1.2) for MQ+AS. The incidence of other illnesses was similar in the groups. Supervised treatments with MQ+AS at the clinic once every 2months is well tolerated and safe and may be more effective in preventing malaria than daily prophylaxis with proguanil. These results justify a larger trial powered to determine efficacy in preventing malaria and malaria-related complications. This trial is registered with ClinicalTrials.gov: NCT01319448 ISRCTN46158146.

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SAFETY AND EFFICACY OF DIHYDROARTEMISININ-PIPERAQUINE FOR TREATING UNCOMPLICATED FALCIPARUM PREGNANCY-ASSOCIATED MALARIA IN GHANA

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Dihydroartemisinin-piperaquine (DHA-PPQ) was approved for treatment of uncomplicated malaria in Ghana in 2008. However, DHA-PPQ is not used in pregnancy due to the paucity of safety data in this population. In light of anticipated DHA-PPQ use by self-medicating pregnant women, we have studied the safety and efficacy of DHA-PPQ compared to artesunate-amodiaquine (ASAQ) in a non-inferiority trial for treatment of uncompliccated malaria in second/ third trimester pregnancies.

Pregnant women who attended antenatal clinics in two districts of the Ashanti Region were screened for falciparum malaria using both a rapid diagnostic test and microscopy. Those testing positive in both were recruited, individually randomized to DHA-PPQ or ASAQ, and followed up actively on days 1, 2, 3, 7, 14, 28 and 42 after the start of treatment, at delivery and 6 weeks post-partum. During this period, assessment of adverse events, assessment of study drug adherence, sampling of blood for haematological and parasitological assessments and data collection on neonatal morbidity and mortality were performed. The primary outcome was parasitological efficacy by day 42. Analysis was primarily per protocol. Uncorrected cumulative parasitological efficacy by day 42 for DHA-PPQ was 91.2% (95% CI: 86.8%, 95.4%) and for ASAQ it was 87.0% (95% CI: 80.6%, 91.7%). By day 28, it was 93.8% (95% CI: 89.3%, 96.9%) for DHA-PPQ and almost 92.0% (95% CI: 86.5%. 95.4%) for ASAQ. There were no reports of hepatic/ renal toxicity or evidence of white blood cell dyscrasia in either study arm. The DHA-PPQ arm had fewer adverse events than the ASAQ arm; vomiting (17% vs 29%; p=0.021), general weakness (40% vs 60%; p<0.001). DHA-PPQ was non-inferior to ASAQ within a margin of 5% and also safe in the second and third trimesters. However, we are cautious in generalizing this conclusion because of the small sample size and our inability to report PCR-corrected efficacy rates. Larger studies are needed to confirm the results of this preliminary study.

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IN VITRO DRUG TRANSPORT OF ANTIMALARIALS; AMODIAQUINE, MEFLOQUINE AND METHYLENE BLUE

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Identification of P-gp mediated drug-drug interactions that interfere with antimalarial drug absorption would inform optimum drug design combinations in malaria. The study aimed to determine the P-gp substrate and inhibitory activities of amodiaquine (AQ), mefloquine (MQ) and methylene blue (MB). Caco-2 cell lines were grown on 0.6 cm^2 filter inserts at the cell density of 65,000 cells/cm^2. Bidirectional drug transport studies were done on 21-24 day cells with transepithelial electrical resistance > 300 $\Omega.cm^2$. AQ, MQ and MB were assayed via HPLC techniques. Rhodamine 123 (Rh123) quantification was done using fluorescence. MQ at 100 micromolar, pH 7.4 showed an apical-basolateral (A-B) permeability of $11*10^{-6}$ cm/sec and basolateral-apical (B-A) permeability of $12*10^{-6}$ cm/sec. Efflux ratios (ER) for 100 and 10 micromolar MQ suggested diffusion, as they were 1.1 and 1.3 respectively. Other antimalarial drug combinations that included MQ were not altered significantly, nor combinations with the P-gp inhibitor PSC-833. AQ at 100 μM, pH 7.4 showed an A-B permeability of $20*10^{-6}$ cm/sec and B-A permeability of $23*10^{-6}$ cm/sec, thus more permeable than MQ, but still not evidence of active efflux as their efflux ratios were suggestive of diffusion. B-A permeability of AQ did increase when combined with PSC833; however the statistical power of the test was low. P-gp substrate activity of MB was studied at 10 μM, pH 7.4 and showed an ER of 3.4 dropping to 1.6 in combination with 4 micromolar PSC833. Changes in transport rates in both directions were statistically significant. P-gp inhibitory activities of the three drugs were studied by combining drugs with a known P-gp substrate, Rh123. Rh123 efflux was not altered significantly when combined with any of the antimalarials (MQ, AQ or MB). We conclude that MQ and AQ are transported mainly by passive diffusion. MQ diffusion is not altered by artesunate. MB is subjected to P-gp mediated drug efflux. These drugs did not exhibit P-gp inhibitory activity.

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THE AVAILABILITY AND COST OF ANTIMALARIAL MEDICATIONS IN DRUG OUTLETS IN RURAL SIAYA COUNTY, WESTERN KENYA

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Although antimalarials are free in public health facilities in Kenya, challenges including stock-outs lead to patients seeking treatment in the private sector or directly from drug outlets. Medicine retailers play a major role in the distribution of antimalarials. The Affordable Medicines Facility - malaria program (AMFm) was introduced in June 2010 to make artemisinin combination therapy (ACT) as affordable as less effective alternatives. This study was conducted to determine the availability and cost of rapid diagnostic tests (RDTs) and antimalarials in drug outlets in a high malaria transmission area in rural western Kenya. We conducted a cross-sectional study from September to October 2013 and mapped all drug outlets within the Kenya Medical Research Institute/Centers for Disease Control and Prevention Health and Demographic Surveillance System area in Siaya County. We administered a standardized structured questionnaire to drug outlet personnel to collect data on outlet characteristics and availability, types and prices of RDTs and antimalarials. We identified 181 drug outlets, and 179 agreed to participate. Of these, 13% were registered pharmacies; 25% informal drug outlets; 46% general shops; 13% home-based outlets and 2% other. Only 17 (10%) had RDTs in stock, and 149 (84%) never stocked RDTs. 165 (92%) had ACTs in stock; of these, 98% had artemether-lumefantrine (AL); 13% had dihydroartemisinin-piperaquine (DHA-PPQ); 47% had sulfadoxine-pyrimethamine (SP); 34% had quinine (QN) and 8% had amodiaquine (AQ). Among the QN-stocking outlets, 48% had tablets and 62% had a parenteral formulation. The mean (SD) price in USD of an adult treatment course of AL, DHA-PPQ, QN tablets, SP, AQ was 1.01 (0.38), 4.40 (1.15), 2.24 (1.18), 0.62 (0.31), and 0.42 (0.20) respectively; the mean (SD) price in USD of RDTs was USD 0.92 (0.64). There was no significant difference in mean price of AL with or without the AMFm logo (USD 1.01 and 1.07 respectively, p= 0.45). The majority of drug outlets did not stock RDTs suggesting that testing prior to treatment per the national guidelines is unlikely for patients first seeking treatment in the outlets. The first-line treatment, AL, is widely available across outlet types. SP and AQ are not recommended for treatment but are less expensive than AL; the lower cost might encourage preferential use by patients. Restrictive policies should be enforced to prevent access and sale of ineffective antimalarials.

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IMPACT OF INTERMITTENT PREVENTIVE TREATMENT WITH DIHYDROARTEMISININ-PIPERAQUINE ON COGNITIVE FUNCTION, SCHOOL ATTENDANCE AND SCHOOL PERFORMANCE IN UGANDAN SCHOOLCHILDREN: A RANDOMIZED PLACEBO-CONTROLLED TRIAL

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Intermittent Preventive Treatment (IPT) is a promising option for malaria control in schoolchildren. However, there is still limited evidence of its benefits on cognitive function and educational outcome in this age group in different transmission setting. Therefore, we have investigated the impact of two dosing schedules of IPT with dihydroartemisinin-piperaquine (DP) on cognitive function, school attendance and school performance in Ugandan schoolchildren living in a high malaria transmission setting. We conducted a randomized, double-blind, placebo-controlled trial in 740 schoolchildren aged 6-14 years. Enrolled children were randomized to receive DP once every month (IPTm), once a school term (four treatments over 12 months) (IPTst) or placebo and followed for 18 months. The outcomes included cognitive function which was assessed using the

Ravan's matrices (for abstract reasoning) and the code transmission test (for sustained attention), school performance in the end of year mathematics and English exams and school attendance. Analyses were conducted on an intention to treat basis. Compared to placebo, IPTm marginally reduced the risk of missing school due to being sick (12.5% versus 9.5% p=0.06), especially if the illness was malaria (5.1% versus 0% p=0.05). No difference was observed between IPTst and placebo on school attendance. No impact of IPT compared with placebo was observed on sustained attention (mean difference [MD] IPTm -0.01 p=0.920, and IPTst -0.11 p=0.693), abstract reasoning (MD IPTm 0.09 p=0.726, and IPTst 0.52 p=0.228), results in mathematics exams (MD IPTm -0.8 p=0.418, and IPTst -1.5 p=0.676) or results in English exams (MD IPTm 0.5 p=0.551, and IPTst -1.2 p=0.797). IPTm reduced the risk of missing school due to malaria. However, IPT as implemented in this study (IPTm and IPTst) was not effective in improving the cognitive function or school performance of schoolchildren.

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ADHERENCE TO CO-FORMULATED AMODIAQUINE-ARTESUNATE VS. ARTEMETHER-LUMEFANTRINE FOR THE TREATMENT OF UNCOMPLICATED MALARIA IN FREETOWN, SIERRA LEONE

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Increasing access to and targeting of artemisinin-based combination therapy (ACT) is a key strategy of malaria control programmes. Approaches, such as co-packaging ACTs into blister packs, have been shown to improve adherence. While co-packaging facilitates correct treatment dosing, it does not reduce the quantity or frequency of tablet intake. To overcome theses limitations, several ACTs are now produced as co-formulated tablets. Although there have been a number of studies looking at adherence to ACTs, the majority have focused on adhernce to artemether-lumefantrine (AL). The aim of this study was to measure the level of caregiver adherence to co-formulated amodiaquine-artesunate (AQAS) compared to AL in Sierra Leone. This open-labelled randomised controlled study was conducted from September 2013 to January 2014 in two public health centres in urban Freetown. Patients 6-59 months presenting for fever at the health facility were randomized to receive either AQAS or AL at the time of prescription. Adherence was assessed using self-report of medication intake and package inspection. Exit interviews of caretakers were conducted at the health facility and follow-up interviews at their home on day four post-prescription. Of 1,950 patients screened, 795 were excluded due to: inappropriate age (29.6%), residence outside of the catchment area (12.3%), prior participation in the study (6.6%), no fever or history of fever (9.1%), and evidence of severe disease (2.0%). Of the 1,155 patients enrolled, 90% completed study follow-up, and 859 (87.5%) received an ACT. Preliminary results from one study site show that there was no difference in adherence to AQAS (96.4%) compared to AL (97.1%) (p=0.56). Reasons for non-adherence were similar for both treatment arms (forgot the last dose or took dose incorrectly). Vomiting was the most cited reason for missing a treatment dose for both groups. Data analysis for the second site is currently on-going. Full results, including comparison of caregiver adherence at both study sites and factors influencing adherence, will be presented.

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POPULATION PHARMACOKINETICS OF ORALLY ADMINISTERED MEFLOQUINE IN HEALTHY VOLUNTEERS AND PATIENTS WITH UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA

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The determination of dosing regimens for the treatment of malaria is largely empirical and thus a better understanding of the pharmacokinetic/pharmacodynamic properties of anti-malarial agents, such as mefloquine, is required to assess the adequacy of current treatment regimens and identify sources of sub-optimal dosing which could select for drug-resistant parasites. Mefloquine pharmacokinetics were assessed in 24 healthy adults and 43 patients with *P. falciparum* malaria. Population pharmacokinetic modelling was conducted using NONMEM. A two-compartmental model with a single transit compartment and first-order elimination from the central compartment most adequately described mefloquine concentration-time data. The model incorporated population parameter variability for clearance (CL/F), central volume of distribution (VC/F) and absorption rate constant (KA), and identified, in addition to body weight, malaria infection as a covariate of VC/F (but not CL/F). Simulations predict that falciparum malaria infection is associated with a shorter elimination half-life (407 c.f. 566hr) and time above MIC (766 c.f. 893hr). This is first population pharmacokinetic study to show falciparum malaria to influence mefloquine disposition. Protein binding, anaemia and other factors may contribute to differences between healthy individuals and patients. As VC/F is related to the earlier portion of the concentration-time profiles, which occurs during acute malaria, and CL/F is more related to the terminal phase during convalescence after treatment, this may explain why malaria was found to be a covariate for VC/F but not CL/F. These findings highlight the importance of patient characteristics when determining optimal dosing regimens for use in clinical practice; more studies will be needed in different populations.

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ANEMIA IN ACUTE AND CONVALESCENT UNCOMPLICATED FALCIPARUM MALARIA TREATED WITH ARTESUNATE-AMODIAQUINE AND COMPARATORS IN SUB-SAHARAN AFRICA

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Anemia is both a complication of malaria and a potential drug adverse reaction. Recovering from malaria should coincide with increased hemoglobin (Hb) levels, but the process is confounded by poorly-understood parasite, host and drug-related factors. Delayed hemolytic anemia was recently reported after parenteral artemisinin for severe malaria. We analysed 5189 patients with acute uncomplicated falciparum malaria from 8 studies conducted at 14 sites in 9 Sub-Saharan Africa countries during 2002-2009. Median age was 4 years (range 6m - 86y). 64% of the patients were 10 yo. 2380 patients were treated with artesunate-amodiaquine (ASAQ, 45.8%), 2809 with comparators (54.2%). In ASAQ groups, high parasitaemia was associated with low Hb both at baseline (r=-0.287) and post-treatment at D3, at D7, D14, and D28 (r=-0.165, -0.483, -0.161, -0.382, respectively, p=0.001 for all, Spearman test). Patients before ASAQ treatment had a mean Hb=10.9 g/dL (sd 2.20). Mean changes between D0-D3 were -0.48 g/dL (-4.0%); D0-D7 -0.45 g/dL (-3.2%); D0-D14 +0.54 g/dL (+7.5%); D0-D28 +0.77 g/dL (+9.8%). By multivariate analysis with random effects on the site (9594 Hb records) Hb increased over time (r=0.04, p=0.001) and in older patients (r=0.06, p=0.001) and decreased with higher parasitaemia (r=-0.39, p=0.001). By

multivariate survival analysis, patients with higher parasitaemia (adjusted hazard ratio, AHR=1.32, 95%CI 1.19-1.46, p=0.001), and children <6 yo (AHR=1.91, 95%CI 1.55-2.34, p=0.001) and anemia (<10 g/dL) before treatment (AHR=1.26, 95%CI 1.09-1.46, p=0.002) were at higher risk for parasite recurrence. On D0 32.6% (502/1541) patients were anemic, 39.6% (610/1541)on D7,16.2% (249/1541) on D28. Between D7 and 28, 2.0% (19/931) became anemic, 37.7% (230/610) remained anemic and 62.3% (380/610) became normal. Similar trends were observed with other combination treatments. Anemia and high parasitaemia concur to increase malaria treatment failure. Hb increases by D14 after initial fall. Delayed anemia did not occur in uncomplicated malaria.

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SCALE UP OF INTEGRATED HOME-BASED MANAGEMENT IN SENEGAL

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Since 2008, the Senegal National Malaria Control Program (NMCP) has implemented home-based management of malaria (PECADOM) using rapid diagnostic tests (RDTs) and artemisinin-based combination therapy (ACTs). In 2012, the NMCP and partners piloted integrated home-based management, including case management of diarrhea with zinc and oral rehydration salts (ORS) and pneumonia with antibiotics (in children under 5 years) as well as malaria in all age groups. Encouraging results prompted the scale-up of integrated home-based management in the southeastern regions of Tambacounda and Kedougou, which suffer from high all-cause under 5 mortality. In 2013, 428 villages in Tambacounda and Kedougou selected a home-based care provider (DSDOM). They received four days of classroom instruction on diagnosis and treatment of diarrhea, pneumonia, and malaria, followed by 15 days of practical experience at the health post nearest the village, supervised by the head nurse. Each provider received a drug box, timer, vest and hat, case register, RDTs, ACTs, co-trimoxazole, ORS, and zinc. Health districts received a budget to provide monthly supervision by the nurse and quarterly supervision by the district health management team. From June to December 2013, DSDOMs saw 12,979 patients: 5,705 patients under 5 years and 7,274 patients 5 years and older. Of these, 10,704 (82%) had fever and 98% were tested with an RDT; 7,310 (70%) had positive tests and 7,652 were treated with an ACT. Of children under 5, 2074 (36%) were diagnosed with malaria, 1,041 (18%) were diagnosed with diarrhea, 53 (5%) were treated with ORS alone, 35 (3%) were treated with zinc alone, and 953 (92%) received both. There were 656 (11%) children under 5 diagnosed with pneumonia and treated with co-trimoxazole. Of the remaining 578 patients, 575 (10%) were diagnosed with upper respiratory infection, and symptomatic relief was recommended. Of the 3,217 patients (25%) referred to the health posts (all ages), 2,791 (22%) had a negative RDT, 30 (0.2%) were less than two months, 25 were pregnant women (0.2%), and 359 (3%) had severe disease. In 2014, integrated PECADOM will be scaled up to an additional nine regions, and DSDOMs will be trained to identify and refer acute malnutrition among children under 5 years. These efforts should contribute substantially to the achievement of Millennium Development Goals 4 and 6, the reduction of under 5 mortality and the fight against malaria and other diseases.

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CHANGE IN MALARIA FALCIPARUM TREATMENT IN A MUNICIPALITY OF ACRE STATE, BRAZIL

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Brazil has around 300.000 malaria cases/year which represents about 60% of cases in Americas. In 2012, Acre state showed a 37% increase in malaria cases compared to 2011.Since 2006 Acre state has been using artesunate+mefloquine (ASMQ) for *P. falciparum* treatment, started as a efficacy study and adopted as treatment as soon as the drug was registered in Brazil. According World Health Organization recommendations to not use mefloquine in high transmission areas due to its longer half-life and possible development of resistance, The malaria advisory committee has suggested in 2011 to change the treatment and in 2012, treatment has been changed to artemether+lumefantrine (AL). All others Amazonia regions were already using al since 2006.The aim of the present study was to evaluate if the change in treatment regimens has contributed to increase cases in Rodrigues Alves municipality in 2013. An observational cross sectional study was perfomed based on primary data and were used as a measure of Odds ratio association to estimate relative risk. Study population was patients treated in health units or by field health workers in routine visits in Rodrigues Alves, with positive thick blood smear for *Plasmodium falciparum*. Data were analysed with Epiinfo 3.5.3. A total of 210 questionnaires were analysed. Respondents were male in 54% and the median age was 15 years (1-80). The slide cure verification on day 7 (d7) was performed in 191 (60%) patients. Treatment with ASMQ has been used in 99 (47%) patients. Three (3%) have not been cured. 111 (53%) have been treated with AL and one (0.9%) has not been cured. The odds ratio was 0.29 and IC95% 0.02-2.84. The results show that individuals treated with AL had 0.29 times more probability to have been cured when compared to subjects treated with ASMQ. However, this difference was not significant statistically, and the protection factor was possible related to a spurious association. In this way it suggests a similar efficacy between the two drugs. On the other hand, possibly there was a reduction of the mefloquine half-life effect to prevent new infections. Monitoring resistance surveillance to antimalarial drugs has prominent role to contain or eliminate the resistant parasites before they spread to higher transmission areas what will put in serious risk the recent progress in malaria control. It is important to create new strategies to detect early decrease of effectiveness of these drugs.

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REPEATED SPOROZOITE EXPOSURES WITH TRIMETHOPRIM-SULFAMETHOXAZOLE (TMP-SMX) PROPHYLAXIS INDUCE HETEROLOGOUS PROTECTION AND EFFECTOR/EFFECTOR MEMORY T-CELL RESPONSES IN RODENTS

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Repeated experimental malaria exposures in individuals receiving antimalarial prophylaxis can induce long-lasting sterile immunity, a strategy known as Chemoprophylaxis Vaccination or "CVac". We previously showed that TMP-SMX given at prophylactic doses can arrest liver stage development of malaria parasites, and that TMP-SMX prophylaxis during repeated malaria exposures induces long-lived sterile CD8 T-cell dependent immunity in mice. We set out to assess this protection against heterologous challenge, and to characterize the CD8 T cell responses observed. Mice receiving prophylactic TMP-SMX were inoculated with *Plasmodium yoelii 17 XNL* sporozoites once a month for 3 months, after which TMP-SMX was discontinued (TMP-SMX Cvac). One to three months later, mice were challenged with *P. yoelii P265 BY or P. berghei*

sporozoites. Splenic and hepatic lymphocytes harvested from unchallenged mice were phenotyped using flow-cytometry. TMP-SMX CVac induced sterile heterologous protection against *P. yoelii P265 BY* (p=0.00009) and *P. berghei* (p<0.0001) at 1 and 3 months, equivalent to the level of protection against homologous challenge that we previously reported. CD8 T cells identified using H-2K^{d} restricted epitope of CS Protein had Effector/Effector Memory Phenotypes at 1 and 3 months (p<0.05) with enhanced responses in the liver. In conclusion, TMP-SMX CVac induces protection against heterologous sporozoite challenge for up to 3 months in mice, characterized by CD8 T cell-Effector/Effector Memory responses, which parallels responses induced by irradiated sporozoite vaccination. Because HIV and malaria geographically overlap, we must understand how the use of TMP-SMX in HIV management impacts malaria infection and immunity in children.

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PHARMACOKINETICS AND *EX VIVO* ANTIMALARIAL ACTIVITY OF ARTEMISININ-PIPERAQUINE VERSUS ARTESUNATE-AMODIAQUINE IN HEALTHY VOLUNTEERS

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Reduced susceptibility to artesunate and artemisinin-based combination therapies (ACTs) such as dihydroartemisin-piperaquine has been reported in western Cambodia and south Vietnam. This is of immense concern as ACTs are currently recommended worldwide for first-line treatment of uncomplicated *Plasmodium falciparum* malaria. In Vietnam, dihydroartemisinin-piperaquine is the ACT of choice for the treatment of falciparum malaria, with little knowledge of the efficacy and tolerability of alternative ACTs. Recently, we reported Artequick (artemisinin-piperaquine) and Coarsucam (artesunate-amodiaquine) to be highly efficacious (PCR-corrected cure rate >98%) and well tolerated in Vietnamese patients with falciparum malaria in south-central Vietnam. Because there is limited pharmacokinetics (PK) data on these two ACTs, we evaluated the PK of artemisinin-piperaquine and artesunate-amodiaquine in two groups of 22 healthy Vietnamese subjects. Group 1 volunteers were administered daily a dose of two tablets of Artequick (each tablet contains 62.5 mg artemisinin and 375 mg piperaquine) for 3 days. Group 2 volunteers received a daily dose of two tablets of Coarsucam (each tablet contains 100 mg artesunate and 270 mg amodiaquine) for 3 days. Serial blood samples were collected up to 42 days and 28 days after the last dose of Artequick and Coarsucam, respectively. Plasma samples were assayed by LC/MS/MS for estimating the PK properties of the partner drugs for each ACT. Furthermore, we carried out a head-to-head comparison of the *ex vivo* antimalarial activity (pharmacodynamics-PD) of the two ACTs using the volunteers plasma samples after drug administration to determine which ACT possessed greater blood schizontocidal activity, *in vitro*. The PK-PD relationship of the two ACTs will be presented and discussed, including their implications for the treatment of malaria.

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EXPLOITING EVOLUTION TO SUPPRESS THE EMERGENCE AND SPREAD OF DRUG RESISTANCE IN MALARIA

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Rising drug resistance threatens to make malaria untreatable, necessitating both the discovery of new antimalarial agents and the development of strategies to identify and suppress the emergence and spread of drug resistance. Drug resistance can rapidly compromise the effective useful lifetime of antimalarial agents. However, drug resistance emerges in the context of a population and is limited by evolutionary fitness costs. We focused on in-development dihydroorotate dehydrogenase (DHODH) inhibitors. We identified eleven point mutations and gene amplification in the PfDHODH target, as well as several unknown resistance mechanisms. These mutant resistant parasites were often hypersensitive to other PfDHODH inhibitors, which immediately suggested a novel combination therapy approach to preventing resistance. Indeed, a combination of wild-type and mutant-type selective inhibitors led to resistance far less often than treatment with either drug alone. The effects of point mutations in PfDHODH were corroborated with purified recombinant proteins. Comparative growth assays demonstrated that two mutant parasites were less fit than their wild-type parent, and the purified protein of those mutants showed a decrease in catalytic efficiency, thereby suggesting a reason for the diminished fitness. Co-crystallography of PfDHODH with three inhibitors suggested that hydrophobic interactions are important for drug binding and selectivity, and that mutations in the active site tunnel have more serious catalytic and fitness consequences than mutations in the active site lid. Fitness limitations result in a small number of mutational escape pathways being heavily favored in *Plasmodium*. These escape pathways can be anticipated and blocked, and doing so increases the efficacy of an individual patient´s treatment and protects the useful lifetime of novel therapeutics.

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MEFLOQUINE VERSUS SULFADOXINE-PYRIMETHAMINE FOR INTERMITTENT PREVENTIVE TREATMENT IN PREGNANCY (IPTP): A MULTIPLE-OUTCOME ANALYSIS ON EFFICACY AND TOLERABILITY

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Evaluation of alternative drugs to replace sulfadoxine-pyrimethamine (SP) for intermittent preventive treatment (IPTp) is urgently needed because of the spread of resistance of parasites to the drug, which raises the question of the short-term efficacy of IPTp-SP to prevent malaria in pregnancy. Very few options are currently available, of which none fulfil all the ideal properties for IPTp. Therefore, alternatives need to be evaluated on basis of a benefit-risk ratio. Mefloquine (MQ) is one of the options that have already been evaluated for IPTp. Here, we reanalysed data from the first Beninese trial on IPTp-MQ using a multiple-outcome approach, which allowed the joint assessment of efficacy and tolerability. The trial was conducted in Ouidah, South Benin, and involved 1600 pregnant women, who were randomised in two groups (IPTp with MQ, 15mg/kg, versus IPTp with SP (1500-75mg) administered twice during pregnancy). For the present analysis, the overall superiority of one drug to the other was defined as: superiority on low-birth-weight (birth weight < 2500 grams), placental malaria or maternal anaemia (Hb < 10g/dL), non-inferiority on all efficacy outcomes and non-inferiority on tolerability defined as severe adverse events (AE) - either cutaneous or neuro-psychiatric - or low compliance with the treatment (i.e., reluctance to receive the second IPTp dose because of AE at the first dose or refusal to receive the second dose whatever the reason). MQ was found to be overall superior to SP (global P value=0,004). By applying different strategies to deal with missing data and/or including stillbirths, spontaneous abortions and multiple pregnancies in the analyses provided similar results. Whatever

the final decision on the use of MQ for IPTp, such a statistical approach outweighing the benefits/disadvantages of a large prevention strategy should systematically be taken into account by institutional policy makers.

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CYP 2D6 METABOLISM IS ESSENTIAL FOR THE ANTIMALARIAL ACTIVITY OF TAFENOQUINE AND NPC-1161B

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Tafenoquine (TQ) is an 8-aminoquinoline (8AQ) that has been tested in several Phase II and Phase III clinical studies and is currently in late stage development as an anti-malarial prophylactic agent. NPC-1161B is a promising 8AQ in late preclinical development. It has been reported that the 8AQ drug primaquine requires metabolic activation by CYP 2D6 for efficacy in humans and in mice, highlighting the importance of pharmacogenomics in the target population when administering primaquine. A logical follow-up study was to determine whether CYP 2D activation is required for other compounds in the 8AQ structural class. In the present study, the anti-malarial activities of NPC-1161B and TQ were assessed against luciferase expressing *Plasmodium berghei* in CYP 2D knock-out mice in comparison with normal C57BL/6 mice (WT) and with humanized/CYP 2D6 knock-in mice by monitoring luminescence with an *in vivo* imaging system. These experiments were designed to determine the direct effects of CYP 2D metabolic activation on the anti-malarial efficacy of NPC-1161B and TQ. NPC-1161B and TQ exhibited no anti-malarial activity in CYP 2D knock-out mice when dosed at their ED_{100} values (1 mg/kg and 3 mg/kg, respectively) established in WT mice. TQ anti-malarial activity was partially restored in humanized/CYP 2D6 knock-in mice when tested at two times its ED_{100}. The results reported here strongly suggest that metabolism of NPC-1161B and TQ by the CYP 2D enzyme class is essential for their anti-malarial activity. Furthermore, these results may provide a possible explanation for therapeutic failures for patients who do not respond to 8AQ treatment for relapsing malaria. Because CYP 2D6 is highly polymorphic, variable expression of this enzyme in humans represents a significant pharmacogenomics liability for 8AQs which require CYP 2D metabolic activation for efficacy, particularly for large-scale prophylaxis and eradication campaigns.

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A CONSTANT RATE INTRAVASCULAR INFUSION MODEL FOR THE EVALUATION OF INCREASES IN HEMATOCRIT AFTER ARTEMISININ-BASED COMBINATION TREATMENTS OF ACUTE FALCIPARUM MALARIA IN CHILDREN

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A constant rate intravascular infusion model, which employed the principles of constant rate intravenous infusion of drugs, was used to evaluate the kinetics of the increases in hematocrit after artesunate-amodiaquine (AA) or artemether-lumefantrine (AL) treatments of acute, uncomplicated falciparum malaria in 112 children. The model assumed baseline hematocrit was zero and a constant rate increase in hematocrit from baseline after treatment, and involved semi-logarithm plots of the difference between hematocrit at plateau and that at earlier times, against the corresponding times. Hematocrit reached a plateau in a median time of 28 days after treatment started. Mean plateau hematocrit was 6.75% (95%CI 6-7.5) and was similar for AA- and AL- treated children [6.92% (95%CI 6.01-7.83), n = 81 *v* 6.32% (95%CI 4.92-7.7), n=31, P 0.45]. Times to plateau were significantly shorter and plateau hematocrit significantly lower in non-anemic compared to anemic children. Declines from plateau were monoexponential with mean half time of 2.49 days (95%CI 2.1-2.79) and were similar in AA and AL-treated children [2.42 days (95%CI 2.07-2.77) *v* 2.68 days (95%CI 2.03-3.3), P = 0.46]. Hematocrit half times were significantly shorter in anemic compared to non-anemic children [2.09 days (95%CI 1.77-2.41, n 59) v 2.94 days (95%CI 2.41-3.46, n 53), P = 0.006) indicating hemopoietic responses may have been 'accelerated' following successful treatment in the former. The constant rate intravascular infusion model permits evaluation of increases in hematocrit following antimalarial treatments and may be used in observational and clinical studies in uncomplicated falciparum malaria.

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COMBINING COMMUNITY CASE MANAGEMENT OF MALARIAL AND SEASONAL MALARIA CHEMOPREVENTION FOR CHILDREN LESS THAN 10 YEARS IN SENEGAL: FEASIBILITY, IMPACT ON MALARIA AND ANEMIA

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Malaria and anemia are commonly associated in tropical regions. Scaling up antimalarial interventions as advocated by the WHO, will contribute to further reduce malaria burden and may induce a significant reduction in the global anemia burden. Malaria control in many epidemiological situations such as in Senegal will require a multi-intervention strategy with the use of combination of antimalarial interventions. Studies are needed, to document the feasibility as well as the impact on malaria and anemia of combining several antimalarial interventions. Relevant studies are also needed to assess potential anemia determinants and risk factors while scaling up antimalarial interventions, for an effective and integrated anemia control. This project aimed to assess the potential benefit of adding seasonal malaria chemoprevention (SMC) to community case management of malaria (CCMm) and to investigate factors associated with anemia among <10 years. The study objectives were covered by undertaking: i) a cluster randomised trial during witch eight communities were randomised to receive either CCMm combined with SMC (intervention arm) or CCMm alone (control arm), (ii) a case control study with anaemic and non.aneamic children. The overall adjusted rate ratio for incidence of malaria attacks in intervention and control communities was 0.15, indicating a protective effect of CCMm+SMC of 85% (95% CI:39.9%-96.3%, p=0.01). The case control study, demonstrated that the main factors significantly associated with anemia in these communities were: malaria parasitaemia (aOR=5.23, 95% CI[1.1-28.48]), sickle cell disorders (aOR=2.89, 95% CI[1,32-6.34]), alpha-thalassemia (aOR=1.82, 95% CI[1.2-3.35]), stunting (aOR=3.37, 95% CI[1.93-5.88], age ranged from 2 to 4 years (aOR=0.13, 95% CI[0.05-0.31]) and age > 5 years (aOR=0.03, 95% CI[0.01-0.08]). The combination of CCMm and SMC will result in a significant reduction of malaria and anemia burden. However, to achieve an effective control of anemia, integrated approaches taking into account other aetiologies of anemia need to be developed.

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RISK FACTORS FOR POOR QUALITY ARTEMISININ CONTAINING ANTIMALARIALS IN TANZANIA'S PRIVATE SECTOR - RESULTS FROM A NATIONALLY REPRESENTATIVE OUTLET SURVEY

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Artemisinin-containing antimalarials (ACAs) are acknowledged to be the most effective treatment for uncomplicated malaria. There have been reports that in some areas up to a third of antimalarials are "fake", especially in the private sector, but few studies have been conducted in a representative fashion. Poor quality (falsified, substandard or degraded) antimalarials can lead to increased mortality and morbidity and contribute to resistance. In Tanzania the private sector is commonly used as a source of treatment for fever. We purchased ACAs from drug retailers throughout Tanzania mainland and measured the quality of each sample. A nationally representative survey was conducted in 2010 as part of the Independent Evaluation of the Affordable Medicines Facility-malaria. Every drug store, pharmacy and general retailer in 49 wards was visited and a sample of every ACA in stock was purchased. The active pharmaceutical ingredient (API) of the artemisinin and partner component was measured using high performance liquid chromatography and mass spectrometry, followed by multivariate analysis to determine risk factors for poor quality. Of the 1,737 ACAs purchased and analysed, 87.1% were in tablet form and a quarter were WHO prequalified products. All samples contained the artemisinin derivative (ART), with 4.1% being outside the acceptable 85-115% API range. Based on multivariate analysis of the ART component, ACAs manufactured in Europe were 4.02 times more likely to be of poor quality (p=0.01), while WHO prequalified ACAs had 0.05 times the odds of being poor quality (p=0.01). When combined with the partner component, 12.1% of samples were of poor quality, while granular ACAs had 8.3 times the odds of being poor quality (p=0.04), and WHO prequalified ACAs had 0.07 times the odds (p=0.002). Weak medicine regulatory systems have been observed in a range of low and middle income countries, reflecting lack of financial resources, manpower and capacity. However, these results indicate that important improvements in quality can be achieved by ensuring that only products meeting WHO pre-qualification are registered and permitted onto the market.

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PLASMODIUM FALCIPARUM MALARIA-ASSOCIATED ANEMIA IN UNDER FIVE-YEAR-OLD NIGERIAN CHILDREN: BEFORE AND AFTER ARTEMISININ-BASED COMBINATION TREATMENTS

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The risk factors for, and recovery from anemia in acute falciparum malaria were characterized in 672 <5year-olds from five geographical areas of Nigeria before and after artemether-lumefantrine or artesunate-amodiaquine treatments. Of these, 294 (43.8%, 95%CI 40.0-47.6) presented with anemia (hematocrit <30%). Before treatment, an age <3 years and duration of illness >3 days were independent risk factors for anemia, and anemia at enrolment and parasitemia ≥100,000/μL after treatment. Malaria-attributable fall in hematocrit was 6.4% (95%CI 6.06-6.84). Mean time to recovery from anemia was 12.7d (95%CI 11.9-13.4), was similar with both treatments, and correlated positively with enrolment parasitemia (P=0.012). Fall in hematocrit per 1000 asexual parasites cleared from peripheral blood after treatment was significantly lower at higher compared with lower parasitemias (P<0.0001). Kinetics of the deficit in hematocrit (from 30%) after treatment began were estimated by a non-compartment model. Declines in hematocrit deficit were monoexponential, with a mean elimination half-life of 0.37d (95%CI 0.35-0.38). Deficit in hematocrit half-lives and efficacy in anemic children were similar for both treatments. Artemether-lumefantrine and artesunate-amodiaquine, influence the risk of anemia after treatment, conserve hematocrit at high parasitemias, and may influence recovery from *Plasmodium falciparum* malaria-associated non-severe anemia in Nigerian children.

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ARTEMETHER-LUMEFANTRINE PHARMACOKINETICS AND PHARMACODYNAMICS IN VERY YOUNG CHILDREN TREATED FOR UNCOMPLICATED MALARIA IN UGANDA

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Artemether-lumefantrine (AL) is the most widely utilized treatment for malaria. Pharmacokinetic (PK) and pharmacodynamic (PD) data are limited in the youngest children, but concern exists over inadequate exposure in young children. Our objective was to describe the population PK/PD of lumefantrine (LR) in children 6 to 24 months of age. Capillary blood samples were collected on filter paper (n=1027) over 21 days during 249 episodes of malaria occurring in 105 children. Samples were assayed using solid-phase extraction followed by HPLC. Population PK analysis of LR with

the software Monolix® (v.4.3) employed a 2-compartment open model with first-order absorption and allometric scaling for CL, Q, V1 and V2. Cox proportional hazards regression, stratified by use of trimethoprim-sulfamethoxazole (TS) prophylaxis, was conducted to explore relationships of LR levels on days 7 and 14 with recurrent parasitemia to days 28 and 42. Point estimates for the typical patient in this population were 173 L for V1, 0.94 L/h for Q, 5,740 L for V2, and 0.021 L/h for CL. The influence of hematocrit on V1 was the only covariate identified as significantly ($p \leq 0.005$) affecting PK, with a positive correlation. Multivariate regression revealed that, in those children not taking TS, higher day 7 LR capillary concentrations were significantly associated with lower cumulative risk of recurrent parasitemia by days 28 and 42 (HR 0.7, $p<0.001$ for both). Children with detectable LR on day 14 (n=15/129) had no recurrent parasitemia by day 28. Additionally, while exposure was not significantly affected by TS usage in the population PK model, day 7 LR levels were significantly higher in children on TS compared to those not on TS (median level 252 vs 209 ng/mL). Our results describe a 2-compartment PK model for LR disposition in children with a significant covariate effect of hematocrit on V1. LR exposure was significantly associated with recurrent parasitemia, and TS significantly impacted that relationship. Additional analyses are underway to inform treatment strategies in the youngest age group.

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RITONAVIR-BOOSTED LOPINAVIR (LPV/R) ANTIRETROVIRAL THERAPY ASSOCIATED WITH LOWER CONCENTRATIONS OF DESETHYLAMODIAQUINE IN MALARIA-UNINFECTED HIV-POSITIVE PEOPLE TREATED WITH ARTESUNATE-AMODIAQUINE

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In sub-Saharan Africa, most HIV-infected (HIV+) individuals on antiretroviral therapy (ART) are exposed to malaria. When infected with malaria they require treatment with artesunate-amodiaquine (ASAQ). Few studies have examined the pharmacokinetics (PK) and safety of ASAQ in HIV+ individuals taking ARTs containing Nevirapine (NVP) or ritonavir-boosted Lopinavir (LPV/r). We conducted an open label clinical trial to compare the maximum concentration (Cmax) and area under concentration-time curve (AUC) of amodiaquine (AQ) and desethylamodiaquine (DESAQ) in antiretroviral naive HIV+ individuals and those taking NVP and LPV/r-based ART. In step 1 of the trial, malaria uninfected adults (n=6/ART group) received half the standard dose of ASAQ (1 tablet of 100/270mg each) at times 0, 24 and 48hrs. In Step 2, another group of malaria uninfected adults (n=25/ART group) received a standard dose of ASAQ (2 tablets of 100/270mg each). We performed data-rich PK blood sampling and assessed treatment emergent hematological and biochemistry abnormalities. In Steps 1 and 2, AQ concentrations were well below the HPLC assay lower limit of quantitation (25ng/mL). From step 1 to Step 2, DESAQ AUC significantly increased 7-fold from 5,724 ng.hr/mL (95%CI: 3,877-10,100) to 40,400 ng.hr/mL (95%CI: 36,285-43,175), $p<0.000001$. In Step 1, there were no significant differences in DESAQ Cmax and AUC among participants in the LPVr-, EFV and ART-naive groups. In Step 2, compared with the ART-naive group, participants in the LPV/r-ART group had lower DESAQ Cmax (267, [95%CI: 178-375] vs 412, [95%CI: 324-678] ng/mL, $p<0.0001$) and AUC (27,893, [95%CI: 17,276-41,383] vs 48,172, [95%CI: 35,402-59,336]) ng.hr/mL, $p<0.0001$). However, no significant differences in Cmax and AUC were observed between the NVP-based and ARV-naïve groups. In step 2, cases of transaminitis (n=2) and neutropenia were commonly observed in the NVP-ASAQ and ART-naïve groups but not in the LPV/r group. Thus, LPV/r-ART reduces DESAQ plasma concentration and may be associated with reduced efficacy of ASAQ. However, higher DESAQ levels may be associated with liver and hematological toxicities.

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HIGH RESOLUTION MELTING (HRM) ANALYSIS HIGHLIGHTS MALARIA MISDIAGNOSIS AMONG MICROSCOPICALLY NEGATIVE FEBRILE PATIENTS IN WESTERN KENYA, AND HAS IMPLICATIONS FOR BETTER DRUG USE

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Microscopy and rapid diagnostic tests (RDTs) are common tools for diagnosis of malaria, but are deficient in detecting low *Plasmodium* parasitemia, often leading to inappropriate medical prescriptions and patient care based on misdiagnoses of clinical presentations. We developed and applied a molecular diagnostic tool (nPCR-HRM) that combines the sensitivity and specificity of nested PCR (nPCR) and direct PCR-high resolution melting analysis (dPCR-HRM) to screen blood samples from febrile patients for low-parasitemia malaria in a rural malaria endemic setting.Blood samples (n=197) were collected in two islands of Lake Victoria, Kenya, from febrile patients without *Plasmodium* detectable by microscopy or RDTs. 18S rRNA gene sequences were amplified from extracted DNA by nPCR-HRM, nPCR, and dPCR-HRM to detect and differentiate *Plasmodium* parasites. Data on administration of antimalarials were also collected. The coupled nPCR-HRM assays detected *Plasmodium* parasitemia in 62 (31·5%, 95% CI 25·0-38·0%) of the samples, among which 53 (26·9%, 95% CI 20·7-33·1) had *P. falciparum*. However, nPCR and dPCR-HRM detected *Plasmodium* parasitemia in only 20 (10·2%, 95% CI 5·9-14·4) and 39 (19·8%, 95% CI 14·2-25·4) of the samples respectively. Among low-parasitemia infections determined by nPCR-HRM, only 42 (67·7%) patients were treated with antimalarials, whereas 110 (81·5%) patients not infected with *Plasmodium* parasites were treated with antimalarials. Our findings demonstrate the limitations of differential diagnostics of febrile illness in rural malaria endemic settings that confound epidemiological estimates of malaria, and lead to inadvertent overprescription of antimalarial drugs to non-malaria febrile patients and under-prescription of antimalarial drugs to low-parasitemia malaria patients. nPCR-HRM enhances low-parasitemia malaria diagnosis and can potentially surmount the deficiencies of microscopy and RDT based results in determining malaria parasitemia, and evaluating malaria epidemiology.

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PRESUMPTIVE DIAGNOSIS MALARIA TREATMENT IN FEBRILE CHILDREN IN PARTS OF SOUTHEASTERN NIGERIA

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Malaria diagnosis in Nigeria has largely been done clinically; based to a larger extent on recognition of symptoms. Parasitological confirmation is a key component of case management of malaria. Clinical diagnosis has poor accuracy and leads to over diagnosis of malaria and poor management of non-malaria febrile illnesses and wastage of antimalarial drugs. Parasitological diagnosis using microscopy was performed among 560 febrile children, 0-12years attending pediatric clinics in Federal Medical Center Owerri and some primary health care centers in Imo State, South Eastern Nigeria after obtaining Ethical clearance. Their blood

samples were collected prior to treatment and were treated for malaria according to the IMCI guidelines and the standard routine practice of the clinic. These children were separated into two age groups: group I (0-≤5) and II (>5-12) for data analysis purpose. Out of 560 children (0-12 years) enrolled, 156(27.9%) were actually positive for malaria parasites, while 404(72.1%) were slide negative. Only *Plasmodium falciparum* was found in the positive samples. Children's age was significantly related to the prevalence of uncomplicated malaria (p=0.000) and a high determinant in explaining 6.4% of the variance in the prevalence of uncomplicated malaria (F=37.915 and p=0.000). Group II (>5-12) had higher parasite prevalence of 44% compared to 20% prevalence found in group I (0≤5) although group I had higher parasitaemia (density count). Only children between 0-≤5 years received antimalarials in compliance with the IMCI guidelines. This gave an overtreatment of 83.1% in children 0-≤5 years based on blood smear microscopy confirmation. The findings highlight the need for the scaling-up of parasitological confirmation of all patients suspected to stand overtreatment with the ACTs. Improving the diagnostic system for effective health care delivery in Nigeria and other poor endemic areas will not only provide a good platform for malaria treatment/monitoring but also reduce rapid onset of drug resistance, wastage of antimalarials and adequate management of other febrile illnesses in children.

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LOOP MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) FOR MALARIA DIAGNOSIS TARGETING THE APICOPLAST GENOME

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Malaria still remains a global health burden with an estimated 627,000 malaria deaths worldwide in 2012. The shortcomings of current diagnostic methods are a concern, and the malERA Consultative Group on Diagnoses and Diagnostics has called for improved malaria diagnostic methods. Current malaria diagnostic methods do not rapidly and accurately detect asymptomatic infections which contribute significantly to transmission. As malaria declines and large numbers of samples need to be screened to target intervention measures appropriately, high throughput amplification of nucleic acids via polymerase chain reaction (PCR) become more relied on, however, they are not easily field deployable. Isothermal amplification methods have been developed to address the major shortcomings of PCR methods. Different targets are being amplified, the most common being the conserved small subunit ribosomal RNA 18S locus. In this study, we report the development and optimization of the apicoplast genome of *Plasmodium falciparum* as a target for molecular diagnosis of malaria using a loop-mediated isothermal amplification (LAMP) assay. *P. falciparum* sequences from 15 Gambian isolates and 8 laboratory clones were aligned against the PlasmoDB reference sequence (emb|X95275.2|). Primers were designed from a highly conserved region of the consensus sequence, approx. 1.5kb segment of the gene coding for a ribosomal RNA protein (AP|0010:rRNA); and validated *in silico*. The assay was optimized for temperature, primer, dNTPs and $MgCl_2$ concentration. 184 archived DNA samples from West Africa and S.E Asia were analyzed against a reference PCR method. End point determination was by naked eye and agarose gel electrophoresis after staining with SYBR Green I®. Preliminary results show Sensitivity of 100.00% (95% CI: 93.88 % to 100.00 %), Specificity of 83.78 % (95% CI: 67.98 % to 93.77 %), Positive Predictive Value of 90.77 % (95% CI: 80.97 % to 96.51%), Negative Predictive Value of 100.00 % (95% CI: 88.68 % to 100.00 %) and Kappa index of 0.86 (95% CI: 0.76 to 0.97). Based on the preliminary results, the apicoplast genome shows to be a suitable target for malaria diagnosis, with diagnostic parameters comparable to the reference PCR method. Being easily field-adaptable, without need for a thermocycling equipment, this assay could facilitate targeted interventions towards malaria control and elimination.

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CHALLENGES OF MALARIA DIAGNOSIS IN PEDIATRIC PATIENTS AT A NIGERIAN HOSPITAL

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This study compares the performance of routine malaria diagnostic tests, and explores the challenges of malaria diagnosis in paediatric patients in an endemic setting in South West Nigeria. This was a Cross sectional study conducted at the children's outpatient and emergency units of the University College Hospital, Ibadan, Nigeria. Patients seen between May and August, 2013 were enrolled in the study. The records of all 532 children aged six months to 12 years who received treatment for an acute febrile illness at the hospital during the study period were reviewed. The proportion of children classified as having malaria by clinical diagnosis, Rapid Diagnostic Test (RDT) and blood smear microscopy were compared. Factors associated with test positivity were explored using multivariate analysis. By clinical diagnosis 45.2% of children were diagnosed as having malaria, 37.6% tested positive to malaria parasite on RDT and 19.3% had positive blood smears on microscopy. Logistic regression showed that with RDTs, younger children were less often found to be positive than older children [OR: 0.594 (0.401-0.879)]. A similar lower probability of positivity was found for younger children on microscopy [OR0.624 (0.391-0.996)]. Positive smears were however recorded 3.9 times more often for those who gave a history of fever compared to those who did not [OR: 3.882 (1.154-13.057)]. The true malaria morbidity among these paediatric patients remains questionable due to the differences in the results produced by the different diagnostic methods. The clinical implication of RDT-positive but microscopy-negative samples may be grave if microscopy results are erroneous. Quality control systems and surveillance of routine malaria diagnostics are imperative to limit misdiagnosis of malaria in this endemic setting.

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EVALUATING THE PERFORMANCE OF ID-FLUORESCENT IN SITU HYBRIDIZATION IN DETECTING MALARIA PARASITES IN WESTERN KENYA

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Diagnosis of malaria parasites requires highly sensitive and specific diagnostic tools that may help rationalize antimalarial drug use in Kenya. In many cases, the identification of malaria parasites concerns their salient features that can be utilized to distinguish between species and subspecies. Conventional techniques including serology and microscopy (Giemsa) have limitations making it difficult to meet these requirements. Currently, detecting very low density infection has been done with alternative methods including nucleic acid amplification tests (NAAT) such as Polymerase Chain Reaction (PCR); Loop mediated Isothermal amplification (LAMP) and Fluorescent *In* Situ Hybridization (FISH). These nucleic acid techniques generally involve the use of DNA and RNA probes and have been shown to be very sensitive and specific. However, these techniques are not easily accessible and relatively expensive. Data to-date shows that *Plasmodium* FISH assays for detection of *Plasmodium* parasites and speciation of *P. falciparum* and *P. vivax* have high sensitivity and specificity and are easy to perform. The FISH assays detect both asexual and sexual stages of the malaria parasites. This current study proposes to evaluate the performance of *Plasmodium* FISH assays for the diagnosis of *P.falciparum* malaria infections, more specifically *P. falciparum* gametocytes and low parasitemia in patients. The technique will be evaluated against

microscopy and RT-PCR. Malaria blood films (MBF) will be obtained from clinical and cultured *P. falciparum* samples. The data collected will be analyzed by use of Chi-square for comparisons. This study will provide an alternative malaria diagnostic technique which is robust, sensitive, specific, inexpensive and reliable.

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SEVERE MALARIA CASE MANAGEMENT PRACTICES IN SELECTED STATES IN NIGERIA: NEED FOR URGENT INTERVENTION

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Severe malaria is a life-threatening medical condition that requires emergency interventions including prompt and effective treatment to prevent death (WHO 2000). The AQUAMAT study showed a relative reduction in mortality of 22.5% with use of parenteral Artesunate compared to quinine in the management of severe P.*falciparum* malaria and the national policy on malaria diagnosis and treatment has been revised based on this evidence. The goal of the study was to determine baseline capacity and management practices for severe malaria in selected facilities in Nigeria with the aim of designing interventions to address specific gaps identified. A cross-sectional study design was used to assess twenty-four health facilities in three states (Benue, Kogi and Oyo States). Data on capacity of health care providers; three months malaria services provided at different service delivery points; and medical supplies were collected. Double data entry method was used for data entry into SPSS software programme and analysis done with STATA version 10.0 Total number of malaria cases reported in the three states over the three months preceding the assessment (May, June and July 2013) was 18, 695 and diagnosis of severe malaria was made in 8.6% of the total malaria cases. Out of the severe malaria cases, about 76% of cases were discharged and mortality was recorded in about 2% of the cases. However, providers in most of the facilities managed severe malaria with injectable artemether (46%), quinine (37%) and artesunate (29%). Majority of the health facilities (96%) practiced parasite-based diagnosis of malaria but 29% monitored the parasite clearance of patients with severe malaria. Seventy percent of the facilities did not have basic supplies for supportive management of severe malaria. In addition, 67% of the health facilities experienced stock out of parenteral artesunate in the previous three months. Case management of severe malaria is still sub-optimal in study sites and to reduce the mortality attributable to malaria, effort must be intensified toward health system strengthening with emphasis on capacity building of health care providers, medical commodity security and improvement in supportive management of severe malaria.

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MALARIA DIAGNOSIS SERVICE AVAILABILITY - MAPPING OF PRIVATE SECTOR SERVICE DELIVERY OUTLETS IN 7 PMI/ MAPS SUPPORTED STATES, NIGERIA

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The private sector's role in health services in Nigeria has increased in recent times, and provides approximately 60% of health services. The MAPS project supports the implementation of parasitological confirmation of malaria cases in public health facilities in selected states. A mapping exercise was carried out to identify the private facilities providing malaria diagnostic services and determine the gaps in existing malaria diagnostic capacity with the aim of designing a strategy for improvement. Data were obtained from questionnaires administered to facility owners and location data collected by handheld GPS device. Overall, 394 private health facilities in urban areas of 7 states were mapped; Hospital/Clinics (79.7%) and Stand-alone Laboratories (20.3%). Majority (28.9%) of personnel were Nurses/Midwives, followed by Auxiliary Nurses (21.9%); Medical Specialist (14.3%) and Medical Officers (10.1%); others were CHEWs (8.1%), Medical Lab Scientist (6.3%), Lab Technicians (5.7%), Lab Assistants (3.1%); and CHOs (1.6%). Only 97 (24.6%) health workers had received training on malaria diagnosis; 43.5% of facilities offered both malaria microscopy and rapid diagnostic test (mRDT) while 17.7% and 38.8% offered only mRDT and microscopy respectively. Quality of microscopy was sub-optimal because of poorly trained personnel, re-cycling of slides and sub-optimal reagents. Overall prevalence of clinically diagnosed malaria in the outpatients was 70.3%, and malaria slide positivity rate was 78.8% using available 3-months outpatients/laboratory statistics. A good pool of human resources exists in the private sector and their capacity needs to be built on malaria diagnosis especially mRDT for non-laboratory staff. Quality of malaria microscopy could be improved with re-training of laboratory scientists and increased access to slides and good reagents. Policy framework that provides access to quality malaria diagnostic materials and creates an enabling environment is needed to support the massive scale-up of public campaigns against malaria control.

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SUCCESS OR FAILURE OF CRITICAL STEPS IN COMMUNITY CASE MANAGEMENT OF MALARIA WITH RAPID DIAGNOSTIC TESTS

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Community case management of malaria (CCMm) by community health workers (CHWs) aims to increase access to correct and timely treatment. Currently the World Health Organization recommends to treat only confirmed malaria cases, rather than to treat presumptively. In general, CCMm with rapid diagnostic tests (RDTs) is considered a good strategy, but no in-depth review of the steps determining its success or failures has been performed. We describe an in-depth review of existing literature to provide a comprehensive overview of the success or failure of critical steps in CCMm with RDTs to identify why certain programs of studies have better results with CCM than others and to identify areas for further research. Our study showed that CHWs performed RDTs correctly, although specificity levels were variable (between 83.2 and 97.9%). Also safety issues such as reusing lancets were seen in certain studies and warrant attention. CHWs showed high adherence to test results but treating patients with a negative RDT for malaria remained an issue in several areas (ranged between 0.2 and 58%). Uptake and acceptance by the community was high, but negative-tested patients did not always follow up referral advice. Information on CHW motivation was limited and needs to be further studied. In general CHWs were not satisfied with the financial compensation. However, when asked about the most motivating aspect of their work, community respect and spiritual outcome were mentioned the most. Stock outs occurred in 4 out of 5 studies that reported on these. It is generaly believed that CCM with RDT also reduced morbidity and mortality compared to CCM without RDT this could not be concluded form this study. Interestingly the preception of the pateints on morbidity showed that CCM with RDT is worse than without RDT. In addition the actual cost benefit of the intervention could not be assessed. Although several studies have looked at it the data is too heterogenic to make a firm conclusion. In conclusion, we showed that trained CHWs can deliver quality care for malaria using RDTs. However, lower RDT specificity could lead to misdiagnoses of non-malarial diseases. Other threats for CCMm are non-adherence to negative test results and low referral

completion. Integrated CCM where the CHW has more treatment options may solve this. Repeated training is key in the successful execution and subsequent treatment by CHWs.

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TESTING, TREATING AND TRACKING MALARIA IN A LOW RESOURCE SETTING: IMPROVED CASE MANAGEMENT THROUGH EMPOWERMENT OF A MEDICINES AND THERAPEUTICS COMMITTEE AT ENYIRESI GOVERNMENT HOSPITAL IN ATIWA DISTRICT, GHANA

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The World Health Organization initiative, T3: Test. Treat. Track, urges malaria-endemic countries and donors to move towards universal access to diagnostic testing and antimalarial treatment, and to build stronger malaria surveillance systems. Health providers in Ghana have practiced presumptive diagnosis and treatment of malaria for many years. Provider adherence to malaria test results is only around 50%. To operationalize the T3 initiative and achieve universal diagnosis and treatment, there is a need to improve provider adherence. Enyiresi Government Hospital, a district health facility, switched from presumptive diagnosis to treating patients based on malaria test results through strong leadership, training, and inter-departmental collaboration. Hospital leaders empowered the medicines and therapeutics committee (MTC) to implement a policy to treat suspected malaria cases only when a positive diagnosis is received from the laboratory. The MTC developed a peer review mechanism and motivated staff across departments including the outpatient department (OPD), consulting room, laboratory, and pharmacy. As a result, the hospital improved testing of suspected malaria cases from 24% in 2010 to 100% in 2012. During this time, the annual number of diagnosed outpatient department (OPD) cases of malaria declined from 10,243 (25% of total OPD visits) in 2010 to 1,707 (5% of total OPD visits) in 2012. Because of ongoing stewardship by the MTC, the hospital began monitoring the number of malaria cases treated and their outcomes and investigations for other causes of fever were redoubled in malaria test negative cases. Consequently, they noted that malaria morbidity and mortality dropped significantly across all age groups. Resources once used for treating clinically diagnosed malaria were refocused on other diagnoses and the community increased trust in medical care at the facility. Successful implementation of the T3 initiative required a strong leadership commitment, collaboration among departments, effective training, and a steady supply of malaria commodities.

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PERSISTENCE OF *PLASMODIUM FALCIPARUM* DIAGNOSTIC ANTIGENS AFTER TREATMENT WITH ARTEMISININS: ASSOCIATION WITH PARASITE STAGE AND MECHANISM OF CLEARANCE

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The most sensitive rapid diagnostic tests of *Plasmodium falciparum* are based on detection of histidine-rich protein 2 (HRP2), but HRP2 persists in the blood after treatment and cannot be used to assess treatment response. We measured antigen levels over a 4-week period in ACT-treated hyperparasitaemic patients with *P. falciparum* in Uganda and Thailand (prior to the emergence of artemisinin resistance). Antigen clearance was quantified by calculation of area-under-curve (AUC) after normalization to baseline antigen level. Whole blood HRP2 levels showed a wide range of AUCs: Uganda median 415 d·% (range 48-2588); Thailand 590 d·% (33-1534). The primary determinant of AUC was admission stage; in patients presenting with >75% tiny/small rings (32/40 in Uganda, 24/38 in Thailand), median HRP2 AUCs were respectively 6.4 and 10.7-fold higher than cases with later stages (p=0.0005, <0.0001). Later stages at baseline strongly predicted rapid HRP2 clearance (AUROC = 0.89 for the combined dataset). LDH clearance was relatively rapid and narrower in terms of AUC range: Uganda median 53 d·% (range 19-205); Thailand 76 d·% (25-141). The stage effect was also reduced with median AUCs in early ring infections only 2.0 and 1.9-fold-higher than with late stages (p=0.1665, 0.0002). All seven infections in Thailand showing long parasite clearance half-lives (>4.5h) had late rings at presentation, but paradoxically rapid HRP2 clearance. These data offer a clear explanation for the persistence of HRP2 in whole blood following artemisinin therapy. We propose that in patients who present with mostly young rings at baseline (the majority), parasites are removed by pitting in the spleen and once-infected erythrocytes return to the circulation where they provide the source for persistently high levels of HRP2 and ongoing positivity of HRP2-based RDTs. In contrast, when patients present with large rings/trophozoites, the spleen is unable to pit these larger parasite forms, the entire infected cell is removed by immune clearance or sequestration, and HRP2 clearance is rapid. Consistent with this model, in these less common infections, parasite clearance can be paradoxically slow.

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PERFORMANCE OF THREE CARESTART™ MALARIA RAPID DIAGNOSTIC TESTS AFTER REDUCTION IN MALARIA PREVALENCE IN BAGAMOYO, TANZANIA

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This study aimed at testing the diagnostic performance of three malaria rapid diagnostic tests (RDTs) (CareStart™ Malaria HRP-2, pLDH [Pf-pLDH/pan-pLDH] and HRP-2/pLDH (Pf/pan) Combo Test) with reference to light microscopy for the diagnosis of falciparum malaria in Tanzania. Blood samples were collected from 627 patients suspected to have malaria at th Miswe, and Yombo (Bagamoyo District) primary health facilities from October 2013 to December 2013. The samples were examined immediately by light microscopy and the CareStart™RDTs at the site, second reading of blood slides was done at reference laboratory. Statistical analysis was performed using Stata version 11. Overall 31 of 381 (8.1%) malaria suspected cases were detected by microscopy compared to 5 of 78(6.4%) by CareStart™ Combo kit, 8 of 80 (10.0%) by both PLDH kit and 18 of 223(8.1) by HRP-2 RDT kit. CareStart™Combo RDT kit's sensitivity and specificity for the diagnosis of malaria were 100.0% (47.8%-100%, 95% CI) and 98.6% (92.6%-100%) respectively, compared to standard microscopy. The sensitivity and specificity of CareStart™ PLDH and HRP-2 were found to be 87.5% (47.3%- 99.7%) and 100 %(81.5%-100%), respectively. The CareStart™Combo RDT had positive predictive value of 83.3% (35.9%-99.6%) compared to 50.0% (23.0%- 77.0%) for CareStart™ PLDH and 62.1% (42.3%-79.3%) for HRP-2 RDTs. All the three CareStart™ RDTs had the high negative predictive values of >=98.5% (91.8%-100%, 95% CI). In conclusion, the performances of CareStart™ RDTs had variation (CareStart™ Malaria HRP-2 and HRP-2/pLDH (Pf/pan) Combo Test) maintained high accuracy compared to pLDH [Pf-pLDH/pan-pLDH] RDT.

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MOLMALQA: A MOLECULAR MALARIA EXTERNAL QUALITY ASSURANCE NETWORK TO SUPPORT CLINICAL TRIALS

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Nucleic acid (NA) tests for malaria parasites are important diagnostic endpoints in many clinical trials of malaria vaccine and drug candidates. While many NA tests are described, there is no formalized external quality assurance (EQA) program that provides site qualification and ongoing EQA for the diverse assays in use. We recruited a network of centers performing controlled human malaria infection (CHMI) clinical trials to participate in an EQA effort. Here, we report on initial and ongoing EQA work. Each clinical site received blinded *Plasmodium falciparum*-positive and -negative samples of known parasite density that were prepared by the coordinating laboratory according to needs of each recipient site. Sites tested the samples and reported the data to the coordinating center. All sites in the first round of this effort demonstrated 100% specificity, achieved limits of detection consistent with each laboratory's expectations and demonstrated as-expected levels of within-laboratory precision (<10% coefficient of variation). Reported parasite densities generally agreed with expected values (<0.5 $\log_{10}$ parasites/mL). Where deviations from expected values were observed, root cause analyses were performed. In one laboratory, such analyses revealed reasons for observed quantitative differences and led to assay recalibration at that site. The MolMalQA effort is recruiting additional participating sites and is developing other reagents and standards to share with the malaria community. These efforts may eventually establish a dedicated long-term EQA program in support of high-quality molecular malaria diagnostics.

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TEST AND TREAT PEDIATRIC FEVERS: META-ANALYSIS OF 15 NATIONAL SURVEYS ASSESSING THE EFFECT OF MALARIA DIAGNOSTIC TESTING ON DRUGS USED BY FEBRILE CHILDREN UNDER FIVE IN DIFFERENT MALARIA RISK CONTEXTS

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In 2010, the World Health Organization revised treatment guidelines to recommend testing all suspected malaria cases prior to treatment. Studies from health facilities demonstrate reduced anti-malarial prescriptions after test introduction, and in some studies, increased antibiotic prescriptions. Many of these studies also indicate widespread non-adherence to test results. Yet, there is no evidence on how testing affects drugs used to treat pediatric fevers at the population level, nor if this effect differs by malaria risk, source of care or child's age. 15 DHS/MIS in 2009-2013 were included that collected fever prevalence, care-seeking, diagnostic test and drug use, and geocoded survey clusters to link malaria risk estimates to datasets (n=16,831 febrile under-fives taken for care). Mixed-effects logistic regression models quantified the influence of testing on three outcomes (any anti-malarial, ACT and any antibiotic use) after adjusting for data clustering and confounding socioeconomic covariates. Preliminary results suggest large country variability in malaria testing's effect on treatment patterns. In Uganda, tested pediatric fevers had 1.36 higher odds of antibiotic use (AOR: 1.36, 95% CI: 1.08-1.70) compared to those not tested, and no significant difference in any anti-malarial (AOR: 1.25, 95% CI: 0.97-1.62) or ACT use (AOR: 0.84, 95% CI: 0.67-1.07). Conversely, in Tanzania, tested pediatric fevers had significantly reduced odds of antibiotic use (AOR: 0.48, 95% CI: 0.31-0.74) and significantly higher odds of using any anti-malarial (AOR: 3.68, 95% CI: 2.38-5.68) and ACT (AOR: 1.95, 95% CI: 1.33-2.88). Further analyses will examine this association in all studied countries individually and in the pooled dataset. Results will be stratified separately by malaria risk, source of care and child's age. Findings could inform future research to better understand reasons for variability in effect across settings or population groups (Final results will be available by June 2014).

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AN ENHANCED CHEMILUMINESCENCE SLOT BLOT ASSAY FOR DETECTION OF MOSQUITO STAGE *PLASMODIUM FALCIPARUM* PARASITE

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Robust, quantitative detection of malarial antigens in preclinical and clinical phases of development as well as surveillance and epidemiological studies, particularly in the mosquito host, necessitates the development of immunoassays with superior sensitivity and reproducibility. In this work, we have developed and characterized a research-grade, quantitative enhanced chemiluminescence-based Slot Blot assay (ECL-SB) for detection of *Plasmodium falciparum* circumsporozoite protein produced in recombinant form (r*Pf*CSP) in *E. coli* and as native protein from Oocysts (*PfOocyst*) developing in the midguts of *P. falciparum*-infected *Anopheles stephensi* mosquitoes. The ECL-SB detects as little as 1.25 pg of r*Pf*CSP (linear range of quantitation 2.5-20 pg; $R^2 = 0.9505$). We also find that the earliest detectable expression of native *Pf*CSP in *Pf*Oocyst by ECL-SB occurs on day 7 post infected blood meal. The ECL-SB was able to detect approximately as few as 0.5 day 8 *PfOocysts* (linear quantitation range 1-4, $R^2 = 0.9795$) and determined that one *PfOocyst* expressed approximately 2.0 pg (0.5-3 pg) of native *Pf*CSP, suggesting that this detection limit agrees with the assay's sensitivity limit for purified r*Pf*CSP. The ECL-SB is highly reproducible; the Coefficient of Variation (CV) for inter-assay variability for r*Pf*CSP and native *Pf*CSP were 1.74% and 1.32%, respectively. The CVs for intra-assay variability performed on three days for r*Pf*CSP were 2.41%, 0.82% and 2% and for native *Pf*CSP 1.52%, 0.57%, and 1.86%, respectively. The ECL-SB also matched microscopic analysis of *P. falciparum* prevalence based upon *Pf*CSP expression on day 8 *Pf*Oocysts in two independent feeding assays, yielding estimates of 83.3% and 75% compared to microscopy's 85.7% and 80%, respectively. Thus, based on its performance characteristics, ECL-SB could be valuable not only in vaccine development and manufacturing, but also to measure the parasite prevalence in mosquito populations and in studies of transmission-blocking activities in endemic areas.

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QUALITY INSPIRED PROJECT - A KEY TO ACHIEVING RESULTS WITH MALARIA INTERVENTIONS

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With an aim to accelerate malaria case management, Tanzania Ministry of Health and Social Welfare (MoHSW) is strengthening its pre-service education program to ensure graduates have the right knowledge and

skills to diagnose and treat malaria. Investment in pre-service education lessens the burden on in-service training since those entering the workforce will have the knowledge and skills they need to provide. Jhpiego, through MAISHA (Mothers And Infants Safe, Healthy and Alive) program, provided technical assistance to the MoHSW to help develop a pre-service malaria case management-updates Learning Resource Package (LRP), which includes: Facilitator's Manual, Participant's Manual, Activity Worksheets and Training Modules addenda. The LRP was developed based on national malaria policy, guidelines and in-service training materials; it is taught using job aids, power point presentations, video demonstration and numerous case scenarios which reflect what actually happens in real life situations at service delivery points. The LRP aims at reinforcing appropriate practices for care of malaria patients and management of commodities with emphasis on parasite-based diagnosis and compliance to results, proper recording and reporting; and management of malaria in special situations and groups. The training package is well organized with laboratory and medical supplies which gives each participant an opportunity for hands-on activity to acquire and strengthen their skills. Checklists to guide Quality Assurance/Quality Improvement (QA/QI) processes have been included in these training materials. The project successfully provided competence-based orientation on malaria case management updates to 210 medical instructors. Annually, it reaches more than 4,000 students from eight Zonal Health Resource Centers and 480 students from Medical Universities. There is a need to incorporate the addenda developed into these training modules for easy use. In the near future, clinical skills-mentorship will be conducted in selected schools using the nationally approved QA/QI checklists.

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CLINICAL SIGNS AND SYMPTOMS CANNOT RELIABLY PREDICT *PLASMODIUM FALCIPARUM* MALARIA INFECTION IN PREGNANT WOMEN LIVING IN AN AREA OF HIGH SEASONAL TRANSMISSION

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Malaria in pregnancy is a major public health problem in endemic countries. Though the signs and symptoms of malaria among pregnant women have been already described, clinical presentation may vary according to intensity of transmission and local perceptions. Therefore, determining common signs and symptoms among pregnant women with a malaria infection may be extremely useful to identify those in need of further investigation by rapid diagnostic test or microscopy. Six hundred pregnant women attending the maternity clinic of Nanoro District Hospital, Burkina Faso were recruited, 200 with suspected clinical malaria and 400 as controls. Cases were matched with controls by gestational age and parity. Signs and symptoms were collected and a blood sample taken for rapid diagnostic test, microscopy and haemoglobin measurement. A multivariate model was used to assess the predictive value of signs and symptoms for malaria infection. The overall prevalence of malaria was 42.6% (256/600) while anaemia was found in 60.8% (365/600) of the women. Nearly half (49%) of the cases and 39.5% of the controls had a malaria infection (p = 0.03). The most common signs and symptoms among the cases were fever (36%,72/200), history of fever (29%,58/200) and headache (52%,104/200). The positive predictive value for fever was 53% (95%CI:41-64), history of fever 58% (95%CI:37-63) and headache 51% (95%CI:41-61). Signs and symptoms suggestive of malaria are frequent among pregnant women living in areas of intense transmission. Common malaria symptoms are not strong predictors of infection. For a better management of malaria in pregnancy, active screening to detect and treat malaria infection early should be performed on all pregnant women attending a health facility.

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PREVALENCE AND PREDICTORS OF MALARIA MISDIAGNOSIS IN HEALTHCARE SEEKING CHILDREN UNDER SIX MONTHS OF AGE IN UGANDA

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There is limited information on malaria prevalence and diagnosis among infants under six months of age. Little is known regarding how clinicians in limited resource settings diagnose malaria in this age group, given different and overlapping clinical manifestations and known protection against infection such as maternal antibodies. We identified the malaria prevalence and diagnosis practices, and predictors of malaria misdiagnosis in outpatients. We utilised data on individual outpatients under six months from 36 primary health care facilities in Uganda from January to December 2010. Multivariate logistic regression models were used to identify clinical and operational factors associated with misdiagnosis of malaria. Of the 23,587 young infant outpatients, 10,426 (44.2%) were diagnosed with malaria of which only 3,189 (30.6%) were laboratory confirmed. Of the 3,884 patients with a negative diagnostic test result for malaria, 1,213 (31.2%) were misdiagnosed with malaria. The odds of misdiagnosis were higher if the patient was one (aOR 3.64, 95%CI 1.26, 10.5), three (aOR 4.47, 95%CI 1.36, 14.7), four (aOR 6.34, 95%CI 2.03, 19.7) or five (aOR 6.86, 95%CI 1.95, 24.1) months old; had presence of clinical fever (aOR 2.64, 95%CI 1.33, 5.26); visited the health facility during a week when multivitamins were out of stock (aOR 1.62, 95%CI 1.12, 2.33) or visited a health facility with medium to high malaria endemicity (aOR 1.47, 95%CI 1.01, 2.16). The odds of misdiagnosis were lower when the patient was also diagnosed with cough/cold (aOR 0.32, 95%CI 0.19, 0.53) or urinary tract infection (aOR 0.16, 95%CI 0.03, 0.76). Triage status, clinic reattendance, health provider training, availability of antimalarial, antibiotic and oral rehydration drugs, and diagnosis with anaemia, pneumonia and diarrhoea did not have an effect on odds of misdiagnosis. In conclusion, we found high rates of malaria prevalence and misdiagnosis in young infants. Clinicians were less likely to trust malaria test results when the infant was older or had fever or when health facility had medium to high malaria endemicity or lacked multivitamins. Clinicians were more likely to trust malaria test results when other diagnoses were made. These findings can inform future interventions to improve the quality of malaria care in infants.

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QUALITY IMPROVEMENT OF MALARIA DIAGNOSTICS WITH RAPID DIAGNOSTIC TESTS WITH THE DEKI READER IN TANZANIAN MILITARY HEALTH FACILITIES

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Malaria is the major cause of mortality and morbidity in Tanzania, where over 90% of the population is at risk for infection. Malaria diagnosis remains problematic, especially in resource-challenged settings. The use

of malaria Rapid Diagnostic Tests (mRDTs) has become a useful alternative to microscopy for diagnostic purposes, however its quality control (QC) is needed to ensure accurate diagnosis. In Tanzania we have successfully used the Deki Reader (DR) as a means of addressing diagnostics quality improvement issues through remote monitoring. The DR is a rugged, mobile *in vitro* diagnostic device which interprets commercially available RDTs. The DR automatically captures and securely transfers patient diagnostic data, along with the RDT image, to a centralized database over mobile phone networks. All results from the field are accessible from anywhere in the world in real-time by logging into a password protected web portal. Since 2012 DRs have been deployed at five camps overseen by the Tanzania People's Defence Forces (TPDF). Malaria RDT data and images were monitored remotely for quality assurance on a regular basis. When quality issues were detected monitors communicated with users at the testing facilities to investigate and troubleshoot problems. In this ongoing study, the DR was instrumental in remotely identifying false positive and negative mRDT interpretations made by users. Errors were due to either misinterpretation of mRDT weak lines or improper RDT preparation. Initially, approximately 5.5% of all mRDTs processed were incorrectly interpreted by users. After 6 months of remote QC monitoring and interaction with users, the problems decreased to 2.5%. Similarly, RDT preparation problems decreased from 2.3% to 0.1%. In summary, the use of DR reduced quality issues with RDTs, decreased cost of physical site visits, improved the malaria surveillance mechanism and improved patient care. The DR has proven to be a valuable tool for quality assurance of mRDT use and may be useful in other similar settings for malaria control// elimination efforts.

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MALARIA DIAGNOSTIC AND MONITORING IN REMOTE RIVERINE VILLAGES IN THE AMAZON BASIN DURING ITINERANT MEDICAL MISSIONS

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Malaria affects primarily people living in remote tropical settings. In the Amazon Basin, malaria is concentrated in the riverine villages with limited access to healthcare and diagnostic capacities. Therefore, innovative strategies are needed to deliver diagnostics and treatment to these communities. We describe the contributions of two itinerant riverine medical missions to malaria control. Malaria diagnostics and treatment was provided at two riverine medical missions: 1) the Amazon Hope ship lead by the VineTrust since 2002, and 2) the Rio Napo ship of the Peruvian Navy, which started trips in the Napo River during 2013, as part of multiple medical and social services. NAMRU-6 supports the diagnoses and molecular characterization of cases providing equipment, materials and training. Blood smears and filter paper samples were collected from all suspect cases for malaria diagnosis by microscopy and PCR, respectively. The sensitivity and specificity of microscopy were calculated using PCR as the gold standard. Diagnosis was provided in 20 trips of the Amazon Hope ship in the Marañon, Amazonas, Ucayali, Puinahua and Tigre river basins. A total of 793 febrile cases were tested by microscopy, and 80 (10%) were malaria positive. Three quarters were due to *Plasmodium vivax*. The mean age was 23 years old and 60% were men. The Rio Napo Ship conducted three trips between July and December 2013, and tested 156 febrile cases for malaria. Microscopy diagnoses identified 46 (29%) positives, 26% due to *P. vivax* and 3% due to *P. falciparum*, respectively. The prevalence of *P. vivax* and *P. falciparum* by PCR was 28% and 5%, respectively. Compared to PCR, the sensitivity of microscopy for *P. vivax* was 89% and for *P. falciparum* was 57%, while the specificity for both species was 98%. Itinerant medical ships can extend malaria diagnostic capacities and surveillance to remote, hard to reach areas. Participation and cost-effectiveness can be improved providing training, basic diagnostic resources and other medical and social services.

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PECADOM PLUS: AN ACTIVE, COMMUNITY-BASED APPROACH TO MALARIA DETECTION AND TREATMENT

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The region of Kedougou has the highest prevalence of malaria in Senegal, and geographic, educational and financial barriers often impede rural populations from accessing care. A home-based care model (PECADOM) has been implemented in Senegal since 2008, addressing many of these barriers to care and care seeking. Volunteer, home-based care providers (DSDOMs) perform rapid diagnostic tests (RDTs) for malaria and administer treatment for cases of uncomplicated malaria, referring complicated cases. However, this passive model relies on patients seeking care from DSDOMs. To reduce barriers to treatment seeking, Peace Corps and the Saraya Health District have piloted an active case finding model in which DSDOMs were paid to conduct weekly sweeps of every household in their village to actively seek patients with symptoms of malaria. RDTs were undertaken on those with symptoms, with free treatment provided for positive cases. The model was implemented in fifteen villages from July through November 2013, the period of highest malaria transmission. Fifteen comparison villages were chosen among villages with the original, passive PECADOM model, and three sweeps were conducted in these villages to estimate and compare prevalence of malaria at the beginning, midpoint and end of the active model pilot. At baseline, point prevalence of symptomatic malaria confirmed by RDT for the total population was 1.1% in both sets of villages (p=.79). During the midline comparison sweeps, the point prevalence was 2.5 times higher (p=.007) in the comparison villages (2.9%) than in the intervention villages (1.1%). At endline, the point prevalence in comparison villages (2.5%) was nearly 16 times higher (p=.003) than in the intervention villages (.16%), where only 6 cases of symptomatic malaria were found. This pilot study shows that this model of active weekly sweeps by community health workers is both feasible and effective in increasing access to care and reducing malaria prevalence. Due to these promising results, PECADOM Plus will be scaled up to the entire region of Kedougou in 2014.

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QUALITY IMPROVEMENT AND NOVEL USE OF MALARIA DIAGNOSTICS IN VIETNAM

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The National Malaria Control and Elimination Program in Vietnam is currently planning to eliminate malaria by 2030. They are currently conducting baseline assessments and developing methods for malaria diagnostic quality improvement. Quality malaria diagnostics are essential for malaria elimination efforts. False negative diagnostic results in both symptomatic and asymptomatic infected individuals will increase the malaria transmission reservoir. False positive results (malaria is not really

present) will result in misapplication of limited resources for malaria elimination operation. NIMPE in collaboration with UCSF are developing a quality improvement (QI) program for malaria diagnostics to expedite malaria elimination. In this program, for both case management and screening, the necessary and most cost-effective approaches for baseline assessments and QI will be developed and implemented with the objective of minimizing false negative results. Malaria microscopy is currently used as standard of care for malaria diagnostic in Vietnam. For microscopy, we will assess the effectiveness and cost effectiveness of the cross-checking system currently in place. In a sample of sites, we will assess the accuracy of reporting at each level and estimate how many cases are not being reported. We will assess the competency of microscopists and identify the most cost effective ways to improve competence. We will inspect a sample of laboratories and make suggestions for improvement. Rapid diagnostic tests have been introduced in areas with high malaria endemicity and remote areas with difficulty in access to health service. Similar activities will be conducted at a sample of these sites. For case confirmation/foci investigation, the simplest, fastest and least expensive approaches will be developed to confirm positive microscopy. The initial results from this effort will be available in August 2014 for presentation. These results will be compared and contrasted with our recent experiences with diagnostics QI in Tanzania.

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TRANSFORMING ARMY DIAGNOSTICS LABORATORY INTO A RESEARCH READY FACILITY, IMPROVEMENT IN QUALITY ASSURANCE; RUVU JKT CASE STUDY

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Malaria continues to be one of the diseases affecting many U.S. and Coalition personnel deployed in Sub Sahara Africa. It is responsible for over 100 million reported cases annually and 1-2 million deaths, especially in children. Diagnosis of malaria is based on clinical symptoms which are non-specific, leading to false diagnosis and over use of anti-malarial drugs, increasing the potential of drug resistance, as well as the number of malaria cases. Within Tanzania microscopy continues to be a challenge with the Tanzania People's Defense Force (TPDF) and Tanzania National Service Program (JKT) medical services. Amethyst Technologies LLC (ATL) with its partners developed a comprehensive malaria microscopy and Rapid Diagnostic Testing assessment program to capture information for strengthening of the quality of Malaria Diagnostics at Ruvu JKT as a potential research site. The need to develop quality malaria diagnosis at Ruvu JKT was critical in getting the site laboratory ready for malaria research. Information on infrastructure, safety, human resources, training, and diagnosis was obtained in order to establish the minimum guidelines and requirements for quality malaria microscopy. The assessment came up with a quality management plan to provide on-going quality improvement of malaria microscopy and enabled the research team to estimate the amount of malaria transmission. We will present data obtained through continues training, onsite corrective actions supporting activities and discuss how these data were used to develop our Quality Management Program at Ruvu JKT for research readiness.

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MODULAR DESIGN OF ANTIPARASITIC CHEMOTHERAPEUTICS USING PRENYLATION AS NEW TOOL IN DRUG DISCOVERY

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One of seven people in the world is affected by Neglected Tropical Diseases. Treatments of these diseases are insufficient and inefficient and require constant development of new drugs and strategies. Within the universe of small organic molecules, the isoprenoids are the most important and numerous families of metabolites. In this regard, it is useful to focus on the design, synthesis and studies of molecular probes that interfere with different biosynthetic routes, particularly the isoprene and sterol pathways. The implemented strategies used modern medicinal chemistry approaches like diversity-oriented synthesis, parallel solution library preparation, conventional multistep synthesis, midthroughput screening, bioinformatics and computational chemistry, allowed to generate more than 100 new compounds. The generated libraries were tested against the parasites responsible of the malaria (*Plasmodium falciparum* chloroquine sensitive and resistant strains), visceral leishmaniasis (*Leishmania donovani*), HAT (*Trypanosoma brucei)* and Chagas´ disease (*T. cruzi*). As result of our effort we have found very promising new hits to develop drugs against malaria and other diseases. Our strategy also included action mechanism studies of the lead compounds. To do that, fluorescent tagged isoprenes were synthesized to be incorporated on the lead structure to perform cellular localization by fluorescent microscopy.

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EVALUATION OF THE PROPHYLACTIC ACTIVITY OF ORAL 8-AMINOQUINOLINE DERIVATIVES IN C57BL/6 MICE USING AN *IN VIVO* IMAGING SYSTEM

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As anti-malarial drug resistance escalates, new safe and effective medications are necessary to prevent and treat malaria infections. The US Army is developing 8-aminoquinoline (8-AQ) analogues with improvements in hemolytic toxicity, which is expected to be safer in preventing malaria in deployed military personnel. To compare the prophylactic efficacy of these compounds, a transgenic *P. berghei* parasite expressing the bioluminescent reporter protein luciferase was utilized to visualize and quantify parasite development by using a real-time *in vivo* imaging system (IVIS) at 24, 48, and 72 hours post infection. Two standard compounds of 8-AQ, tafenoquine (TQ) and primaquine (PQ) were treated with single or multiple regimens. As an additional endpoint, blood stage parasitemia was assessed periodically over the next 30 days by flow cytometry. C57BL/6 Albino mice were infected intravenously with 50,000 sporozoites by tail vein and treated orally with three multiple doses of TQ and PQ at their minimal curative doses of 5 and 25 mg/kg, respectively. The outcome of this treatment showed no bioluminescence liver signal and no blood stage parasitemia was observed, suggesting both drugs showed 100% causal activity at the doses tested. Single dose oral treatment with 5 mg TQ or 25 mg of PQ, however, yielded different results as only TQ treatment resulted in causal prophylaxis in *P. berghei* sporozoite-infected mice. TQ is highly effective for causal prophylaxis in mice at a minimal curative single oral dose of 5 mg/kg, which is a five-fold improvement in potency versus PQ. We also tested these drugs in a more sensitive C57BL/6 wild-type strain (black) where mice were infected with only 10,000 sporozoites and treated orally with three multiple doses of TQ at 3 mg/kg or with PQ dosed orally at 15 mg/kg. None of the mice demonstrated a bioluminescence liver signal and no blood stage parasitaemia was observed suggesting both drugs showed 100% causal

activity at the doses tested. Accordingly, we conclude TQ is highly effective for causal prophylaxis in C57BL/6 wild-type mice at a minimal curative single oral dose of 3 mg/kg, which is also a five-fold improvement in potency versus PQ where the minimal curative dose required is 15 mg/kg. Both stains are working well to screen the prophylactic activity of oral 8-aminoquinoline derivatives in liver-stage, but the C57BL/6 wild-type mice are more sensitive than C57BL/6 albino animals.

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LONG-TERM PROPHYLAXIS AND PHARMACOKINETIC EVALUATION OF A SINGLE INJECTION OF INTRAMUSCULAR DECOQUINATE IN MICE INFECTED WITH *PLASMODIUM BERGHEI* SPOROZOITES

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This study was conducted to evaluate the prophylactic efficacy and pharmacokinetics of an intramuscular (IM) depot formulation of decoquinate (DQ) into mice infected with *Plsmodium berghei* sporozoites. Due to the poor bioavailability of oral DQ, a novel drug dispersion technique was utilized to create DQ microparticles suspended in an oily vehicle to retard drug release suitable for long-term malaria prophylaxis. To evaluate the depot formulation, pharmacokinetic studies in normal animals and antimalarial efficacy in liver-stage malaria mice were conducted following single IM-DQ injections at 60, 120 and 240 mg/kg for 2, 4, 6, or 8 weeks prior to infection with *P. berghei* sporozoites. The liver stage efficacy evaluation was monitored by using an *in vivo* imaging system (IVIS). Efficacy results showed that 100% of animals were protected from *P. berghei* infection after injection with a single dose of depot DQ at 120 and 240 mg/kg administered 8 weeks prior to sporozoite inoculation. The 120 mg/kg IM dose is the minimal prophylactic dose required to obtain causal prophylaxis of malaria sufficient for a period of 8 weeks. In addition, a significant increase in the elimination half-life of the depot DQ (1,484 hrs) was achieved compared to that of orally dosed plain DQ (8.3 hrs). The depot IM formulation with slow-release mode provided release of very low and constant drug concentrations in the plasma and liver resulting in a minimal inhibition concentration (MIC) at 5-6 ng/ml in plasma and 70-90 ng/g in the liver. General safety and injection site tolerability were also assessed longitudinally to examine any issues with drug toxicity that may potentially preclude the use of this formulation in man. Modest body weight changes were found after IM dosing not only in the animals treated with IM-DQ, but also in mice treated with the vehicle control which may be a consequence of animal handling. Depot DQ injections up to a single intramuscular dose of 4,800 mg/kg, 40-fold higher than the dose required for treatment, were well tolerated in mice and final evaluations of toxicity will be conducted through histopathological monitoring. Further development of a depot IM-DQ formulation for malaria prophylaxis will be needed to deliver even longer periods of malaria prophylaxis protection beyond the 8 weeks achieved in this study.

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EXPERIMENTAL STUDY OF THE RELATIONSHIP BETWEEN *PLASMODIUM* GAMETOCYTE DENSITY AND INFECTION SUCCESS IN MOSQUITOES; IMPLICATIONS FOR THE EVALUATION OF MALARIA TRANSMISSION-BLOCKING INTERVENTIONS

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The evaluation of *Plasmodium* transmission blocking interventions (TBIs) such as vaccines or drugs to control malaria widely uses mosquito membrane-feeding assays. In these experiments, the intensity of *Plasmodium* infection within the anopheline vector is expected to affect the measured efficacy of the molecules to block transmission. Gametocyte density in the host blood is a broad determinant of the infection success in the mosquito; however, uncertain estimates of parasite densities and intrinsic characteristics of the infected blood can induce variability. To control this variation in TBI evaluation, a feasible method is to dilute infectious blood samples, ensuring that gametocyte numbers are directly comparable between parallel samples. To examine this in detail, we describe the effect of diluting *Plasmodium* gametocyte infected blood and the subsequent impact on mosquito infectivity and TBI efficacy, for two parasite-vector combinations: *Plasmodium falciparum/Anopheles gambiae* and *Plasmodium berghei/Anopheles stephensi*. We additionally examined how blood dilution influences the observed blocking activity of mAb 13.1 against Pbs28 of *Plasmodium berghei*. In the natural species combination (*P. falciparum/An. Gambiae),* blood dilution revealed a positive linear relationships between gametocyte density and oocyst load. Similar relationships was observed in the *P. berghei / Anopheles stephensi* system when using heat inactivated blood as a solvent, whereas diluting infected mice blood with fresh uninfected blood substantially increases infectiousness. This suggests that highly infected mice blood contains inhibitory factors or reduced blood moieties, which impedes infection and may lead to misinterpretation of TBI evaluation assays. In the lab-based system, the transmission blocking activity of mAb 13.1 was confirmed to be density-dependant. This data highlights the need to perform evaluations of TBI candidates carefully, at gametocyte densities and infection intensities that are broadly translatable.

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ANTIMALARIAL ACTIVITY OF NOVEL HARMINE-DERIVED HEAT SHOCK PROTEIN 90 INHIBITORS

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The emergence of multi-drug resistant *Plasmodium falciparum* strains poses a serious challenge in the control of malaria and could have catastrophic outcomes. This necessitates development of effective, safe and affordable new drugs. Previous studies have shown that the natural beta-carboline alkaloid compound, harmine, is a promising antimalarial targeting the *P. falciparum* heat shock-90 (PfHsp90) enzyme. In this study, we have developed a microwave-assisted synthetic route to generate analogs of harmine and have tested their antimalarial effect both *in vitro* and *in vivo*. Out of 45 harmine-derived compounds tested, two (17A and 21A) bound to PfHsp90 with EC50 values of 12.2 ± 2.3, and 23.1 ± 8.8μM, respectively. These compounds also inhibited *P. falciparum* W2 with IC50 values of 1.3 ± 0.027 and 1.4 ± 0.07μM, respectively. *In vitro* cytotoxicity assay showed that both compounds are not toxic. The efficacy of these compounds *in vivo* was assessed using *P. berghei* infection of BALB/c mice. Three daily injections of infected mice with 100mg/kg of

each of 17A and 21A showed significant reduction in parasitemia as compared to the vehicle control. Compounds 17A and 21A resulted in reduction of parasitemia by 51.5% and 56.1%, respectively. Mice treated with 17A and 21A showed a median survival time of 11 and 14 days, respectively while the vehicle control mice showed a median survival time of only 8.5 days. Log-rank test indicated that the survival of mice treated with 21A was significantly higher than vehicle control mice. In summary, we have identified a novel, non-toxic harmine derivative able to bind PfHsp90, inhibit *P. falciparum* W2 *in vitro* at micromolar concentrations and reduce parasitemia in the *P. berghei* mouse model. 21A also improved survival of infected mice, a trait not observed in the parent molecule. This could be attributed to the changes made on harmine backbone. Further dose-ranging studies of 21A are required coupled with development of focused libraries based on the current lead structure.

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DEVELOPMENT OF ANTIMALARIALS WITH SERCAP PROLIFE FROM DOS DERIVED COMPOUNDS

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In the last decade, hundreds of small molecules with previously unknown antimalarial activities were identified, but the drug discovery and development pipeline still lacks compounds with non- erythrocyte stage activity and target diversities. We screened and identified a novel series of compounds derived from Diversity-Oriented Synthesis (DOS) with potent activity against blood-stage *Plasmodium falciparum*. Importantly, the compounds are equipotent against a panel of *Plasmodium* field isolates with resistance to clinically relevant therapeutics, suggesting that these compounds have a novel mechanism of action compared to current therapeutics. The compounds are active in *in vitro* assays for liver-stage parasites (*P. berghei*) and late-stage (IV & V) gametocytes (*P. falciparum*). Intriguingly, the compounds also show *in vitro* activity against the liver stage (small forms) of P. cynomolgi. Finally, compounds in this series showed excellent *in vivo* efficacy in a *P. berghei* mouse model. This profile is consistent with the target product profile envisioned for Single Exposure Radical Cure and Prophylaxis (SERCaP) for treatment of uncomplicated malaria.

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DEVELOPMENT OF A NOVEL PRECLINICAL ANTIMALARIAL CANDIDATE

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Malaria is a devastating disease, leading to several hundred million clinical cases and over 600,000 deaths each year. There is an urgent need to develop new drugs to treat this disease, to counter resistance to current drugs and to expand the range of clinical indications which can be tackled. This includes the need for single-dose treatment, transmission blocking, chemoprevention and treatment for relapsing malaria. Here we report the discovery and development of a potential new antimalarial agent. The starting point for this project was a phenotypic screen carried out against *Plasmodium falciparum* at the University of Dundee, UK. Several series were identified and one of these was optimized to a compound which fulfilled the Medicines for Malaria Venture criteria for a late lead compound. This compound was extensively profiled in a large number of assays and has now been progressed into preclinical development with the aim of entering human clinical trials. This pre-clinical candidate shows promise as a possible single-dose treatment in combination with another antimalarial and demonstrates both transmission blocking and chemoprevention potential.

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PROGRESS TOWARDS MALARIA ERADICATION: NEW SCREENING ASSAYS AND NEW COMPOUNDS WITH ANTI-GAMETOCYTE ACTIVITY

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Drugs able to inhibit *Plasmodium falciparum* gametocytes and thus malaria transmission are strongly needed to achieve the goals of the malaria elimination/eradication agenda. Here, we describe two microtiter screening assays, based on different viability markers and read-outs: the pLDH assay, based on the spectroscopic detection of the gametocyte lactate dehydrogenase (pLDH) activity, and the LUC assay, performed using a parasite strain which expresses a novel luciferase enzyme (LUC1-G) under the control of a promoter specific for gametocytes. The assays have been validated and show good Z' factor and signal to background ratio. Both assays have been used to screen the "MMV-Malaria box" library. A primary screening at a single dose of 3.7 µM was performed with the pLDH assay, measuring parasite viability at two different time points: 72h post-treatment (72 h assay) and 144 h. In the latter case, the compounds were removed from the cultures, which were incubated for further 72h (72+72h assay). The dual checkpoint assay allows the identification of gametocytocidal compounds with fast versus slow speed of action. Seven out of 400 Malaria box compounds inhibited gametocyte viability by more than 50% already after the first 72h incubation; fifteen compounds showed an activity higher than 75% at 144h. Based on the results of the primary screening, thirty-six compounds with activity higher than 50% at 144h were selected for dose-response experiments and IC50 determination in both assays. Seven compounds showed an IC50 lower than 1 µM with both methods. A good correlation between the IC50 results with the two assays was obtained. In conclusion, the pLDH and LUC assays can be used as fast and cheap screening methods for the identification of novel gametocitocydal compounds and to investigate their mode of action (fast versus slow). The evaluation of potential transmission blocking agents selected from other libraries or newly synthesized is on going and results will be ready in the Fall of 2014.

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PHOSPHOLIPASE A2 INHIBITION IN *PLASMODIUM FALCIPARUM*: A POTENTIAL NOVEL ANTIMALARIAL STRATEGY

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Plasmodium falciparum causes millions of infection worldwide, including severe disease, mainly in children residing in sub Saharan Africa. Currently, artemisinin based combination therapies (ACT) are the treatment of choice for the majority of malaria endemic countries. However, resistance

to artemisinin has been reported, which may impact the future efficacy of ACTs. Thus, it is imperative to identify new drug candidates with antimalarial activity. We present data for a potentially novel antimalarial drug target. Phospholipases A2 (PLA2) hydrolyze phospholipids to lysophospholipids, fatty acids, and arachidonic acid, a precursor to leukotrienes and prostaglandin production, which are mediators of inflammation. This enzyme family includes secretory (sPLA2), calcium independent (iPLA2) and cytosolic PLA2 (cPLA2) members that carry out a range of functions, including antimicrobial activity, signaling and metabolism. We identified a putative PLA2 encoded in the *P. falciparum* genome. The open reading frame includes the GXSXG and DXG/A conserved catalytic motifs and its predicted molecular weight of 78kDa is consistent with either the iPLA2 or cPLA2 classification. To determine if PLA2 inhibition of *P. falciparum* alters parasite growth we carried out *in vitro* drug susceptibility assays against a panel of PLA2 inhibitors. We have found that AACOCF3, which inhibits cPLA2 has a 50% of inhibitory concentration (IC50) in multiple *P. falciparum* strains including, 3D7 (10.8 μM), Dd2 (15.2 μM) and HB3 (7.4 μM). PLA2 inhibitors against sPLA2 or against iPLA2, did not inhibit parasite growth. Detailed microcopy studies demonstrate that the antimalarial effect of AACOCF3 takes place during the trophozoite stage of the parasite's life cycle. To determine the target pathway of pfPLA2 we will carry out metabolic labelling with 13C-choline, in the presence and absence of AACOCF3 in infected and uninfected erythrocytes. This data will describe a previously unexplored aspect of parasite biology and may inform a novel antimalarial strategy

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PHYTOMEDICINES FROM *WARBURGIA UGANDENSIS* AND *ZANTHOXYLUM USAMBARENSE* AGAINST *PLASMODIUM KNOWLESI*

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Malaria affects about 500 million people annually with a mortality of up to 2.7 million. The high cost of effective treatment hampers malaria control. In Africa, about 75% of the population either does not have access to or cannot afford conventional medicine and therefore traditional medicine for malaria treatment is an alternative, as reported previously. *Warburgia ugandensis* Sprague (Canellaceae) and *Zanthoxylum usambarense* (Engl.) Kokwaro (Rutaceae) are commonly used as traditional medicine by many communities in Kenya (Njoroge and Bussmann, 2006). Both plants grow in both highland and lowland areas especially in forests around Nairobi, Masaai Mara, Samburu, Southwest of Mt. Kenya and Kakamega, as reported previously. Hot and cold decoctions from leaves and stem barks of *Warburgia ugandensis* are culturally used to treat tooth decay, asthma and bronchitis while *Zanthoxylum usambarense* is used to treat malaria, upper respiratory tract infections, tooth decay and sore gums. Phytochemical studies on *Zanthoxylum usambarense* revealed that it contains alkaloids of tetrahydroprotoberberine type, as reported previously. Moreover, its *in vitro* antimalarial activity against *Plasmodium falciparum* has been reported.The aim of this study was to determine the anti-plasmodial activities of extracts from *Warburgia ugandensis* and *Zanthoxylum usambarense* against the fifth human malaria parasite,*Plasmodium knowlesi*. *Plasmodium knowlesi* is widely distributed in parts of Kenya, Malaysia, the Philippines, Myanmar and Thailand, and can also be fatal, presenting an urgent need for more focused investigations on its control. Eight plant extracts were screened for *in vitro* anti-plasmodial activity *against Plasmodium knowlesi*, in a 96-well plate incubated at 37°C on a RPMI culture medium supplemented with Baboon serum. Inhibitory concentrations (IC_{50}) values of between 3.14 and 75 μg/ml, up to 69% chemosuppression of parasites growth and over 80% survivorship of treated mice were observed. The two medicinal plants, *Warburgia ugandensis* and *Zanthoxylum usambarense* possess bioactive compounds against malaria parasites and could be exploited for further development into malaria therapy.

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LEAD OPTIMIZATION OF BROAD-SPECTRUM ANTIMALARIAL ACRIDONES

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We have previously reported the discovery of a novel antimalarial acridone chemotype that displays efficacy against sporozoite-induced *Plasmodium* infection in addition to efficacy against blood stage parasites. We have been successful in producing extremely potent new lead candidates with pico molar IC_{50} values against MDR resistant parasites, as well as full protection of liver stage infection at comparable dosage with primaquine. Details of the design, chemistry, structure-activity relationships (SAR), safety, metabolic studies, and mechanism of action will be presented.

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TARGET IDENTIFICATION EFFORTS OF NOVEL ANTIMALARIALS FROM DOS LIBRARY

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Given that resistances to all drugs in clinical use have arisen, discovery of new anti-malarial small molecules that act on novel targets and/or exhibit novel mechanisms of action is urgent necessity. Using blood stage *Plasmodium falciparum*, we screened approximately 100,000 small molecules of a diversity-oriented synthesis (DOS) library, which were designed to provide more structural complexity than traditional compound library. Among these unique compounds not related to existing pharmacophores, BRD3842 and BRD3444 were selected as lead candidates based on their good potency at not only blood stage but also liver and gametocyte stages and *in vivo* efficacy using *P.berghei*. BRD3842 has a spirocyclic carboline structure with one sterocenter, showing a characteristic biphasic dose response curve in blood stage assay (EC50s: 60nM & 700nM); the enantiomer is not active. When 100nM was applied, BRD3842 inhibited parasite growth at schizont stage and reduced production of next generation, while the compound at higher dose (5μM) arrested the parasites at the ring/early troph stage. BRD3444 has an azetidine-fused eight membered ring structure with three stereocenters, two of which are critical to its activity based on SSAR analysis. The EC50 for blood stage is 15nM, and the compound are potent to all stages including early ring stage that artemisinin is not very effective. In order to identify their targets and mechanism of action by genomic approach, we performed resistant selection by exposing parasites with the compounds for short period intermittently. Resistant lines to each compound were evolved in two (BRD3842) or three (BRD3444) independent flasks, exhibiting 3.5- and 10-fold shifts and 3.4-, 13- and 67-fold shifts in EC_{50} values correspondingly. Multiple clones were then isolated from each selection, and whole genome was sequenced and analyzed to identify non-synonymous SNPs. Further studies including copy number variations analysis, target validation, drug phenotype analysis will be discussed.

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PROGRESS IN THE PRECLINICAL DEVELOPMENT OF ANTIMALARIAL DM1157

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We are developing a class of hybrid molecules made from a chloroquine-like moiety and a chemosentisizer (reversal agent) against chloroquine-resistance (CQR). These molecules have been shown to have low-nanomolar *in vitro* IC_{50} values against all strains of malaria tested so far, whether chloroquine-sensitive or -resistant, often surpassing the activity of even chloroquine against chloroquine-sensitive strains of *P. falciparum*. Here we report on the selection of a lead compound, DM1157, and its progress in the preclinical development pathway. This update will cover results from *in vitro* evaluations (including off-target activity), rodent toxicology and pharmacokinetics, and the initial second-species work. Also, aspects of the scale-up chemistry for GMP-production will be discussed.

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TARGET IDENTIFICATION OF TWO CHEMICALLY DIVERSE DRUG-LIKE COMPOUNDS USING CHEMOGENOMICS

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We aim to identify the mode of action of the most prominent compounds from whole-cell *Plasmodium falciparum* screens of chemically diverse libraries. We have used a chemogenomic approach whereby resistant parasites are generated by *in vitro* selection under drug pressure and whole genome sequencing is employed to identify the genetic basis of resistance. Independent selections acquiring mutations in the same gene or pathway indicate candidate determinants of the resistance phenotype. Study compounds were selected based on availability, purity, potency in a multi-drug resistant strain, and lack of known mechanisms of action such as mitochondrial function and folate biosynthesis. To further eliminate overlap with known targets, we performed cross-resistance testing against drug resistant lines with well-characterized mutations in dihydroorotate dehydrogenase, heat shock protein 90, prolyl tRNA synthetase and PfATPase4. Two chemically distinct drug-like compounds, MMV006767 and MMV007564, did not exhibit cross-resistance in any of the assays employed indicating they inhibit novel targets. To gain more insight into the compounds' mechanism of action, MMV006767- and MMV007564-resistant lines were generated *in vitro* by intermittent and continuous selection methods. Resistant parasites emerged with 3- to 12-fold EC_{50} shifts. Whole genome sequencing revealed that MMV007564-resistant lines from 3 independent selections have novel mutations in the Pf cyclic-amine resistance locus (*Pfcarl*). PfCARL, a conserved protein with unknown function, has recently been shown to be the target of imidazolopiperazines – a class of compounds distinct from MMV007564. Given their diverse chemotypes, these results suggest that PfCARL can act as a common resistance mechanism in the parasite. Our findings may also reveal new structure-activity relationship for GNF156 – an imidazolopiperazine currently in Phase II clinical trials. Further characterization of the mode of action of MMV007564 as it relates to PfCARL and whole genome sequencing for MMV006767-resistant lines are underway.

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INHIBITORS OF UBIQUITIN E3 LIGASE AS POTENTIAL NEW ANTIMALARIAL DRUG LEADS

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Malaria is a deadly disease caused by parasites of the genus *Plasmodium* and transmitted by female *Anopheles* mosquito. Malaria is curable if diagnosed early and treated appropriately. According to a recent WHO estimate, every year 219 million cases (range 154–289 million) and 660 000 deaths (range 490 000–836 000) occur every due to malaria. As the parasite has developed resistance for the most of the currently used antimalarial drugs, there is an urgent need to identify new molecular targets and to find new leads against those molecular targets. The ubiquitin/proteasome pathway is the principal system for degradation of proteins in eukaryotic. Ubiquitin is a highly conserved 76 amino acid polypeptide that covalently attaches to target proteins through the concerted action of three classes of enzymes: the ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2) and ubiquitin-protein ligase (E3). The endoplasmic reticulum associated degradation (ERAD) system of *Plasmodium falciparum* is essential for the survival and maintenance of the parasite. The ERAD system is composed mainly of three proteins: ubiquitin-activating E1 enzyme (UBA1), ubiquitin-conjugating E2 enzyme (UBC), and *Plasmodium* ubiquitin E3 ligase (HRD1). The protein encoded by Pf14_0215, a putative E3 ubiquitin ligase, has been localized to reticular structures in the trophozoite and schizont stages of the parasite. In the late schizont stage PF14_0215 proteins reside within globular structures surrounding each budding merozoite. PF14_0215 E3 ubiquitin ligase resides in the ER membrane and is essential for the parasite survival. We screened a set of selected standard E3 ubiquitin ligase inhibitors *in vitro* against *Plasmodium falciparum* cultures. Among these, JNJ- 26854165 and HLI 373 showed significant activity against both D6 and W2 strains of *P. falciparum*. The compounds were simultaneously tested against Vero cells for mammalian cell cytotoxicity. The active compounds JNJ-26854165 and HLI 373 were significantly less toxic to mammalian cells as compared to *Plasmodium*. Thus, these lead compounds showed a good selectivity as antimalarials. Further investigations on the E3 ligase inhibitors promise new understanding of the importance of E3 Ligase functions in the malaria parasite, and may provide new classes of antimalarial drug leads.

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MAPPING OUT OF ANTIMALARIAL DRUGS ON STOCK AT THE MARKET IN A RURAL DISTRICTS OF GHANA

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Antimalarial drugs are a very important component of any policy for effective reduction of morbidity and mortality related to the malaria disease. The availability of efficacious and high quality antimalarials and their correct use can mitigate the risk of morbidity and mortality among the people of sub-Saharan Africa who have the highest risk of contracting and dying from malaria. Chemical (medicine) shops are major source of care for most developing countries where anti-malarial drugs can be purchase at the counter. The paper seeks to identify the different kinds of anti-malarial drugs on the market for malaria treatment in a rural district in Ghana. A structured questionnaire was used during two seasons (peak and low malaria transmission seasons) to collect information on anti-malarial drugs from all 58 chemical shops within the Dangme West district now (Shai Osudoku and Ningo Prampram districts). Pictures of the anti-

malarial drugs were taken, the active ingredients, and also the source of the drugs documented. GIS locations of the shops were also recorded to ascertain the proximity of the shops to households in the communities. Majority (72.0%) of the chemical and pharmacy shop owners are males. Only 7.0% of the shops are pharmacy while the remainder is licensed chemical shops. The total numbers of antimalarial drugs counted were forty nine (49). Among the stock, 4.2% were quinine, 31.9% of them were monotherapies such as artemether, Amodiaquine, Artesunate etc. Altogether, 59.4% of the artemisinin combination therapies (ACTs) were artemether + Lumefantrine, 25.0% were Artesunate + Amodiaquine. Other antimalarials observed were 9.4% Sulfadoxine + Pyrimethamine and 3.1% of of Artesunate + Sulfamethoxypyrazine + Pyrimethamine. About 47% of the anti-malarial drugs were pediatric formulations. GIS mapping shows that majority of the households are within a periphery of 5km to a chemical shop. The national antimalarial drug policy recommends the use of ACTs for malaria treatment however; all sorts of anti-malarial drugs which are not ACTs are in stock at the chemical shops in Ghana. Chemical shops are closer to households and play a very important role in the treatment of malaria hence there is the need to train chemical sellers to stock and administer the recommended antimalarials.

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AN EFFICIENT PRIMARY HUMAN HEPATOCYTE CULTURE PLATFORM FOR ASSESSING DRUG AND VACCINE EFFICACY AGAINST *PLASMODIUM* PARASITES

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Malaria remains one of the world's most resilient public health problems, fighting the spread of this disease has been hampered due to a poor understanding of the parasite's unique biology and inadequate *in vitro* culture protocols for the liver stages of *Plasmodium falciparum* and *P. vivax*, two causative agents of malaria. Here we describe a novel dual-chambered microfluidic device designed to serve as an improved liver stage culture platform. Devices are manufactured from polydimethylsiloxane and feature a PDMS membrane with 10 µm pores separating two microfluidic channels. Primary human hepatocytes are concentrated and injected into a collagen-coated device channel to initiate culture. By incorporating *in vivo*-like cell confinement within an *in vitro* system, hepatocyte architecture is maintained; leading to production of albumin, clotting factor IX, and bile salts over three weeks of continuous culture. Optimized infection conditions with as few as 10,000 parasites yield several developing liver forms within each device. Arrays of devices, each containing dilutions of drugs or immune sera, form an inhibition of invasion and development assay useful for assessing the efficacy of novel compounds and vaccine targets. Future studies include high-resolution imaging of time-lapsed intracellular parasite development to better understand the parasite's liver forms and low-to-medium throughput drug screening.

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IN-VITRO SUSCEPTIBILITY OF *PLASMODIUM FALCIPARUM* TO EIGHT ANTIMALARIAL DRUGS IN CAMBODIA, 2012-2013

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Artemisinin (ART)-resistant *Plasmodium falciparum* was first reported in western Cambodia in 2009 and now threatens the efficacy of all ART-based combination therapies (ACTs). While ART resistance is established in western Cambodia, there is no firm evidence of piperaquine (PPQ) resistance there. To monitor for resistance to PPQ and other antimalarials, we conducted a clinical efficacy study of dihydroartemisinin-piperaquine (DHA-PPQ) for the treatment of uncomplicated *P. falciparum* malaria and measured in-vitro drug susceptibilities for parasites in 2012-2013 from Pursat, Preah Vihear and Ratanakiri in western, northern and eastern Cambodia, respectively. Using a SYBR Green I fluorescence assay, we calculated the in-vitro IC_{50}s of 197 parasites to eight antimalarials: chloroquine (CQ), mefloquine (MQ), quinine (QN), PPQ, artesunate (ATS), dihydroartemisinin (DHA), pyronaridine (PYN), and atovaquone (ATV). Geometric mean IC50s (GMIC50s) for CQ, QN, PPQ, and DHA are significantly higher in Pursat and Preah Vihear than in Ratanakiri ($p \leq 0.005$). In contrast, GMIC50s for MQ are significantly lower in Pursat than in Preah Vihear and Ratanakiri, and have decreased in Pursat over the past 2 years. Significant positive correlations between $GMIC_{50}$s for PPQ and those for CQ, QN, ATS, and DHA were observed. These data suggest that the recent replacement of ATS-MQ with DHA-PPQ as first-line treatment for malaria in Cambodia may be selecting for PPQ resistance and against MQ resistance.

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EMERGING PIPERAQUINE RESISTANCE AND RISING MEFLOQUINE SUSCEPTIBILITY LINKED TO *PLASMODIUM FALCIPARUM* MULTIDRUG RESISTANCE GENE 1 (PFMDR1) GENOTYPES IN ISOLATES COLLECTED FROM CAMBODIA IN 2008-2013

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Growing reports of clinical failures with the current first-line artemisinin combination therapy (ACT) in Cambodia, dihydroartemisinin-piperaquine (DHA-PPQ), are alarming. We conducted *ex vivo* drug susceptibility testing of *Plasmodium falciparum* isolates to help inform public health policy in selecting an alternative ACT to replace DHA-PPQ in Cambodia. We performed histidine-rich protein-2 (HRP-2) ELISA *ex vivo* testing in a panel of antimalarials (DHA, artesunate, mefloquine, chloroquine, piperaquine, lumefantrine, quinine, and atovaquone) and molecular marker genotyping of 753 isolates collected in 2008-2013 from western, northern, and southern Cambodia. We found temporal and regional trends in *ex vivo* susceptibility and pfmdr1 amplification results corresponding with the 2010 national malaria treatment guideline change replacing artesunate-mefloquine (AS-MQ) with DHA-PPQ. Northern isolates during 2010-2013 had a significant reduction in PPQ susceptibility with geomean IC50s more than doubling (12.8 to 29.6 nM), whereas MQ IC50s concomitantly decreased from 67.1 to 26 nM. Isolates with multiple pfmdr1 copies and the 184F mutation had significantly reduced susceptibility to DHA, AS, MQ, quinine, lumefantrine, and atovaquone, whereas isolates with a single pfmdr1 copy plus 184F mutation showed reduced susceptibility to chloroquine and PPQ. The frequency of northern isolates with pfmdr1 amplification declined over 2009-2013, corresponding with diminishing MQ use. We found a high level of chloroquine (CQ)-resistance, as indicated by all evaluable isolates being of the *P. falciparum* CQ resistance transporter (pfcrt) gene CVIET mutant haplotype. Nearly 20% of enrolled patients had plasma active against *P. falciparum* blood stages in an *ex vivo* bioassay indicating prior malaria drug use and correlating well with patient-reported malaria history. Overall, our findings suggest PPQ resistance is emerging, while MQ sensitivity is increasing and linked with pfmdr1 deamplification, and support reintroduction of artesunate-MQ in areas in Cambodia where DHA-PPQ is failing.

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POPULATION GENETICS OF *PLASMODIUM FALCIPARUM* SURVIVING ARTEMISININ TREATMENT IN CHILDREN TAKING PART IN AN EFFICACY CLINICAL TRIAL IN KISUMU COUNTY, WESTERN KENYA

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The genetic basis for artemisinin resistance is beginning to be understood with most data coming from Southeast Asia. In order to establish how widely applicable the identified genetic markers are in predicting artemisinin resistance, comparable data is needed from other regions where artemisinin is the drug of choice for treating malaria. This study aims to establish whether similar molecular mechanisms are driving artemisinin resistance in western Kenya as those seen in Southeast Asia. A total of 75 samples from a recently completed *in vivo* efficacy study and archived samples (before ACT introduction) were used. We measured six hourly parasite counts in the patients to obtain clearance rates. DNA purified was genotyped with respect to twelve polymorphic microsatellite markers to determine multiplicity-of-infections in order to identify samples containing >1 parasite clone. Ninety one single nucleotide polymorphisms distributed across the *P. falciparum* genome were genotyped using the Sequenom platform as previously published. Effect of parasite genotype on clearance rates will be assessed by correlating the 91-locus genotypes to parasite clearance half-lives. Heritability was assessed by comparing variance of parasite clearance within and between clonally identical parasites recovered in >2 subjects and phylogenetic analysis carried out to determine parasite relatedness. Parasite clearance half-life with a geometric mean of 2.6 h (1.19-4.70) was reported with the slower clearance patients showing >3.05h slope half-life. Of the 91 SNPs assessed, 85% (78) gave robust genotype data which were correlated with parasite clearance half-life to show a positive correlation. Of the 75 samples genotyped, 26 had single clone infections to be included in heritability analysis. Genetically indistinguishable parasites (multilocus parasite genotypes) infecting >1 person will be identified. Genetic profiles of the parasites at baseline and those from *in vivo* efficacy study will be analyzed; phylogenetic analysis. Tracking of genetically determined artemisinin resistance in *P. falciparum* is critical in monitoring emergence/ spread of Artemisinin resistant parasites in Kenya. Findings will inform authorities to develop containment strategies as artemisinin resistance emerges in Kenya.

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FACTORS IN TROPICAL RURAL AREA STUDY SITES THAT CAN EFFECT DRIED BLOOD SPOT DRUG ASSAYS IN THERAPEUTIC ANTI-MALARIAL DRUG MONITORING

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Dried blood spots (DBS) as a sampling technique has been in use for decades. It is now increasingly used in preclinical and clinical studies, especially in antimalarial drug studies. DBS require a small volume of blood, and it is a simple and cheap technique to collect blood samples. It is particularly useful in rural areas with no laboratory facilities for sample processing, storage and shipping of blood samples. DBS offer an additional advantage in pediatric studies, where it is ethically challenging and difficult to collect large volumes of blood. However, the high humidity in tropical regions will significantly extend the drying time of blood spots on filter paper and could have an impact on the sample integrity. The storage conditions at the study site and conditions during transportation can also affect the integrity of the samples. These are some examples of pre-analytical issues that are difficult to control in rural areas. At the analytical laboratory, the samples enter a controlled environment and storage conditions are often investigated to minimize drug loss as part of analytical method development. We will demonstrate how environmental conditions during drying and storage can affect the outcome of antimalarial drug measurements and what precautions that needs to be in place to minimize these errors. If used properly, DBS sampling is a useful technique for therapeutic drug monitoring in rural areas and has many advantages over the conventional venous blood sampling technique.

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IMPACT OF SEASONAL MALARIA CHEMOPREVENTION ON THE MOLECULARS MARKERS OF RESISTANCE AND THE DEVELOPMENT OF ANTI MALARIA IMMUNITY IN SENEGAL

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The Chemoprevention of seasonal malaria with AQ + SP was adopted in areas with seasonal malaria transmission. The emergence and spread of antimalarial drug resistance poses a serious public health problem. Prophylactic or therapeutic failures LEAD re-emergence of malaria accompanied by an increase in transmission, morbidity and mortality especially among children. The spread of resistance to SP but also to AQ resulting in mutations located on pfdhfr, pfdhps, pfcrt and pfmdr1 genes could affect the effectiveness of this strategy. A child born of immune mothers are protected during the first months of his life, by passive transfer of maternal immunity. The child is exposed to infection and begins to develop his own immunity, a process that will take several years before becomes effective. Hence the need to check whether SMC will have an impact on immunity in children. As well as objectives of this thesis we i) examine the prevalence of molecular markers of resistance of *Plasmodium falciparum* to SP and AQ and ii) evaluate the impact of seasonal malaria chemoprevention on the acquisition of antibodies directed against malaria. To do this, blood samples on filter paper were made during cross-sectional surveys conducted after the intervention in three health districts in central Senegal in 2008, 2009 and 2010. We determined the mutation associated with resistance to SP (pfdhfr and pfdhps) and AQ (pfcrt and pfmdr1) by PCR and secondly measuring the production of antibodies against antigens of MSP1 and AMA1 of *P. falciparum*. The results show about the prevalence of molecular markers of resistance that the triple mutation pfdhfr were high between 2008 and 2010. The quintuple mutation pfdhfr / pfdhps were not observed either in control areas or in SMC area after three years of implementation. However, the absolute prevalence of resistance markers was lower in areas with SMC. Mutations pfdhfr 164L and 540E pfdhps were not found. Regarding immunity, it was found that children living in control areas produced more antibodies (MSP1 and AMA1) than those in areas with strategy. So it seems that the SMC retards the development of immunity in these children. In view of our results, it is likely that the SMC has no impact on the prevalence of molecular markers of resistance to SP and AQ but appears to delay the acquisition of anti malarial immunity in children undergoing strategy.

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POLYMORPHISMS OF *PFMDR1*, *PFCRT* AND *PFNHE1* MS4760 GENES IS ASSOCIATED WITH LOW VITRO QUININE SENSITIVITY IN KENYAN ISOLATES

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In combination with antibiotics, quinine is recommended as the second-line treatment for uncomplicated malaria, first-line treatment for severe malaria and for treatment of malaria in the first trimester of pregnancy. Quinine (QN) has been shown to have high clinical failures and yet the mechanisms of action and resistance are not been fully elucidated. However, parasite QN reduced sensitivity is linked to polymorphisms in multiple genes including multidrug resistance 1 (*Pfmdr1*), chloroquine-

resistance transporter (*Pfcrt*), multidrug resistance protein (*Pfmrp*) and the sodium/hydrogen exchanger gene (*Pfnhe1*). In this study, we investigated the association between *in vitro* quinine susceptibility with genetic polymorphisms in *Pfmdr1*codons 86 and 184, *Pfcrt* codon 76*,* and *Pfnhe1* ms4760 in field isolates from western (lowland and highlands) and coastal regions of Kenya. *In vitro* activity was assessed as the drug concentration that inhibits 50% of parasite growth (IC_{50}). DNA was extracted and polymorphisms in *Pfmdr1*, *Pfcrt* and *Pfnhe1* genes were determined by sequencing. Associations between the *in vitro* QN sensitivity [phenotypic] and the polymorphisms of the *Pfmdr1*, *Pfcrt* and *Pfnhe1* gene [genotypic] were established. Data revealed there was significant association between polymorphisms in *Pfmdr1*-86Y, 184F and *Pfcrt*-76T with quinine susceptibility ($p = 0.0001$). 82% of parasites resistant to quinine carried mutant alleles at these codons (*Pfmdr1*-86Y, 184F and *Pfcrt*-76T) whereas 74% of parasites susceptible to quinine carried the wildtype allele. Fifteen different profiles of ms4760 in *Pfnhe1* gene were observed, five of these had not been described. In addition, quinine IC_{50} of parasites with *Pfnhe1* ms4760 3 DNNND repeats was significantly higher compared to those with 1 or 2 repeats ($p = 0.033$ and $p = 0.0043$ respectively). The validity of these genetic markers is probably only relevant in the context of the genetic background of the isolates. Clinical efficacy studies are required to confirm the validity of these markers and the importance of parasite genetic background.

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USE OF MOBILE PHONE TECHNOLOGY FOR REAL-TIME REPORTING OF FEVER CASES AND MALARIA TREATMENT FAILURE IN AREAS OF DECLINING MALARIA TRANSMISSION IN TANGA, NORTHEASTERN TANZANIA: A PILOT STUDY

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Early detection and management of febrile illnesses need to be improved for communities to benefit from the declining trends of malaria. Capturing events on malaria, drug resistance or associated disease in real-time, and pipelining them to appropriate clinics and/or laboratories for proper management is essential for patient care and disease control. Currently mobile phone technology is common in developing world and most developing countries are using mobile phone technology to collect and transferhealth information and services to remote populations. This study is assessing the applicability of mobile phone technology in facilitating detection and management of fever cases by village health workers (VHWs) in north-eastern Tanzania. This is a prospective pilot study involving three villages in Muheza district (Mamboleo, Magoda and Mpapayu) and one dispensary of Magoda. VHWs are suing mobile phone technology in surveillance of febrile episodes (axillary temperature ≥37.5^{0}C or/and history of fever past 24hours) through active (ACD) and passive case detection (PCD). Cases found positive by malaria rapid diagnostic test (mRDT) are given first dose of artemether/lumefantrine (ALu) at the dispensary. VHWs visit each patient at home to supervise intake of remaining doses. In May 2013, the population was estimated to be 2934 individuals in 678 households. Between 15th November 2013 and 15th March 2014, a total of 528 febrile cases aged 6.5 months -94 year were attended. Most of the cases (82.3%) were detected through PCD; 21.0% were positive by mRDT and 19.3% by microscopy. Of those with malaria, 89.5% had completed 7 days of follow-up and 10.5% were recurrent infections. No severe cases requiring a referral were reported. In conclusion, preliminary findings show that mobile phone based data collection tools have been successfully used in surveillance and timely reporting of fever episodes and monitoring of drug utilization through VHWs.

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DOXYCYCLINE REDUCED *IN VITRO* SUSCEPTIBILITY IN *PLASMODIUM FALCIPARUM* KENYAN FIELD ISOLATES IS ASSOCIATED WITH *PFTETQ* KYNNNN SEQUENCE POLYMORPHISM

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Doxycycline is widely used for malaria prophylaxis by international tourists visiting malaria endemic countries. Its efficacy profile as an antimalarial in Kenya is not well defined due to limited available information regarding reference *Plasmodium falciparum* doxycycline molecular markers. We determined doxycycline *in vitro* susceptibility profiles in Kenyan isolates and further assessed their association with *Plasmodium falciparum metabolite drug transporter* and *Plasmodium falciparum GTPase tetQ protein gene* polymorphisms from 2010 to 2013.*In vitro* susceptibility testing using SYBR green I dye was employed for determination of doxycycline 50% inhibitory concentrations. While quantitative real-time polymerase chain reaction (qPCR) was used to determine copy number variation in 96 *P.falciparum* Kenyan isolates. Direct sequencing was used for determination of the number of KYNNNN motif repeats within the *PftetQ protein gene*. There was a marked reduction in median doxycycline 50% inhibitory concentrations from 18,956nM [15,234-50,141] in 2010 to 3445nM [1,744-12,775] in 2013, indicative of increasing susceptibility to the drug. However 15% of the isolates with doxycycline IC_{50} above the resistance threshold of 35,000nM, had a higher odds of having <3 KYNNNN motif repeats polymorphism of *Plasmodium falciparum GTPase tetQ protein gene* relative to those with IC_{50} < 35,000nM was (Odds ratio [OR], 15 [95% confidence interval (CI), 3.0-74.3]; P < 0.0002). While the odds of having increased copy number of both *Plasmodium falciparum GTPase tetQ protein* gene and *Plasmodium falciparum metabolite drug transporter* gene was (odds ratio [OR], 0.4100, 95% confidence interval (CI) [0.04-3.7]; P= 0.65) and OR, 0.205 [95% CI, 0.02-1.6; P=0.172] respectively for the 96 samples. *PftetQ* KYNNNN polymorphism is associated with a reduced doxycycline susceptibility phenotype in Kenyan isolates.

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ANALYSIS OF CANDIDATE GENETIC LOCI ASSOCIATED WITH DELAYED PARASITE CLEARANCE FOLLOWING TREATMENT WITH ARTEMISININ BASED COMBINATION THERAPY IN *PLASMODIUM FALCIPARUM* ISOLATES FROM WESTERN KENYA

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In a recent study conducted in South East Asia (SEA), regions on chromosomes 10, 13 and 14 of *Plasmodium falciparum* were identified that showed association with slow parasite clearance rates (CRs) when subjects were treated with artemisinin based combination therapy (ACT). We conducted a study to analyze chromosome 10, 13 and 14 regions as recently published to establish the genetic baseline of *P. falciparum* in western Kenya. Historical samples collected through surveillance studies from 1995-2013 and samples from an ACT efficacy clinical study conducted in western Kenya in 2013-2014 were analyzed. Parasite clearance rates for subjects in the ACT efficacy trial were calculated using the WWARN online parasite clearance estimator. All subjects from the ACT trials achieved parasite clearance within 42 hours of treatment, with a median CR constant of 0.27(range 0.15 - 0.58). More than 60% of the SNPs that were showed to be association with slow CR in SEA were present in samples from western Kenya. Interestingly however, the prevalence was highest in samples collected before the introduction of ACTs in Kenya, and decreased steadily over time. In the efficacy study, 24% of these SNPs were present. There was no increase in PfMDR copy

number observed. This data indicate unlike what was observed in SEA, these markers are not associated with ACT CRs, and are more likely associated with pressure from chloroquine which was removed from circulation at the time when reduction in the prevalence of the SNPs is observed.

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PFCRT HAPLOTYPE DIVERSITY IN CAMEROON AND EMERGENCE OF THE S(AGT)VMNT TYPE

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Evolution and spread of chloroquine-resistant-malaria parasite *Plasmodium falciparum* have posed great challenge in malaria control efforts all over the malaria endemic countries of the globe. Although chloroquine (CQ) has been banned from national malaria control programs of many endemic countries; due to high efficacy, easy avaibility and affordability, CQ still continues to be non-officially used in many countries including Africa. The main objective of this study is to unravel distribution of different Pfcrt genotypes in central, littoral, eastern, southern and in international bordering part of Cameroon. This is because, (i) chloroquine (CQ)-resistant (CQR) malaria parasite *Plasmodium falciparum* is of wide occurrence in Cameroon, (ii) mutations in the 72nd - 76th amino acid positions of the Pfcrt gene are known to confer resistance to CQ and (iii) only a single CQR haplotype (C72V73I74E75T76) has so far been reported in Cameroon. We have followed molecular approach with DNA sequencing of the 2nd exon of the Pfcrt gene to identify Single Nucleotide Polymorphisms (SNPs) in 180 *P. falciparum* field isolates sampled in five different locations in Cameroon. The CQR-Pfcrt CVIET haplotype was mostly abundant, followed by the wild CVMNK haplotype. Five hitherto-unreported CQR-Pfcrt haplotypes were detected for the first time in Cameroonian *P. falciparum*, including the surprise appearance of the S(agt) VMNT haplotype. Observations on the high haplotype diversity of the CQR-Pfcrt haplotypes coupled with appearance of the S(agt)VMNT type is daunting and can pose a greater challenge to the malaria control program of Cameroon than before.

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PARASITOLOGIC AND MOLECULAR ASSESSMENT OF EFFICACY OF SOME ANTIMALARIAL DRUGS USED DURING PREGNANCY IN LAGOS, NIGERIA

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The prevention of malaria in pregnancy (MIP) reduces the adverse effect of malaria on both mother and foetus. Resistance to sulphadoxine-pyrimethamine (SP) has led to discontinuation of the use of SP in treatment of malaria, but used only in intermittent preventive treatment of malaria during pregnancy (IPTp). It is therefore essential to continuously monitor the efficacy of SP during pregnancy. Other non-recommended drugs but commonly used for antimalarial chemoprophylaxis during pregnancy (e,g, chloroquine and pyrimethamine) were assessed using molecular markers of resistance. Protective efficacy of SP in IPTp and the equivalence of monthly IPTp-SP to two-dose IPTp-SP was assessed in a longitudinal study from the second trimester to delivery in Lagos. A total of 1084 pregnant women were screened, but 259 were recruited (122 and 137 in two-dose and monthly-dose arms respectively) into the longitudinal study. The protective efficacy of SP was similar in both study arms (P>0.05): 98.3%, 99.1%, 100% and 100% at months 1,2,3 and 4 respectively in the two-dose arm versus 98.5%, 100%, 100% and 100% at months 1,2,3 and 4 respectively in the monthly-dose arm. The outcome of pregnancy (low birth weight and live births) was similar in the two study arms (P>0.05) irrespective of gravidity and age of the women. The frequency of Pfcrt haplotypes were 24.1, 53.7% and 22.2% for CVMNK, CVIET and CVIET+CVMNK respectively. The dhfr haplotypes were 26.7%, 6.7% and 66.7% for ACNCSVI, ACICNVI and ACIRNVI respectively. The pfmdr1 haplotypes were 53.6%, 17.9%, 21.4% and 7.1% for NYSND, YYSND, NFSND and YFSND respectively. SP was effective in IPTp and 2-dose was equivalent to monthly-dose SP regimen in Lagos. The high levels of resistance markers for pyrimethamine and chloroquine indicates that antimalarial treatment/chemoprophylaxis in pregnancy with either pyrimethamine or chloroquine may not be effective.

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THERAPEUTIC EFFICACY AND SAFETY OF ARTEMETHER/LUMEFANTRINE FOR TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA AND PARASITE GENETIC FACTORS ASSOCIATED WITH PARASITE CLEARANCE OR TREATMENT FAILURE

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Following deployment of artemisinin based combination therapy (ACTs), regular testing for efficacy is recommended by the World health Organisation (WHO). This study assessed the efficacy and safety of Artemether/Lumefantrine (AL) and parasite genetic factors associated with parasite clearance or treatment failure in an area where the transmission has significantly declined in recent years. This was an open-label, single-arm trial, involving 88 children (6 months to 10 years) with uncomplicated falciparum malaria attended at Mkuzi Health Centre in Muheza, Tanga. . Follow-up was for 28 days and the primary end point was parasitological cure on day 28. Secondary end points included: haemoglobin improvement at day 28 and occurrence and severity of adverse events. Parasite sequencing is being performed at Sanger Institute, UK. Of the 163 patients screened, 88 were enrolled in the study. Geometric mean parasite density was 18,551 asexual parasites/µl (range: 256-200,000) and mean haemoglobin level was 10.4±1.8g/dL. Nine patients (10.2%) were lost during follow up and there was no early treatment failure. Before PCR correction; 78.4% (n=40) under-fives and 75.0% (n=21) children aged ≥5years hadadequate clinical and parasitological response (ACPR) . Late clinical failure (LCF) was seen in 5.6% of under-fives (n=51) and 3.6% children aged ≥5years (n=28). Furthermore, 15.7% and 21.6% of the patients had late parasitological failure (LPF) among under-fives and children aged ≥5years respectively. After PCR correction, ACPR was 100% in both groups. Reported adverse events included abdominal pain (11.9%), cough (59.7%), diarrhoea (1.5%), fever (23.8%), headache (1.5%), and skin rashes (1.5%). The association between treatment outcome and parasite genetic factors will be performed later. AL was safe and efficacious for the treatment of uncomplicated malaria. Since Mkuzi/Muheza has been a hotspot of drug resistance in Tanzania, surveillance needs to be continued to detect future changes in parasite sensitivity to ACTs.

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INDEPENDENT EMERGENCE OF THE SUPER RESISTANCE-CONFERRING MUTATION AT *DHPS* CODON 581 IN EAST AFRICAN *PLASMODIUM FALCIPARUM* POPULATIONS

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Sulphadoxine-Pyrimethamine (SP) is no longer applied as treatment against malaria, due to a variety of mutations in *Plasmodium falciparum* populations conferring resistance to the drug. However, SP has been shown to still provide protection as preventive treatment against malaria. WHO now recommends SP in preventive treatment in pregnant women and in infants, as well as in combination with Amodiaquine for seasonal malaria chemoprevention. Unfortunately, the parasites still harbor the resistance-conferring mutations, which in East Africa consist of a triple mutation in the *Pfdhfr* gene, and a double mutation in the *Pfdhps* gene. Now it is being shown in East Africa that a third mutation has appeared in the *Pfdhps* gene, terminating the efficacy of SP as preventive treatment. Emergence of this "super resistance"-conferring mutation threatens the hitherto successful control measure of intermittent preventive treatment in pregnancy. In this study, we have analysed the origin of the "super resistant" triple mutant parasites in Ethiopia, Uganda and Tanzania, with regard to the previously established double mutants. We have applied microsatellite analysis of the genetic region flanking the *Pfdhps* gene, to analyse the lineage of the double and triple mutants. In Ethiopia both double and triple mutants were derived from a single lineage which was distinct from those in Uganda and Tanzania. This correlates well with previous studies showing distinct parasite populations in Northeast and Southeast Africa. In Uganda and Tanzania, we also found triple mutants which were derived from the previously characterized Southeast African lineage. However, a novel microsatellite allele incorporated into the Tanzanian triple mutant lineage since 2004, exceeding in numbers the previously described Southeast lineage, illustrates the local expansion of a new triple mutant lineages. We conclude that the A581G mutation has occurred independently and multiple times on local *Pfdhps* double mutant backgrounds.

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QUANTITATIVE ANALYSIS OF THE ANTIMALARIAL DRUG PYRONARIDINE USING DRIED BLOOD SPOTS

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The fixed-dose antimalarial drug combination of artesunate and pyronaridine (PYRAMAX®) has recently been developed and introduced in the treatment of falciparum and vivax malaria. Pyronaridine have a long terminal elimination half-life and it is possible to detect and quantify the drug for several weeks after the last dose. To be able to measure pyronaridine from a small quantity of blood, a sensitive quantification method was developed for therapeutic drug monitoring and pharmacokinetic studies. Pyronaridine have a high blood-to-plasma ratio and this also makes whole blood the most suitable sample matrix. Therefore, a method to measure pyronaridine in dried blood spots was developed and validated. This method use 50 µl of whole blood applied as blood spots on a filter paper to be dried at ambient temperature. The extraction process followed by separation and detection by electrospray LC-MS/MS will be presented as well as the stability tests of pyronaridine in dried blood spots. The developed method was validated according to the US FDA bioanalytical method validation guideline. Dried blood spots, as a sample collection technique, have become an increasingly useful tool in rural areas where transportation is problematic and where laboratory facilities are minimal. The accurate and sensitive method presented here requires a very small sample volume, which can be collected by finger or heal pricks. The work presented here may be particularly advantageous in studies involving small children, in whom it is ethically and practically impossible to collect large volumes of blood.

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THE EFFECT OF ANTIMALARIAL DRUGS ON INTRACELLULAR PH IN *PLASMODIUM FALCIPARUM*

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The acidic digestive vacuole (DV) of *Plasmodium falciparum* is the site of action of several antimalarial drugs and its membrane contains transporters that are implicated in the regulation of drug resistance. The low pH of the DV lumen is necessary for hemoglobin degradation and the detoxification of the toxic byproduct heme and its maintenance is thus crucial to the survival of the parasite. The quinoline drugs chloroquine (CQ), mefloquine (MQ) and quinine (QN) have previously been shown to raise DV pH using spectrofluorimetry, however, no accurate quantitative measurements have been performed. In this study, CQ-resistant Dd2 and CQ-sensitive 3D7 parasites were transfected with a DNA construct encoding the ratiometric pH-sensitive green fluorescent protein pHluorin alone or pHluorin with a targeting peptide that mediates trafficking of the fluorescent protein to the DV. Quantitative pH determinations were carried out using confocal fluorescence microscopy of single live parasite-infected erythrocytes under physiological conditions. MQ had the strongest effect on DV pH at a concentration of 500 nM and caused the organellar pH to rise by 0.7 pH units, whereas CQ and QN addition resulted in a pH increase by 0.3 - 0.5 pH units. To test whether the acidification of the cytosol in response to CQ, MQ and QN may be due to H^+-coupled drug export from the DV into the cytosol mediated by *P. falciparum* chloroquine resistance transporter (PfCRT), the pH effects of PfCRT inhibitor verapamil (VP) were measured in combination with a quinoline drug. VP prevented the cytosolic acidification caused by CQ and QN in Dd2 parasites, supporting the hypothesis that these drugs are expelled from the DV in symport with protons in drug-resistant strains. In contrast, VP did not alter the pH effects of MQ, which is probably not transported by PfCRT. The specific V-ATPase inhibitor concanamycin A at a concentration of 100 nM abolished the transvacuolar pH gradient between DV lumen and cytosol within 20 min, indicating that the V-ATPase might play a central role in maintaining this gradient.

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PREVALENCE OF *DHFR* AND *DHPS* MOLECULAR MARKERS IN *PLASMODIUM FALCIPARUM* PARASITES IN PREGNANT WOMEN OF NCHELENGE DISTRICT, NORTHERN ZAMBIA

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Sulphadoxine pyrimethamine (SP) is the recommended drug for Intermittent Preventive Treatment in pregnancy (IPTp) in most African countries including Zambia. However, malaria is still one of the leading causes of morbidity and mortality in pregnant women despite reports of greater than 50% of women taking at least two doses of SP in IPTp. Studies have shown that resistance to SP is associated with mutations in the *dhfr* and *dhps* gene of the *Plasmodium falciparum* parasite. Few studies have been done to determine the prevalence of these mutations

in parasites found in pregnant women in Zambia. This cross-sectional study was conducted in Nchelenge, Northern Zambia in February-April 2013. Nchelenge is a hyper-endemic area with estimated perennial malaria prevalence of 50%. Three Rural Health Centers were randomly selected and a census survey carried out at each health center. A questionnaire was administered and malaria testing done using RDT and microscopy, with collection of a dried blood spots. Parasite DNA was extracted from dried blood spots using the chelex method followed by nested PCR. Positive samples then underwent mutation specific enzyme restriction digestion. The overall results (n=385), showed a mean age of 25. The prevalence of malaria was 22%. Multivariate analysis showed that there was an association between malaria and anaemia (AOR: 2.94) and women aged >25 years old (AOR: 0.40) were less likely to have malaria. The prevalence of *dhfr* codon S108N was 94%; codon C59R was 93.4%; and codon A16N was 5.54% *dhps* codon 436 was present at 97% and no mutation was found on *dhps* codon 540. This study showed a high number of mutations in the *dhfr* and *dhps* genes than previously reported. The high malaria endemicity in the general population of this area may have contributed to the high prevalence of resistant parasites in pregnant women. Other studies have shown that in highly endemic areas resistant parasites tend to spread quickly. These findings call for close and more detailed monitoring and evaluation of IPTp in Zambia to ascertain its effectiveness.

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THE EFFICACY OF ARTEMETHER-LUMEFANTRINE FOR THE TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN THE GAMBIA: 2013 FOLLOW UP STUDY

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Artemisinin-based combination therapy (ACT) remains the main stay of case management of uncomplicated *Plasmodium falciparum* malaria. Because of increasing threat of artemisinin resistance, routine efficacy monitoring is recommended and World Health Organization guideline recommends a change in the choice of a first-line ACT when its treatment failure rate exceeds 10% in a 28 or 42-day *in vivo* therapeutic efficacy study. In 2008, The Gambia introduced artemether-lumefantrine (AL) for treatment of uncomplicated *P. falciparum* malaria and here, we report on the therapeutic efficacy monitoring of AL in the Western region of the country in the 2013 malaria transmission season. In a one-arm prospective study, 129 children aged >1 to 12 years with uncomplicated *P. falciparum* mono infection of between 1,000 to 100,000 asexual parasites/µl were treated with a weight-based oral AL and followed up for 28 days for clinical and parasitological response. Parasite density was determined using Giemsa stained thick blood films on day 0 and on days 1, 2, 3, 7, 14, 21, 28 or on any other day a child had symptoms of malaria. Filter paper spots were obtained during each of the follow up visits for polymerase chain reaction (PCR) analysis to determine if a recurring parasitemia was a re-infection or a recrudescence. Blood samples were obtained on day 0 and on whichever day a parasitemia >1,000 parasites/µl was detected for *ex vivo* parasite susceptibility drug testing. In the per protocol study population (n=100), PCR uncorrected results showed that proportion of children with adequate clinical and parasitological response by day 28 was 93% (93/100). Of these, 56% and 100% had complete parasite clearance by days 3 and 7 respectively. All the cases of parasitemia detected by day 3 were low grade (<1,000 parasites/µl). All the treatment failures (n=7) encountered were late parasitological failures between days 14 and 28. These results suggest that AL remains efficacious for the treatment of uncomplicated *P. falciparum* malaria in the Western region of The Gambia. A high proportion of low grade parasitemia by day 3 was however observed.

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MALARIA AND MIGRANTS IN THAILAND: TREATMENT-SEEKING (AND OTHER) BEHAVIORS

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Artemisinin-resistant malaria has emerged as an urgent concern in South East Asia. One strategy to address the problem is to improve the access of mobile and migrant populations (i.e. those considered at highest risk of malaria) to health services. This study sought to understand the knowledge, attitudes and practices (KAP) of different migrant groups in Thailand, in order to design strategies to increase migrants' health access. In September 2013, 386 migrants who visited selected health facilities along the Thai-Myanmar border participated in a KAP retrospective study. The results showed that net usage among migrants who crossed borders daily was lower than migrants who stayed in Thailand for longer periods. Less than half (48.3%) of fever cases in the past 3 months sought treatment at public health facilities, even though 36.4% of those who were tested for malaria were positive. Lower net usage, as well as delayed treatment seeking for fever, was also reported among rubber plantation workers. New intervention packages (such as insecticide-treated net delivery and behaviour change communication delivered at official and unofficial border points) must be devised to reach migrants as well as those who work in rubber plantations, as both groups are more likely to sleep without mosquito nets, and delay seeking treatment.

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THERAPEUTIC EFFICACY OF CHLOROQUINE FOR THE TREATMENT OF *PLASMODIUM VIVAX* IN SOUTH ETHIOPIA

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The development and spread of chloroquine resistance *Plasmodium vivax* threatens the health of millions of people and poses a major challenge in the control of malaria .Monitoring of drug efficacy in two years interval is an important tool in establishing rational antimalarial drug policies. The current study assessed therapeutic efficacy of chloroquine for the treatment of *Plasmodium vivax* in South Ethiopia. A one-arm prospective evaluation of clinical and parasitological responses for uncomplicated microscopically confirmed vivax malaria was conducted to monitor the therapeutic efficacy of chloroquine from September to December 2012 in South Ethiopia. A total of 90 patients above 6 months and greater than 5kg with symptomatic, microscopically confirmed, uncomplicated P.vivax and fulfill the inclusion criteria were selected and included. All eligible patients were treated with CQ and followed for 28 days. PCR was conducted to differentiate re-infection from recrudescence. Pvmdr1 gene, the orthologue of pfmdr1, were sequenced and analyzed for the presence of mutations at positions 976 and 1076. Result: Of the 2500 febrile patients, 92 *P. vivax* positive cases were enrolled and treated with chloroquine. Out of 92 vivax cases 90 completed 28 days follow up period. Parasites reappeared on 12.2 % (11/90) patients. Among 11 patients, 9 were late treatment failures and 2 were early treatment failures. On the day of recurrent parasitaemia, the level of chloroquine/desethylchloroquine (CQ-DCQ) was above the minimum effective concentration (>100 ng/ml) in 9, but lower in 2 patients. We found that 8.9 % (8/90) of the isolates carried the F976 single nucleotide polymorphism. There was no mutation at codon 1076 in all *P. vivax* isolates in our study area. In conclusion, reappearance of the parasite within the 28 days follow-up period might

be due to parasite resistance to chloroquine. This finding alarms the need to launching a monitoring program of chloroquine for the treatment of *P. vivax*.

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STRUCTURAL AND FUNCTIONAL ANALYSIS OF THE SUGAR PHOSPHATASE PFHAD1, A REGULATOR OF THE MEP PATHWAY FOR ISOPRENOID BIOSYNTHESIS IN *PLASMODIUM FALCIPARUM*

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The emergence of drug resistance to currently available antimalarial drugs is a major challenge in malaria control efforts, and thus, understanding the mechanisms that underlie drug resistance is of great importance. The methylerythritol phosphate (MEP) pathway for isoprenoid precursor biosynthesis is an attractive target for novel antimalarial drug development, and the small molecule compound fosmidomycin has been validated as a competitive inhibitor of the MEP pathway enzyme deoxyxylulose 5-phosphate (DXR). Fosmidomycin was employed in a forward genetics approach to gain understanding into regulation of the MEP pathway. Fosmidomycin-resistant *Plamodium falciparum* strains were selected *in vitro*, and genetic analysis revealed that mutations in PfHAD1 are associated with fosmidomycin resistance. PfHAD1 is a previously uncharacterized enzyme with homology to haloacid dehalogenase-like sugar phosphatases. The crystal structure of PfHAD1 was solved in order to define the structural basis for loss-of-function mutations in PfHAD1. The identified point mutations map to tightly packed hydrophobic inner core regions or catalytic regions of PfHAD1, resulting in either protein misfolding or interference with substrate binding. Biochemical investigations show that PfHAD1 dephosphorylates a variety of sugar phosphate compounds, including intermediates of glycolysis, which feed into the MEP pathway. Several of these sugar phosphates have been co-crystallized with PfHAD1 in order to investigate the structural basis for its diverse substrate specificity. Finally, metabolic profiling reveals that fosmidomycin-resistant parasite strains lacking PfHAD1 have substantial increases in MEP pathway metabolites. Together, these results demonstrate that PfHAD1 regulates substrate availability to the MEP pathway and that loss of PfHAD1 function confers fosmidomycin resistance in *P. falciparum*.

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DIVERSITY OF *PLASMODIUM* MALARIA AND MOLECULAR MARKERS OF SULPHADOXINE RESISTANCE IN TANZANIA

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Intermittent preventive treatment with sulfadoxine-pyrimethamine (IPTp-SP) is widely deployed in the control of pregnancy-associated malaria. However widespread SP resistance threatens its effectiveness. This study aimed at assessing diversity of *Plasmodium* species as well as identifying molecular markers of sulphadoxine resistance in the current situation of changing malaria epidemiology. A cross-sectional study was conducted in three districts of Tanzania with different transmission intensity; Muheza and Muleba (hypoendemic), and Nachingwea (hyper-endemic/ holoendemic). Patients with a history of fever or fever at presentation (≥37.50) were screened using malaria rapid diagnostic tests (mRDTs) and confirmed by microscopy. Molecular markers of sulphadoxine resistance were detected by nested-PCR followed by sequence specific oligonucleotide probes-enzyme- linked immunosorbent assay (SSOP-ELISA). A total of 466 (56.9%) patients were tested positive by mRDTs and 397 (48.5%) microscopy. Nachingwea had higher positivity rate compared to other sites, at 29.0% and 27.7% by mRDT and microscopy respectively. *Plasmodium falciparum* species was the predominant specie at all sites while P.ovale was only detected at Muleba and Nachingwea. Prevalence of P.falciparum gametocytes was higher in Muleba (13.7%) followed by Nachingwea (7.2%) and the lowest was observed in Muheza (0%). Additionally, fever was a strong predictor of malaria parasite rates at Nachingwea ($p<0.001$). Prevalence of sulphadoxine resistance involving Pfdhps codon 540E (96.6%) and 581G (53.4%) were higher in Muheza compared to the other sites.This study showed that Nachingwea had high malaria positivity rate compared to other sites. High Sulphadoxine resistance observed at Muheza could be attributed to local use of SP and it may spread to other areas. Further studies to monitor the spread of sulphadoxine resistance in other regions are warranted

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INVESTIGATION OF STRUCTURE-ACTIVITY RELATIONSHIPS OF ANTIMALARIAL DRUGS THROUGH NOVEL *PLASMODIUM FALCIPARUM* CHLOROQUINE RESISTANCE TRANSPORTER (PFCRT) HAPLOTYPES

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Mutation in the *Plasmodium falciparum* resistance chloroquine transporter (PfCRT) protein causes chloroquine resistance and alters susceptibility to various antimalarial drugs. Mutant PfCRT transports chloroquine out of the parasite digestive vacuole; however, the molecular details of drug-receptor interaction are poorly understood. The antiviral drug amantadine has moderate potency against *P. falciparum* and acts to reduce the level of chloroquine resistance determined by the *pfcrt* haplotype. In accordance, chloroquine-resistant parasites show enhanced sensitivity to amantadine. We used continuous, step-wise amantadine selection on three chloroquine-resistant lines of the *P. falciparum* 106/1 strain. Each of these lines contains a unique *pfcrt* allele differing only by a non-synonymous SNP in codon 76, resulting in PfCRT K76I, K76N or K76T, but otherwise contains an identical genetic background. Stepwise selection with amantadine resulted in parasite lines with six novel *pfcrt* alleles. Parasites showed resistance to amantadine, increased chloroquine sensitivity, and changes in susceptibility to other digestive vacuole-targeting drugs. The results shed light on new topographical regions of the PfCRT protein and amino acid residues involved in drug interactions and digestive vacuole morphology. Two additional amantadine-selected parasite clones, derived from 106/1 K76N and K76I, showed amantadine resistance and altered quinoline susceptibility without accompanying mutations in *pfcrt*. These parasite lines are currently under investigation. Our results increase the understanding of how digestive vacuole-targeting drugs interact with the PfCRT protein and explain patterns of cross-resistance in the malaria parasite.

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INVESTIGATION OF THE THAI MULTIDRUG RESISTANT *PLASMODIUM FALCIPARUM* C2A STRAIN IN THE *AOTUS* MONKEY MODEL

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Antimalarial drug resistance is the biggest threat to the current malaria eradication agenda (MaLERA) based on oral artemisinin-based combination therapies (ACTs). ACTs are the standard of care for uncomplicated malaria, but reports of clinical treatment failures (defined as a reduced parasite clearance rate or persistence of parasites on the third day of treatment) of artesunate (AS) / mefloquine (MQ) combinations are rapidly emerging from South-East Asia. The Thai *Plasmodium falciparum* C2A clone is a Multiple Drug Resistant (MDR) isolate obtained from a patient in Thailand in 1992 before deployment of this ACT, first introduced in 1994 and adopted in 2005, though, artemisinins had been in use in western Cambodia since the late 70's (Witkowski et al, 2013). In a previous *in vivo* adaptation study in Aotus monkeys, we observed that even in the absence of MQ pressure, C2A appeared to gradually loose its susceptibility to MQ as passage levels increased. In order to further characterize this observation we assessed the efficacy of MQ and AS alone or in combination in infected splenectomized Aotus monkeys. Our results confirmed the previous findings of resistance to oral MQ at 40 mg / Kg given once or to oral AS at 33 mg / Kg x 3 days alone or in combination with MQ at 40 mg / kg once. Strikingly, in the AS alone group, clearance occur in 1 / 2 animals on day 7 post-treatment (PT) but recrudesce on day 8 PT (slow clearance phenotype) and persisted in the other one until day 10 PT when it was finally rescue treated. As a comparison, AS at 20 mg / Kg orally x 3 days against infections of the Vietnam CQ resistant FVO strain, clears infection within 1-2 days PT in spleen intact Aotus. Only when AS was administered IV at 20 mg / Kg x 3 days in combination with oral MQ at 40 mg / Kg, infections were cured in 4 / 5, but cleared and recrudesce in 1 / 5 rescue treated animals. Additional *in vitro* assays of C2A demonstrated reduced susceptibility to MQ, CQ, artemisinin, dihydro-arteminsinin, and atovaquone-proguanil, arteether and AS compared to the sensitive D6 strain, the *in vitro* adapted TM90-C2A line or passage III Aotus adapted C2A when compared to the later monkey passage X. Analysis of drug resistance loci and copy number variation is underway to determine the genetic basis for the observed drug resistance profile. We anticipate that these studies will shed light on the early spread and evolution of multi drug resistance and reduced artemisinin susceptibility in South-East Asia.

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EFFECT OF DRUG PRESSURE ON THE PREVALENCE OF SULFADOXINE-PYRIMETHAMINE RESISTANT HAPLOTYPES AND SELECTIVE SWEEP CHARACTERISTICS IN MALAWI

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The reemergence of chloroquine sensitive parasites in Malawi following the switch to sulfadoxine-pyrimethamine (SP), raised hope that abandoned drugs might later find clinical utility. Malawi replaced SP with artemisinin-based combination therapy (ACT) in 2007 in response to failing efficacy. Here we examine the effect of SP pressure on prevalence of SP-resistant haplotypes and the characteristics of associated selective sweeps. Pyrosequencing and microsatellite genotyping were performed on samples from three time periods in the history of SP use: high SP-use, when SP was the first-line treatment; the transition period from SP to ACTs; and low SP-use, five years after the switch to ACTs. An increase in the prevalence of dhfr triple and dhps double mutants occurred between the high SP use and transition periods ($p<0.0001$), but there was no significant change in haplotype prevalence 5 years after reduction of SP pressure. The prevalence of the dhps triple mutant 437G/540E/581G increased despite reduced SP pressure ($MHp<0.0001$). A reduction in the prevalence of less resistant dhfr and dhps was also observed. Microsatellite analysis identified sweeps flanking dhfr triple and dhps double mutant haplotypes. Changes in sweep characteristics were seen at both distal and proximal markers. At most dhps flanking loci there was a decline in expected heterozygosity after the high SP use period, however changes at proximal markers suggest that change in SP pressure may not affect all markers equally. Changes flanking dhfr were not as pronounced as dhps, suggesting that drug pressure affects sweeps at different rates. Our data suggest that SP-resistance may be longer lived than chloroquine resistance. Maintenance of SP resistant genotypes and sweep characteristics could be due to continued pressure from residual SP use for intermittent preventive treatment and trimethoprim-sulfamethoxazole prophylaxis in persons living with HIV individuals, and/or to a lack of fitness cost of resistance.

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ADDRESSING ARTEMISININ-RESISTANT MALARIA BY IDENTIFYING MALARIA HOTSPOTS IN CAMBODIA

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During the past decade Cambodia has made significant strides toward malaria reduction and now, elimination. The last remaining cases will be the hardest to reach, as they are located in remote border areas. Efforts are also threatened by the emergence of artemisinin-resistant malaria (ARM). The PMI/USAID Control and Prevention of Malaria (CAP-Malaria) Project developed a model to effectively identify malaria hotspots and treat remaining malaria cases in remote villages through household active case detection of ARM and a comprehensive response. Key strategies include individual index malaria case detection serving as the focal point for subsequent comprehensive response to surrounding areas. Village Malaria Workers (VMWs) are trained to diagnose malaria in their communities and take a blood smear. Following patient treatment through directly observed therapy (DOT), the VMW takes a second blood smear after 72 hours of the treatment initiation which is analyzed at a health center laboratory. Cases that are still positive on day-3, and therefore an early warning sign for ARM, receive further investigation and case management, including follow-up to day 28. In addition, 40-50 people surrounding the index case are screened for malaria regardless of symptoms and treated if infected, and any gaps in community coverage of long-lasting insecticidal nets (LLINs), indoor residual spraying (IRS), and malaria knowledge are addressed by a team including the VMW, health center staff, and district malaria officials. Between September 2010 and December 2013, 1,733 malaria patients were diagnosed with malaria by VMWs from 11 targeted health facilities in catchment areas where evidence of ARM has been found. Among the residents from four catchment areas, 1,585 *Plasmodium falciparum* and mixed infection malaria cases, 109 (7.7%) were positive on day-3. During the three year time period, however, the number of day-3 positive cases detected decreased, even while overall malaria enrolled cases increased. Potential reasons for the drop in day-3 positive cases could include the systematic DOT provided by the VMWs, ensuring treatment adherence and improved quality of the drug supply.

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REAL-TIME PCR AND HIGH RESOLUTION MELTING (HRM) ANALYSIS OF POLYCLONAL *PLASMODIUM FALCIPARUM* INFECTIONS

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Recurrent *Plasmodium falciparum* parasitaemia in treated malaria patients results from either recrudescence of parasite clones which survived drug action, or acquisition of new clones that emerge from the liver during follow up after antimalarial treatment. One molecular method used to distinguish between the two is PCR-correction using size polymorphisms of various genes and microsatellites . The recurrent infection is commonly categorized by comparing the size of polymorphisms in genetic markers of the genes on enrolment samples (day-0) and on follow-up day on which parasite are detected by microscopy (day of parasitological failure). We have previously reported that by increasing the parasite genotyping to multiple time-points (day-0, day-1 and day-2), recrudescent clones are identified more often than by the conventional approach. However, most currently used gel-electrophoresis methods, particularly when multiple time-points are incorporated, are time-consuming, laborious and the interpretation of results can be ambiguous. The development of an alternative, rapid, simple and accurate method based on Real-time PCR and High Resolution Melting (HRM) analysis will be reported. Its usefulness for *ex vivo* drug sensitivity and complexity of infection studies will be discussed.

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K13-PROPELLER, MAL10 AND MAL13 AND POLYMORPHISMS IN *IN VIVO* ARTEMISININ SUSCEPTIBLE *PLASMODIUM FALCIPARUM* PARASITES FROM BOUGOULA-HAMEAU, MALI

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Artemisinin resistance has been documented in South-East Asia and may already be spreading in that region**.** Molecular markers are important tools in monitoring the spread of antimalaria drug resistance. Recently, two SNPs on chromosomes 10 and 13 (MAL10-688956 and MAL13-1718319) were shown to be useful markers of delayed parasite clearance in surveillance for artemisinin resistance in South-East Asia. In addition, mutations in the PF3D7_1343700 kelch propeller domain ('K13-propeller') were associated with artemisinin resistance *in vitro* and *in vivo*. The prevalence of these molecular markers of artemisinin resistance is unknown in sub-Saharan Africa. We therefore extracted DNA from dried blood spots of pre-treatment falciparum malaria infections in Bougoula-Hameau, Mali where we recently showed that artemisinin monotherapy was highly efficacious with a median parasite elimination slope half-life of 2 hours. Polymorphisms of MAL10-688956 and MAL13-1718319 were genotyped by Nested PCR followed by restriction enzyme digestion with *NsiI* and *MslI*, respectively. Mutations in K13-propeller were determined by direct sequencing of Nested PCR amplicons. One hundred children aged 1-10 years were included in this study. Sample processing and statistical analyses are underway and results will be presented at the meeting. This study will provide information on the prevalence of molecular markers of artemisinin resistance in a population of sub-Saharan African malaria parasites fully susceptible to artemisinin.

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IDENTIFICATION OF MALARIA-INFECTIOUS INDIVIDUALS BY FIELD-BASED DIAGNOSTIC METHODS: LOOP-MEDIATED ISOTHERMAL AMPLIFICATION AND LIGHT MICROSCOPY

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Malaria elimination strategies will require sensitive diagnostic tools capable of detecting most human falciparum infections in endemic communities. Malaria loop-mediated isothermal amplification (LAMP) is a field-friendly assay that has greater diagnostic accuracy compared to microscopy and consequently might have a key role in mass screening campaigns. Here, we assessed the infectiousness of 130 randomly-selected individuals living in a malaria endemic area of Burkina Faso by membrane feeding assays (N=307 assays) and related infectivity results to malaria infection status determined by two field-based methods (LAMP and light microscopy), and by quantitative-nucleic acid sequence-based amplification (QT-NASBA). In 72.0% of samples, *Plasmodium falciparum* infection was detected by LAMP. Parasites were identified by microscopy (asexual or sexual parasites) and 18s NASBA in 45.0% and 94.8% of samples, respectively. In 75.5% of feeding assays where at least one mosquito was infected, parasites were detected by LAMP, similar to light microscopy (74.4%). Our results confirm previous findings that LAMP is more sensitive than microscopy for detecting falciparum infection. A similar proportion of infectious individuals had parasites detected by LAMP and microscopy, suggesting that high parasite density, higher than microscopy detection threshold, is an important predictor of infectiousness. Further research is warranted to verify whether this pattern also occurs at low transmission intensities, where most infectious individuals have low-level infections.

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UNEARTHING THE HIDDEN INTRICACIES BETWEEN FISHING AND MALARIA: A CASE STUDY OF RUSINGA ISLAND, WESTERN KENYA

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Establishment of underlying factors of malaria exposure remains key for identification of ideal preventive measures.It is apparent that malaria risk behaviours are rooted in wider aspects of local livelihoods, and socio-cultural beliefs and practices. This research work draws on empirical data derived from in-depth, one-to-one semi-structured interviews, focus groups and questionnaire administration to participants in villages in Rusinga Island to explore the nature of these practices and their potential impact on malaria exposure risk. Participants were fish crew, both men and women. By eliciting local understandings of malaria-related behaviours, we explore how malaria risks are played out in people's everyday lives. Our findings reveal the problem of non-usage of bednets by community members resulting from their livelihood and socio-cultural practices and events. These practices contravene the consistent and sustained use of the bednet which are called for by public health policies. In particular, we try to explain how in reality livelihood activities and lifestyle play a role in exposure to malaria by Rusinga fish crew around dawn and dusk.

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A COMMUNITY-BASED SURVEY ON KNOWLEDGE, ATTITUDE AND PRACTICE OF DIAGNOSIS AND TREATMENT FOR VIVAX MALARIA IN SOUTH BENGKULU AND MINAHASA DISTRICTS, INDONESIA

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Plasmodium vivax malaria globally threatened an estimated 2.49 billion people in 2010. In Indonesia, *P. vivax* is transmitted among over six million pregnancies and 130 million individuals, with overall prevalence of 3.84%. On the contrary to a conventional philosophy, growing evidence suggests that *P. vivax* demonstrate as debilitating illness and life-threatening for adults and children in eastern archipelago. Management of *P. vivax* malaria is challenging due to the presence of relapsing liver stage (hypnozoites), which can be killed by the only available drug, Primaquine (PQ) and administration with blood schizontocides acts as radical cure of vivax malaria. According to current recommendations, suspected fever cases should be confirmed by microscopy or rapid diagnostic tests (RDTs) prior to treatment. However, a vast majority of fever cases in Indonesia are clinically diagnosed and Artemisinin Combination Therapy (ACT) is not properly administered. PQ failure has been attributed to inadequate treatment regimen and dosage, and patients' lack of compliance. Further, PQ can induce hemolytic toxicity in individuals who are deficient in the enzyme glucose-6-phosphate dehydrogenase (G6PD). Treatment and diagnostic practices essential for optimal PQ efficacy are inadequate in Indonesia, as indicated by high levels of drug resistance. To date research into malaria-related knowledge and behaviours has been widely conducted with household surveys. These measurements reveal information on determinants of malaria diagnosis and treatment processes. Cross sectional surveys were conducted between April and August, 2013. Three questionnaires were implemented in the household and primary health centers (PHCs). Total number of respondents was 562. Clinicians and laboratory technicians were queried in ten selected PHCs. Data was analyzed by STATA ver. 9. Overall, the processes involved in vivax malaria diagnosis and treatment must be evaluated in order to provide control programs with a "pulse of community. Most people stated an understanding about malaria, symptoms, severity level and consequence of untreated malaria. However, despite a call for malaria elimination program, community awareness related to early diagnosis and effective treatment should be strengthened. Health providers require multi-faceted interventions to support better laboratory confirmation and treatment towards vivax malaria infections.

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SINGLE DOSE OF PRIMAQUINE FOR MALARIA ELIMINATION IN A POPULATION WITH HIGH PREVALENCE OF G6PD DEFICIENCY

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Malaria caused by multi-drug resistant *Plasmodium falciparum* (including artemisinin resistance) is common in the population living along the Thai-Myanmar border. In the context of malaria elimination to combat the spread of artemisinin resistance, the Shoklo Malaria Research Unit has evaluated the safety of single dose of 0.25 mg base/kg of primaquine with an ACT in a population of over 2000 subjects. Blood collection for hemoglobin levels and sensitive detection of malaria parasite were performed every 3 months during one year in 4 different villages. G6PD was characterized by Fluorescent Spot Test in all study participants and genotyping performed for the most common local mutations in subjects with abnormal phenotype. Hemoglobin levels of subjects treated with PMQ before and after primaquine administration were analyzed. Results will be presented showing the effect of the single low dose primaquine in G6PD normal and deficient participants.

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PROTEIN MICROARRAY ANALYSIS OF HUMAN ANTIBODY RESPONSES IN *PLASMODIUM VIVAX* RELAPSE VS. REINFECTION IN THE PERUVIAN AMAZON

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Accurate estimations of parasite prevalence, malaria disease incidence, and hence prioritization of control efforts require new, cost-effective, and field-deployable methods. Because determining the presence of malaria parasitemia is time-intensive, often insensitive, and expensive, interest has grown for using serological tools to monitor infection status and transmission dynamics. Because *Plasmodium vivax* is only available in relatively low parasitemia from humans and in limited quantities from non-human primates, microarray seroepidemiology using *P. vivax* asexual stage parasite lysates has only infrequently been done. The field activities of this study were carried out from 2005-2008 in the Santo Tomas, San Jose de Lupuna, and Padrecocha villages of the Peruvian Amazon. Genotyping of *P. vivax* isolates was performed using PCR-restriction fragment length polymorphism (PCR-RFLP) analysis of the *P. vivax* merozoite surface protein-3alpha (PvMSP3α). Relapse was defined as identical RFLP patterns of the primary and subsequent infection parasites. Reinfection was defined as different RFLP patterns of the primary and subsequent infection parasites. Of 111 subjects, 96 had 2 episodes, 10 had 3 episodes, and 5 had 4 episodes over a 24-month follow-up period. Using PvMSP3α PCR-RFLP genotyping of the recurrent infections, 38 were classified as relapse; 88 were classified as reinfection; and 5 showed no RFLP band and thus were not classified. Using microarray analysis, the antigens differentially recognized by *P. vivax*-infected subjects were more likely to have significantly higher signal for subjects with PvMSP3α genotypes different than the previous infection than for subjects with PvMSP3α genotypes similar to the previous infection. Proteins recognized by Human IgG Antibodies are more likely to have amino acid changes than proteins coded by the genome as a whole. Proteins recognized by sera are more likely to have SNPs than the genome as a whole, suggesting selection arising from host-pathogen interactions, immune or otherwise. The protein microarray analysis results will provide tools for applying sero-epidemiology to surveillance, control and elimination; establishing tools for decay kinetics.

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EFFICACY OF INDOOR RESIDUAL SPRAYING (IRS) WITH ACTELLIC® 300CS IN SENEGAL

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In areas of low malaria transmission, such as western Senegal, where scaling up of control measures has been effective in reducing malaria incidence, additional measures are required to eliminate the disease. Targetting additional control measures to communities with persisting high transmission of malaria may be more effective than blanket control measures. As part of a cluster randomized trial of a targetted control strategy, efficacy of Actellic 300CS (pirimiphos methyl) for IRS delivered by district spray teams was evaluated. Hotspot villages were identified on the basis of the number of cases of malaria the previous year. In August 2013, IRS with Actellis300CS was delivered to all households in these villages by community health workers, trained and supervised by staff of the department of Public Health (Service National d'Hygiène). Efficacy was assessed in 4 villages over 4 months. In each village, 5 treated rooms were selected, and untreated rooms selected as controls, and bioassays were done on each of three walls per room to measure knock-down and 24-hour mortality of lab reared *Anopheles gambiae* and of wild mosquitoes locally caught. A household survey was conducted to assess completeness of coverage and acceptability. Results and conclusion: After two, three and four months the 24-hour mortality, adjusted for control mortality using Abbott's formula, was 92% (95%CI 85%,96%), 72% (63%,80%), and 37% (29%,45%) respectively. Sensitivity tests to different insecticides using local *Anopheles* showed 100% sensitivity to pirimiphos methyl in all four sites, and to bendiocarb and permethrin in three sites with lower sensitivity in one district, with resistance to DDT in all sites. These results show that IRS with Actellic 300CS was highly effective but the duration of efficacy was shorter than expected.

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APPLICATION OF MOLECULAR BARCODE AND PRE-AMPLIFICATION TECHNIQUES IN TRACKING *PLASMODIUM FALCIPARUM* POPULATION STRUCTURE CHANGES DURING SCALE-UP MALARIA INTERVENTIONS IN SOUTHERN AFRICA

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Malaria is receding from many endemic countries in the wake of scaled-up interventions for possible elimination of the disease. However, the decline is heterogeneous within and between countries and the resilient scourge persists as low-grade submicroscopic infections among semi-immune members of the population. This transitioning epidemiology not only poses risk of possible resurgences typical for malaria, but also presents challenges for detection and tracking of intervention impact on the surviving parasite reservoir. Here we demonstrate application of molecular barcode and pre-amplification technology for the successful detection and documentation of *Plasmodium falciparum* population structure changes in relation to control interventions in areas of southern Africa. Dry blood spot (DBS) samples were collected through prospective cross-sectional surveys, between 2003 and 2013, in three areas of southern Africa exhibiting contrasting malaria epidemiology under control, namely Choma district in southern Zambia (successfully declining malaria), Mutasa district in eastern Zimbabwe (rebounding malaria) and Nchelenge district in northern Zambia bordering the Democratic Republic of Congo (persistently holoendemic malaria). Following DBS screening by realtime quantitative PCR (with preamplification for low-grade infections), *P. falciparum* infections were genotyped using a 24 SNP molecular barcode assay. Analysis of population structure across the 24 barcode markers demonstrated significant loss of genetic diversity and population structure decline for Choma (M-W U = 178.0, p = 0.023) but not for Mutasa (MW U = 10, p = 0.487) or Nchelenge, where the genetic diversity was even higher than Mutasa (MW U = 35.0, p = 0.029). Barcoding and pre-amplification techniques afford an instrumental tool for parasite identification, tracking and documenting the impact of interventions on parasite populations during malaria control and elimination programmes when parasitaemia is expected to decline to submicroscopic levels.

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MALARIA AND HEALTH NEEDS ASSESSMENT AMONG MIGRANT FARM WORKERS IN AMHARA REGION, ETHIOPIA: A VENUE-BASED APPROACH

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Mobile populations present unique challenges to malaria control and elimination. Each year, around 350,000 people travel to areas of North Gondar Zone, Ethiopia to work on large-scale farms, doubling the population of areas with the heaviest malaria burden in Amhara Region. The farming season overlaps the major malaria season (September to December). Little is known about these migrant workers, including migration patterns, access to, and use of malaria prevention measures, housing accommodations, and health care seeking behavior. In order to assess these factors and prevalence of malaria and anemia, a venue-based survey of migrants who traveled for farm labor in two districts of North Gondar was conducted in July 2013 at the beginning of the farming season. A total of 605 migrants ≥18 years of age were recruited from three venue types: farms (58% of survey population), roads between farms (16%) and towns (26%). Most (73.1%) had started farm work at the time of survey and 99% were male. Mean age was 22.8 years, with 74.7% less than 25 years. Nearly all (95.8%) came from Amhara, with half (51.8%) of those coming from other districts in North Gondar. Most (77.4%) migrants arrived in June or July, and 46.3% intended to leave in September. Around half (52.1%) lived in temporary shelters, while 20.8% had no sleeping accommodations. Malaria was the leading identified health concern. LLINs were available to 12.0% of participants. Reported net use last night was 74.6% among those with access to a net and 8.8% overall. *Plasmodium* prevalence by RDT was 12.0%, with 9.6% *Plasmodium falciparum*, 1.7% *P. vivax*, and 0.7% mixed infections. Anemia (Hb <13 mg/dl) was detected in 28.3% of migrants. Around one-third (30.3%) reported having fever within the past two weeks, of whom 31.3% sought treatment. Distance to a healthcare facility and cost were important reported barriers to seeking treatment. These results are being utilized to develop interventions tailored to this population.

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COMMUNITY PERCEPTIONS AND PRACTICES TOWARDS MALARIA CONTROL MEASURES IN RWANDA: A DESCRIPTIVE QUALITATIVE STUDY

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Long lasting insecticidal nets (LLINs), indoor residual spraying (IRS) and malaria case treatment with artemisinin based combination therapy have been proved to reduce malaria significantly. Elimination requires consideration of other factors yet underexplored. Our objective was to explore behavioral and environmental predictors associated with malaria transmission, to draw operational recommendations. In December 2013, we conducted nine focus group discussions, in Ruhuha, Southern East of Rwanda with 81 participants. Various cooperatives members, community health workers, health care professionals, lay community, schools teachers, youth representatives, as well as local and religious leaders were included. Discussions were recorded, transcribed in English and coded using Nvivo 10. Study protocol was approved by Rwanda National Ethics Committee. Malaria ranked first among the top five diseases in the area. High knowledge on transmission and symptoms was observed. The notion of malaria elimination by the community was acknowledged but challenges noted. A recent outbreak of bedbugs was found hindering the use of LLINs in addition to the hot weather characterizing the dry season. IRS was generally perceived as no longer killing insects including mosquitos unlike spreads them. Seeking care at the health center as first choice was related with having a community based health insurance (CBHI) while buying medicines in pharmacies was commonly seen among those without a CBHI or who claim spending much time at the health center. A recent wealth categorization has had an impact on the amount to be contributed to get a CBHI. A large number of participants argued that the categorization was unfairly done and leading some families unable to afford the CBHI. In conclusion, joint efforts are needed to tackle issue of bedbugs for proper use of LLINs at household level. Further studies are needed to test the efficacy of IRS and look for appropriate chemicals if needed. Lastly, to uphold high coverage of CBHI, government is suggested to revise the wealth categorization.

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EVALUATION OF THE LOOP MEDIATED ISOTHERMAL DNA AMPLIFICATION (LAMP) FOR MALARIA DIAGNOSE IN A *PLASMODIUM VIVAX* ENDEMIC SETTING

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Current malaria diagnostic tests, including microscopy and antigen-detecting rapid tests, cannot reliably detect low-density infections. Molecular methods such as polymerase chain reaction (PCR) are highly sensitive but remain too complex for field deployment. We assessed the field applicability of a new molecular assay for malaria diagnosis based on loop-mediated isothermal amplification (mLAMP) in *Plasmodium vivax* endemic setting of Colombia. A prospective cohort of patients was used to assess the diagnostic performance of the mLAMP. After signing the informed consent, volunteers were diagnosed using microscopy and LAMP at point of care (POC). To assess the field performance of mLAMP for detection of submicroscopic infections, a cross-sectional survey was conducted in 3 regions of Colombia with different epidemiological profiles. The mLAMP results were interpreted by visual reading under ultra violet light. Real time PCR and nested real time PCR in discordant samples was used as reference. LAMP sensibility and specificity was comparable to real time PCR for both *P. vivax* and *P. falciparum* detection at point of care diagnosis. 278 febrile patients were enrolled at Tierralta, Cordoba, the mLAMP sensitivity of for both *P. falciparum* and *P. vivax* was 100% and 90% respectively compared with PCR. All false-negative LAMP results involved samples of *P. vivax* with parasitaemia levels detectable by 3-well nested real time PCR but very low or undetectable by qPCR. In the cross-sectional, survey 980 volunteers in 10 sentinel sites at 3 different malaria endemic regions of Colombia were enrolled. LAMP detected 14.8 times more cases than microscopy with a prevalence across all sentinel sites of 6.02% (n=59) and 0.4% (n=4) respectively. *Plasmodium falciparum* infections accounted for 23.9% and *P. vivax* infections for 76.1%. No cases of mixed infections were identified by PCR. The proportion of asymptomatic infections among mLAMP confirmed cases was 98.0%. Malaria LAMP in a *P. vivax* low endemic setting achieved sensitivity similar to that of single-well nested PCR in a reference laboratory. LAMP showed detection limits comparable to PCR under minimum infrastructure condition under field conditions and dramatically increase the detection of asymptomatic malaria providing a new tool for diagnosis, surveillance, and screening in elimination strategies.

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A NOVEL CROWD-SOURCING TECHNIQUE FOR PREDICTING DENSITIES AND DISTRIBUTION OF DISEASE-TRANSMITTING MOSQUITOES IN RURAL TANZANIA

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Lack of reliable techniques that can be used for large-scale programmatic monitoring of distribution and densities of disease-transmitting mosquitoes is a major challenge to public health authorities, especially in rural and remote communities where high-tech GIS and remote sensing facilities are not readily applicable for regular use. We developed and evaluated a new community-based participatory mapping approach that relies simply on the knowledge and experiences of residents to rapidly identify areas where disease-transmitting mosquitoes are most abundant. The method is proposed for use in spatial targeting of mosquito control interventions. Such simplified methodologies for mapping vector densities will be particularly necessary for optimal placement of new interventions to complement existing ones such as long-lasting insecticide treated nets (LLINs) for malaria control. This new crowd-sourcing technique consisted of 5 steps. We initially mapped three test villages comprehensively identify major land marks (step 1). We then selected 60 community members monthly, taught them basic map-reading and offered them gridded maps of their own villages so they could identify locations where they think mosquitoes are most abundant, by simply ranking the grids on scale of 1-5 (step 3). The data generated was interpolated in ArcGIS using inverse distance weighting method and classified to show places where people thought there were high, medium and low mosquito densities (step 4). Finally, mosquito sampling was done using an effective odor-baited sampling tool, to verify outdoor mosquito densities in locations pre-identified by community members as having high, medium and low mosquito densities, and to validate this crowd-based prediction method. Maps were derived from community knowledge and opinions on the mosquito density distributions. For twelve months in three villages, entomological surveys depicted the same vector densities and distribution pattern as the crowd-sourcing technique. This study thus provides evidence that we can rely on community knowledge and experience to identify suitable areas where mosquitoes are most abundant and where to locate outdoor complementary interventions. Such a method will be cheaper, quicker and easier even for planning and implementing large-scale vector control operations.

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EVALUATION OF THE EFFECTIVENESS OF MALARIA CONTROL ACTIVITIES BY SIMULTANEOUS NATIONWIDE CROSS-SECTIONAL AND CASE-CONTROL SURVEYS IN MADAGASCAR

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The Malaria Control Program of Madagascar has set since 2007 an objective to pre-eliminate malaria. In order to help decision making to reach pre-elimination, we aimed at evaluating the actual effectiveness of malaria control activities. We conducted a nationwide survey in 2012-2013 in 62 sites representing all transmission patterns. This survey included (1) a cross-sectional study to measure the effectiveness of each control intervention on reducing the transmission, and (2) a concurrent case-control study to measure the effectiveness on reducing the morbidity. We present here the results related to organized vector control activities, i.e. Long Lasting Insecticidal Nets (LLIN) distribution and Indoor Residual Spraying (IRS) campaigns. The cross-sectional survey included 15,734 individuals among which 3.7% had a positive Rapid Diagnostic Test (RDT). LLIN daily use was 52.3% in areas covered by universal distribution and IRS coverage was 64.8% in targeted areas. 818 uncomplicated clinical malaria cases were compared to 7,767 controls living in the same villages. Multilevel analysis of factors associated with a positive RDT or with the occurrence of an episode of non-complicated malaria revealed that LLIN daily use had a 45% protective effectiveness (PE) against infection (aOR 0.55 [95%CI 0.42, 0.72]) and a 48% PE against morbidity (aOR 0.52 [0.28, 0.96]). The PE of IRS was evaluated to be 23% against infection (aOR 0.77 [0.53, 1.13]) and 49% against morbidity (aOR 0.51 [0.39, 0.66]). In areas where both activities are implemented, coverage of LLIN was 21.3 percentage points lower than in areas where LLIN only were deployed. Combining IRS with LLIN provided almost no gain in preventing infection, but the PE of LLIN use against morbidity increased from 51% (aOR 0.49 [0.20, 1.20]) to 66% (aOR 0.34 [0.16, 0.74]) when IRS was added, although non significantly. Our results show that, taken separately, LLIN and IRS perform satisfactorily but that their concurrent use might have a limited benefit as compared with efforts to improve the coverage of a single intervention.

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IDENTIFICATION OF ASYMPTOMATIC MALARIA INFECTION IN BORDER CROSSING POPULATIONS IN CAMBODIA

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Cross-border population movement can hinder elimination of malaria by importing new infection, maintaining hot-spots of high transmission, and contributing to the development and spread of drug resistance. Cross-border movement is common in Cambodia yet there is limited surveillance at border points. To investigate malaria infection in border-crossing populations, three border crossing points were chosen as study sites, one from each of Cambodia's borders with Thailand, Vietnam and Lao. From August 2013 to February 2014, 3,206 border crossers were tested for malaria (by RDT and PCR) and fever, and were interviewed regarding *a priori* risk factors for infection. Univariate and multivariate logistic regression were performed to investigate potential exposure variables against the outcome of malaria infection. Based on RDT analysis there were 103 cases of malaria (67 Pf, 34 Pv and 2 mixed) giving an overall prevalence of 3.21% (PCR prevalence and drug resistance analysis in progress and available April 2014). Prevalence differed between boarder sites (0.09% Thailand, 0.99% Vietnam and 8.04% Laos). Sixty-nine (67%) cases were asymptomatic. Main risk factors for infection determined by multivariate logistic regression were previous episode of malaria (OR 5.52, p<0.0001), Forest-goers (OR 5.26, p<0.0001), <15 years old (OR 4.94, p=0.003), presence of fever (OR 4.03, p<0.0001), and working as security/armed forces (OR 3.11, p=0.02). Knowledge of two or more prevention methods gave a protective effect from infection (OR 0.45, p=0.008). The Lao border point had greater prevalence of forest-goers, high-risk occupations, previous malaria episodes, and low knowledge of prevention methods; leading to higher prevalence of malaria infection. Cross-border malaria will hinder elimination where border-crossing populations have high prevalence of risk factors for infection. Lessons learned from this project have set the basis for a comprehensive cross-border surveillance platform in the Greater Mekong Subregion. Similar border points should continue to be identified and targeted surveillance put in place for countries across the GMS where population movement is common.

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CHANGING TRENDS IN MALARIA AMONG MOZAMBICAN PREGNANT WOMEN: EFFECT ON ANTIMALARIAL IMMUNITY AND CLINICAL IMPACT OF *PLASMODIUM FALCIPARUM* INFECTIONS

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Understanding the determinants and consequences of malaria declines and resurgences as well as the timescales over which antimalarial immunity is gained and lost, has become a priority in the context of current goals of malaria elimination and eradication. Epidemiological changes provide a unique opportunity to investigate how anti-malarial immunity is lost following reductions in transmission and how this might impact disease burdens in potential resurgences. Here we present the results from a study that combines epidemiological and immunological description of malaria among pregnant women delivering between 2003 and 2012 at antenatal clinics in the Manhiça District hospital in Southern Mozambique. *Plasmodium falciparum* qPCR-positivity decreased from 33% in 2003 to 2% in 2010 (p<0.001) and increased to 6% in 2012 (p=0.026), with antimalarial IgGs mirroring these malaria trends. Parasite densities in peripheral blood and in the placenta were higher in 2010-12 than in 2003-5, whereas prevalence of peripheral infections that were submicroscopic followed an opposite trend. An attenuation of the parity effect on parasite densities and on IgGs against placental-type parasites was observed during 2010-12 compared to 2003-5. Malaria infection was associated with a larger reduction in maternal haemoglobin levels and in the birthweight during 2010-12 compared to 2003-5. These results suggest that a sustained reduction in the exposure to malaria parasites leads to a weakening of immune regulation of parasite densities that can increase the potential for occurrence of high-density infections and malaria-related harmful effects. These findings reinforce the importance of sustaining efforts when moving from control to elimination to avoid rebounds of malaria associated with the reduction in naturally acquired immunity.

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PROGRESS TOWARD DEVELOPMENT OF A LOW DENSITY INFECTION-DETECTION TEST TO SUPPORT ACTIVE DETECT-AND-TREAT INTERVENTIONS AIMED AT REGIONAL MALARIA ELIMINATION

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As successful malaria control programs dramatically reduce malaria prevalence, strategic and programmatic changes are required to eliminate malaria transmission altogether. Development of new, game-changing, and possibly disruptive malaria infection-detection technologies can enable more efficient elimination interventions in the most challenging malaria-endemic environments. For active detect-and-treat interventions, the essential product characteristics required are: low limit-of-detection, rapid turn-around times, ease of use, high sensitivity, portability, and appropriate pricing. PATH's Diagnostics for Malaria Elimination Toward Eradication (DIAMETER) project is focused on bridging the gap between the rapidly evolving set of detect-and-treat tactics and the existing diagnostic capabilities. We aim to ensure that elimination interventions are not hampered by test performance. The goal of the DIAMETER project is to catalyze rapid access to the most cost-effective and temporally-effective elimination infection-detection tools in the product development pipeline. Our phase I activities have produced an elimination market analysis, a six-country field investigation of user requirements, and a landscape of technologies in the product development pipeline. Our phase II activities focus on development of infection-detection tests (IDTs) for qualitative detection of low-density *Plasmodium falciparum* infections. We envision that IDTs will be used by malaria elimination programs to identify and treat subclinical, low-parasite-density populations that serve as reservoirs of parasite biomass. We will present our phase I findings, the process used to develop the IDT target product profile, and the landscape of promising platform technologies that can be leveraged to achieve the required performance characteristics.

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G6PD DEFICIENCY IN LATIN AMERICA: PREVALENCE, VARIANTS AND IMPLICATIONS FOR MALARIA ELIMINATION

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Glucose-6-phosphate dehydrogenase deficiency (G6PDd) is the most prevalent human genetic disease worldwide. Predominance of *Plasmodium vivax* malaria in Latin America (LA) is especially relevant, since *P. vivax* radical cure requires the use of primaquine (PQ). It is important to consider that PQ may cause severe drug-induced hemolysis and may lead to acute intravascular haemolysis in G6PDd individuals. This could further hamper malaria control efforts. Despite clinical and epidemiological significance of G6PDd and malaria interaction, G6PDd prevalence has not been well measured in LA populations. A systematic review using existing bibliographic and biomedical databases was made to better understand the distribution of G6PDd in Latin America and the Caribbean. The review resulted in low prevalence rates in Argentina, Bolivia, Mexico, Peru and Uruguay. Studies performed in Curaçao, Ecuador, Jamaica, Saint Lucia, Suriname and Trinidad, as well as some surveys carried out in some areas of Brazil, Colombia and Cuba showed high prevalence (>10%) of G6PDd. G6PD encoded by the *G6PD A-^{202A}* mutation was the most broadly distributed across LA, being identified in 81.1% of deficient individuals surveyed. It was observed a virtual absence of G6PDd among Amerindians, suggesting that PQ use is safe in this population. In order to avoid unnecessary exposure to hemolysis inducing drugs, as PQ, it is crucial to develop a rapid and accurate G6PDd diagnostic test to be used in LA field conditions. Issues related to the radical cure of *P. vivax* infection and an eventual elimination of malaria in LA, such as massive drug treatment, must be linked to information about the genetic background of the population. Although considering the inexistence of multicenter studies with similar methodologies, it is possible to estimate roughly that G6PD deficiency in LA is a recognized phenomenon, however with the evidence that some populations are probably not affected, such as the indigenous.

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QUALITY OF ARTEMISININ COMBINATION THERAPIES IN SUB SAHARAN AFRICA AND CAMBODIA, ASSESSED USING LABORATORY ANALYTICAL TECHNIQUES

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Artemisinin combination therapies (ACTs) are recommended as first line treatment for malaria by the World Health Organisation (WHO) and officially implemented in 79 of 88 countries. Reports of ad hoc surveys from SE Asia showed that up to 50% of the artesunate monotherpy sold was falsified. Alarmingly the first case of falsified artesunate (that almost resulted in a fatality) and ACTs were also reported from Africa where the predominant parasite species is the potentially fatal *Plasmodium falciparum*. Resource poor countries do not have the technical (medicines control laboratories), financial, or human resources required to inspect and police the drug supply. The lack of reliable estimates of poor quality drug prevalence and its causes makes it difficult for national regulatory authorities to determine the need and scale of interventions to assure drug quality. We purchased and carried out qualitative and quantitative content analyses on over 10,500 ACTs in total from Cambodia, Ghana (Kintampo), Tanzania, Nigeria (Enugu and Ilorin) and Equatorial Guinea (Bioko Island) following varying sample collections methods to provide effective surveillance of ACTs available in a given geographical region. The percentage active pharmaceutical ingredient (% API), determined by laboratory chromatographic analysis, was used to classify the medicines as good quality (85% - 115%), as recommended by the United States Pharmacopeia for the analysis of single tablet samples. Poor quality samples included substandard samples (115% of both APIs), degraded samples (improper storage and/or transport) and falsified samples (absence of stated APIs). Most of the samples contained the stated APIs, with the exception of samples from Enugu (1% out of 2865), Ilorin (0.8% out of 1449) and Bioko (7.3% out of 683) which did not contained the stated APIs. Of concern are the monotherapy tablets on sale in countries with falsified ACTs. The results were disseminated to the country-specific ministry of health, as well as relevant manufacturers and logged on the WHO Rapid Alert System for the surveillance and monitoring of Substandard/ Spurious/Falsely Labelled/ Falsified Counterfeit, SSFFC, medicinal products.

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SURVEILLANCE SYSTEMS TO FACILITATE MALARIA ELIMINATION

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Robust and responsive surveillance systems are critical for the success of malaria control and elimination. In elimination settings surveillance must be an intervention, where immediate action is taken in response to case

identification. An ideal surveillance system for malaria elimination should include: rapid and complete case reporting, incorporation of data from other relevant systems, central data storage and management, automated and expert data analysis, customized outputs and feedback, and timely and targeted response. Spatial information enhances a surveillance system, ensuring that cases are tracked to the household level and are mapped over time. Data-sharing and coordination across borders to address imported malaria are vital and the incorporation of new technologies can improve the speed, accuracy or quality of data. Ideally, a malaria elimination surveillance system needs to take information from many sources, in multiple formats, conduct some degree of automated analysis and disseminate tailored reports to multiple levels to guide action. While many countries' surveillance systems include one or several of these ideal elements, no single system contains all components. Understanding countries' successes and challenges designing and implementing surveillance systems facilitates the refinement or creation of systems in the future. Many parts of the ideal system for malaria elimination surveillance have already been developed, but have yet to be linked together in a coherent system. To facilitate this process, malaria elimination programs should support the implementation and refinement of existing systems and ensure political and financial commitment for this process. Malaria elimination programs should also strive to: develop systems to improve the access and utility of surveillance databases, establish and pilot cross border databases, and support the creation of standard indicators for malaria surveillance. Ultimately, investment in a timely and targeted surveillance system is necessary to achieve elimination goals.

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THE DIMINISHING RETURNS OF ATOVAQUONE-PROGUANIL FOR ELIMINATION OF *PLASMODIUM FALCIPARUM* MALARIA: MODELLING MASS DRUG ADMINISTRATION AND TREATMENT

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Artemisinin resistance is a major threat to current efforts to eliminate *Plasmodium falciparum* malaria which rely heavily on the continuing efficacy of artemisinin combination therapies (ACT). It has been suggested ACTs should not be used in mass drug administration (MDA) in areas where artemisinin resistant *P. falciparum* is prevalent and that atovaquone-proguanil (A-P) might be a preferable alternative. However, a single point mutation in the cytochrome b gene confers high level resistance to atovaquone, which arises frequently during treatment and this would affect the potential efficacy of atovaquone-proguanil as a tool for elimination. A deterministic population level mathematical model was developed based on data from Cambodia to explore the possible effects of large-scale use of A-P compared to ACT for mass drug administration and/or treatment of *P. falciparum* malaria, with and without adjunctive primaquine (PQ), with the aim of local elimination. The model showed the initial efficacy of ACT and A-P for MDA to be similar. However, each round of A-P MDA resulted in rapid acquisition and spread of atovaquone resistance. Even a single round of MDA could compromise its efficacy sufficient to preclude its use for treatment or prophylaxis. A switch to A-P for treatment of symptomatic episodes resulted in a complete loss of efficacy in the population within 4-5 years of its' introduction. For malaria elimination, A-P for MDA or treatment of symptomatic cases should be avoided. A combined strategy of ACT+PQ MDA, long-lasting insecticide treated bed nets and high coverage with ACT for treatment of symptomatic episodes would be preferable.

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USING ROUTINE AGGREGATED MALARIA CASE DATA FROM SWAZILAND TO PRODUCE FINE SCALE RISK MAPS

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Mapping malaria risk is an integral component of effective and efficient resource allocation. As transmission declines and infections become increasingly rare, countries have to move from using infection prevalence data to case data for stratification, mapping and prediction of transmission risk. While some programs collect information on the household locations of individual cases, facilitating fine scale risk mapping, doing so is resource and time intensive. Routine health facility data is more convenient to collect, but its utility for extrapolating risk across surrounding regions is presently unclear. Using routinely collected case data from health facilities in Swaziland between 2011-2013, here we explore the use of a method termed point sampling, in conjunction with random forest models, to produce fine scale risk maps (~300m resolution). Using known household locations of cases to validate models, results show that all three variations of the approaches tested here have very good predictive ability (AUC values ≥0.83) with the optimal approach incorporating the spatial distribution of the population. Predictions from the best performing model also showed good correspondence with a reference risk map generated using the household locations of cases (mean error = 0.01, absolute error = 0.02). Cross-scale models utilizing routine health facility level data can provide fine scale prediction of malaria risk in low transmission settings and hold promise for other cross-scale disease prediction problems. Bayesian approaches to cross-scale prediction will also be discussed.

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RETREATED BUT NOT DEFEATED: THE ROLE OF HEALTH SYSTEMS IN IMPROVING THE EFFECTIVENESS OF ARTEMISININ COMBINATION THERAPIES (ACTS) FOR MALARIA CONTROL AND ELIMINATION

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Artemisinin Combination therapies (ACTs) are highly efficacious first-line antimalarials. However their use in sub-Saharan Africa, both as treatment and control measure, is hampered by weak health systems and a poorly controlled diversity of antimalarial sources. We extended an existing mathematical model of malaria transmission to include health systems factors: dimensions of access to sources of ACTs in the private, public and tertiary sectors, and quality of care for malaria and non-malarial febrile illness (NMFI). Data from the IMPACT 2 study in Tanzania was used to parameterise the model. Our aim was to estimate the impact of overcoming health systems barriers in 3 settings, differing by epidemiology and prevailing health systems. Outcomes included malaria mortality and parasite prevalence (transmission risk). Primary level interventions (private and public sectors) had most impact on transmission. In low-prevalence scenarios, modelling single interventions, e.g. ensuring 100% treatment seeking for fever, eliminated parasites. Improving quality of care, e.g. introduction of diagnostic-led therapy with adequate stocks of ACTs was as effective in all settings as a policy of presumptive treatment; reducing parasite prevalence in under-fives (U5s) in moderate transmission settings by up to 86% depending on the sector of implementation. In contrast, a policy of presumptive treatment for U5s only was not

effective in any region, likely due to reservoirs of infection in older age-groups. Strengthening public facilities was ineffectual in contexts with strong private sector preference. The model outcomes demonstrate optimal packages of interventions at a national level are not always ideal interventions at a regional level. Improving the effectiveness of ACT delivery is highly dependent on local epidemiology, existing health systems provision and population preferences for either primary health facilities or private drug shops. Context-specific planning and consideration of local priorities e.g. reducing ACT wastage on NMFI versus reducing transmission, may improve progress towards international targets to decrease clinical disease and mortality, and in low transmission settings, to approach elimination.

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CONSEQUENCES OF THE TIMING OF GESTATIONAL ANEMIA ON NEWBORN'S HEMOGLOBIN CONCENTRATION IN A MALARIA ENDEMIC AREA

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Because of the high prevalence of gestational anaemia (GA) in developing countries, anaemia-related adverse effects on birth outcomes are expected to have an important public health impact. We studied the relationship between GA and newborn's anaemia, taking into account the timing of GA within the MiPPAD trial of intermittent preventive treatment in pregnancy (IPTp) (http://clinicaltrials.gov/ct2/show/NCT00811421). Study design Prospective cohort of pregnant women followed up from early pregnancy until the time of delivery. Study site, population and procedures The study was conducted in the district of Allada (southern Benin) where malaria is perennial. The study population was composed of HIV negative women less than 28 weeks of gestational age and their newborns. Socio-demographic data were collected on inclusion (before first IPTP administration, IPTp1), clinical and biological data at each visit (IPTp1, second IPTp administration -IPTp2- and delivery). Children's clinical and biological data were collected at birth. Statistical analysis Logistic and linear regressions were performed to evaluate at each time point the relation between maternal and newborns' Hb levels. Direct and indirect associations between Hb levels in pregnancy and at birth, mediated by Hb levels at different times of pregnancy, were then tested using a path analysis. Newborn anaemia was common (63.5%) in the 862 analyzed mother-newborn pairs. Linear regression showed a significant association between maternal and newborns' Hb levels at delivery only (P = 0.004). Path analyses confirmed the strong direct association between maternal Hb at delivery and newborns' Hb, and also found an indirect effect of maternal Hb at IPT1 and IPT2 (P = 0.006 and P = 0.005). In conclusion, the strong association between mothers' and newborns' Hb status at delivery shows that late GA has important consequences on the baby. The indirect effect of Hb levels at earlier stages of pregnancy suggests that preventive measures such as IPTp and micronutrient supplementations also play a role to lower the consequences of GA and should be reinforced.

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SPACE-TIME MAPPING OF MALARIA AND ITS CO-DISTRIBUTION WITH STUNTING AMONG CHILDREN BELOW FIVE YEARS IN SOMALIA USING BAYESIAN GEO-STATISTICAL APPROACH

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Several studies in developing countries have shown that malaria is associated with greater malnutrition morbidity and mortality and that there is a transient weight loss in young African children following a malaria attack. The majority of studies that have investigated the association of malaria and malnutrition were done in clinical settings and there are limited population-wide investigation of the co-epidemiology of malaria and malnutrition. In 2011, the rate of malnutrition in Somalia was cited to be the highest worldwide. *Plasmodium falciparum* prevalence in Somalia also varies from very low to moderate transmission. This study sought to map the distribution of malaria and stunting among children under the age of five years at similar spatial and temporal resolutions and determine their co-distribution using a large household cross-sectional survey data undertaken bi-annually from 2007 to 2010 in Somalia. We developed a Bayesian hierarchical space-time model through stochastic partial differential equation (SPDE) approach using R-INLA library to produce risk maps of malaria and stunting at 1 x 1 km spatial resolution and predict to each year of study from 2007 to 2010. To determine co-distribution of the two health issues, we developed a shared component model over space and time in a geo-statistical framework. We observed that the prevalence of malaria was high in the Central South zone with a prevalence of 30% followed by the Puntland, 15% and in Somaliland with less than 5%. This same pattern was observed in the rate of stunting where Central South zone had a mean rate of 30% followed by Puntland, 20% and lowest in Somaliland with mean rate of 18%. Results showed that the shared component distribution (representing social economic and environmental determinants) had a larger effect on malaria and malnutrition in the southern and central part of Somalia. Multivariate mapping models provide a better understanding of co-morbidity between health outcomes. In particular, the analyst of multiple disease outcomes can assess the underlying common and divergent spatial distributions of the diseases to optimally integrate disease management required to address the multiple burden of diseases.

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HOT SPOT OR NOT: A COMPARISON OF SPATIAL STATISTICAL METHODS TO PREDICT PROSPECTIVE MALARIA INFECTIONS

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Within affected communities, *Plasmodium falciparum* infections may be skewed in distribution such that single or small clusters of households consistently harbour a disproportionate number of infected individuals throughout the year. Identifying these hotspots of malaria transmission would permit targeting of interventions and a more rapid reduction in malaria burden across the whole community. This study set out to compare different statistical methods of hotspot detection using different indicators for prediction of infection the following year. Two full surveys of four villages in Tanzania were completed over consecutive years, 2010-2011. In both surveys, infection was assessed using nested polymerase chain reaction. In addition in 2010, serologic markers of exposure were assessed. Baseline clustering of infection and serological markers were assessed using three geospatial methods: spatial scan statistics, kernel analysis and weighted local prevalence analysis. Methods were compared in their ability to predict infection in the second year of the study using random effects logistic regression models, and comparisons of the area under the receiver operating curve (AUC) for each model. Sensitivity analysis was conducted to explore the effect of varying radius size for the kernel and weighted local prevalence methods and maximum population size for the spatial scan statistic. Guided by AUC values, the kernel method and spatial scan statistics appeared to be more predictive of infection in the following year. Hotspots of PCR-detected infection and seropositivity to AMA-1 were predictive of subsequent infection. For the kernel method, a 1km window was optimal. Similarly, allowing hotspots to contain up to 50% of the

population was a better predictor of infection in the second year using spatial scan statistics than smaller maximum population sizes. Clusters of AMA-1 seroprevalence or parasite prevalence that are predictive of infection a year later can be identified using geospatial models. Kernel smoothing using a 1km window and spatial scan statistics both provided accurate prediction of future infection.

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FINE SCALE PARTICIPATORY MAPPING OF MALARIA INFECTION CLUSTERS BY RELATING ROUTINE DIAGNOSTIC RESULTS OF PATIENTS ATTENDING HEALTH FACILITIES TO THE NAMES OF THEIR LOCAL LEADERS IN URBAN DAR ES SALAAM, UNITED REPUBLIC OF TANZANIA

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Standard methods for assessing malaria infection burden such as cross-sectional and incidence cohort surveys are limited by sample size in terms of the sensitivity and the spatial resolution they can achieve, especially as transmission declines. This study investigated whether diagnostic data collected routinely at health facilities can provide an affordable alternative for identifying malaria transmission hot spots in urban Dar es Salaam, Tanzania. The study was performed in two adjacent wards of Buguruni and Vingunguti whose total population is 178,000. Anonymised, routinely collected clinical, and test result data for patients tested for malaria were collated from June 2012 to January 2013 from a laboratory registry book at Buguruni Health Centre, located close to the boundary between the two wards. Additionally, patients were asked to provide the name of their local *Ten Cell Unit Leader,* the elected local representative responsible for residential housing clusters. Geographic coordinates of these local leaders' houses were mapped with GPS receivers so that patients' residential locations could be traced and mapped in the absence of further data or a residential address system. Geographic information systems and spatial scan statistics were deployed to detect clustering of malaria cases. Among 2,407 patients diagnosed for malaria, 1,941 (80.6%) provided the name of their respective local leader, 1,121 (57.7%) could successfully be traced to their residential location. Only 240 patients (21.4%) tested positive for malaria. Six geographic clusters of high malaria infections (*hot spots*) and four clusters of low malaria risk (*cold spots*) ($p<0.05$) were identified. Cluster radii for hot spots and cold spots varied from 0 to 276 meters, and 160 to 477 meters respectively. Participatory mapping by recording simple points of reference that community member can readily relate to (e.g. names of local leaders) during routine health facility visits can be used to map hot and cold spots of malaria infections on fine geographic scales as an affordable alternative.

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MALARIA CASE-FINDING AND TREATMENT STRATEGIES IN AN INTERNALLY DISPLACED PERSONS (IDP) CAMP IN THE DEMOCRATIC REPUBLIC OF CONGO

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Social upheaval and chronic human insecurity related to an internecine civil and international war in the Democratic Republic of Congo (DRC) have led to a 70% increase in mortality relative to pre-war levels, and large-scale population displacement. The principal causes of mortality are preventable and treatable infectious diseases such as malaria, and internally displaced persons (IDPs) in temporary shelters may be at elevated risk. Accurate case finding and treatment of malaria is a cornerstone of malaria control efforts; therefore, we explored several possible strategies for active case-finding and treatment in an IDP camp at Mugunga, in Eastern DRC. Beginning with a consecutive sample of 100 febrile patients under 5 years of age from the IDP camp presenting to a nearby clinic for management of a fever episode, we detected 19 cases of uncomplicated malaria diagnosed using HRP2-based rapid diagnostic test, who were then treated with artemisinin combination therapy (ACT). We engaged community health workers in the IDP camp to screen the household members of all 100 children for malaria. The median household size was 5 (range 3 to 9) and there was a median of 1 (range 1 to 2) children under 5 years of age per household, representing a total of 377 screened individuals. We detected 29 cases of malaria through this active case-finding procedure who had not presented for medical treatment, who were then treated with ACT. Fourteen cases were asymptomatic, while 1 participant reported fever, 6 headache, and 8 myalgia. Based on this data, we examined several hypothetical strategies for anti-malarial drug administration in this cohort including: [1] mass drug administration without screening; [2] screen all IDPs with RDT and treat positive cases; [3] screen selectively based on symptoms, then treat positive cases; [4] screen selectively based on household contacts of a child with uncomplicated malaria, then treat positive cases. Under these 4 strategies, the number of missed cases, unnecessary treatments, and cost per case detected and treated were: [1] 0, 348 (92%), $46; [2] 0, 0, $23; [3] 14 (49%), 0, $7; [4] 24 (83%), 0, $25. Although imperfect because nearly half of cases remain undetected, population-level symptom-based screening and treatment appears to be a low-cost strategy to detect and treat cases that might not otherwise present to local health clinics.

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MALARIA REPORTING THROUGH ELECTRONIC INTEGRATED DISEASES SURVEILLANCE AND RESPONSE (EIDSR) IN KAGERA REGION OF TANZANIA

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Malaria surveillance is an important component of malaria control program. Currently, malaria and other disease data are collected through a paper based system that faces challenges of delayed reporting and poor data quality. . We describe the results of weekly reporting of health facility based malaria surveillance data through electronic Integrated Disease Surveillance and Response (IDSR) The Tanzania Ministry of Health and Social Welfare (MoHSW) and the Department of Computer Science - University of Dar es Salaam (UDSM) designed a system of data transfer through the use of mobile phone technology. A five day training of health facilities was undertaken in March 2013 on tallying of data from outpatient registers, recording of data on weekly reporting booklets, and electronic transmission of data. The key malaria indicators collected were: number of people tested for malaria; number tested positive; and number treated presumptively for malaria (clinical malaria cases). Malaria data were transmitted to the District Health Information System (DHIS2) on a weekly basis which enabled establishment of malaria early epidemic detection system (MEEDS), preparedness and response to unusual increase in malaria cases. A total of 289 health facilities in 8 districts of Kagera region participated in the training. In the first 10 weeks of implementation (week 12 to 20 of 2014),87.6% of all expected reports were transmitted on time (by Monday 3pm). Over the same period, a total of 201,431 suspected malaria cases were reported of whom 184,191 (91.4%) were tested for malaria while 17,240 (8.6%) were clinical malaria cases. Malaria

positivity among those tested was 46.4%. Even at the very early stages of the system implementation, electronic disease surveillance has proven advantageous over the former paper based system. This data show an improvement in completeness and timeliness of reporting malaria illnesses, allowing the National Malaria Control Programme (NMCP) to better tailor its programmatic activities and potentially detect and respond to outbreaks in a more timely manner.

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GENETIC DIVERSITY OF *PLASMODIUM FALCIPARUM* FROM WESTERN KENYA HIGHLAND AREAS PRONE MALARIA EPIDEMICS

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Transmission of *Plasmodium falciparum* malaria in the east African highlands is unstable and frequently epidemic, and recent analyses of globally sampled *P. falciparum* showed high levels of genetic diversity positively associated with variation in transmission intensity. In highland areas of unstable malaria transmission, parasite genotyping can provide valuable information on genotype-specific immunity, drug efficacy in the population, and potential etiologies of increased incidence. *P. falciparum* genotyping was conducted on filter paper blood samples from individuals with clinical malaria in two adjoining highland areas in western Kenya. Genotyping was performed at the antigen locus merozoite surface protein-2 (MSP-2), and on a panel of 12 microsatellite loci. At both sites, more than 70% of individuals had infection with >1 parasite genotype. Multiplicity of infection (MOI) was detected at similar frequencies by MSP-2 and microsatellite genotyping. However, microsatellite genotyping detected significantly greater parasite diversity than MSP-2 genotyping, indicating that microsatellite genotypes are more useful for interrogating parasite population diversity and structure. Neither MOI nor genetic diversity differed by age. All individuals with repeated symptomatic infections had infection with a new genotype rather than recrudescence, implying that treatments given to these individuals in an earlier episode of infection were effective and/or there was active immunity against earlier infections. There was also no difference in these metrics between the two areas, indicating a common *P. falciparum* life history and potentially the effectiveness of similar control measures.

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MOLECULAR MARKER TRENDS IN *PLASMODIUM FALCIPARUM* DIHYDROFOLATE REDUCTASE AND *PLASMODIUM FALCIPARUM* DIHYDROPTEROATE SYNTHASE GENES IN KENYAN ISOLATES BETWEEN THE YEARS 2008 TO 2012

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Sulfadoxine-pyrimethamine (SP), an antifolate was replaced by artemether-lumefantrine as the first-line malaria drug treatment in Kenya in 2004 due of the wide spread of resistance. However, SP still remains the recommended drug for intermittent preventive treatment in pregnant women and infants (IPTP/I) owing to its safety profile. This study assessed the prevalence of mutations in dihydrofolate reductase (Pfdhfr) and dihydropteroate synthase (Pfdhps) genes associated with SP resistance in samples collected in Kenya between 2008 and 2012. Field isolates collected from Kisumu, Kisii, Kericho and Malindi district hospitals were assessed for genetic polymorphism at various loci within the two genes by sequencing. Among the Pfdhfr mutations, codons N51I, C59R, S108N showed highest prevalence in all the sites combined at 95.5%, 84.1% and 98.6% respectively. Pfdhfr S108N prevalence was highest in Kisii at 100%. A temporal trend analysis showed steady prevalence of mutations over time except for codon Pfdhps 581 which showed an increase in mixed genotypes. Triple Pfdhfr N51I/C59R/S108N and double Pfdhps A437G/K540E had high prevalence rates of 86.56% and 87.86% respectively. The Pfdhfr/Pfdhps quintuple, N51I/C59R/S108N/A437G/K540E mutant has been shown to be the most clinically relevant marker for SP resistance was observed in 75.71% of the samples.

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ASSOCIATION BETWEEN LENGTH OF NIGHT TIME EXPOSURE AND *PLASMODIUM FALCIPARUM* MALARIA INFECTION RISK IN DAR ES SALAAM, TANZANIA

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Little is known about the role of time as a risk factor in areas that have various forms of malaria interventions. Socioeconomic demands, human behaviours and extended periods of outdoor activities are major contributing factors for malaria transmission in areas where other interventions like long lasting insecticidal nets (LLINs) are widely used. We investigated the associations between sleeping time and *Plasmodium falciparum* parasite prevalence (PfP) among residents in Dar es Salaam, Tanzania. From March 2009 to May 2012 a cross-sectional study was conducted among individuals aged 3-months to 98 years. A total of 4000 people were tested for malaria paracitemia by using rapid diagnostic test (RDT). Individual level information on length of time a person was potentially exposed to mosquitoes after 6:00pm (i.e. time before going inside and under a bed net), demography, house quality and bed-net use were also collected. Hierarchical model was used to assess malaria infection and identify time, social economic status (SES) and net use as risk factors. A total of 411 people (10%) surveyed tested positive for malaria parasitemia. People with outdoor exposure of 5 hours had the highest parasitemia prevalence of (PfP=14.4%) followed by children of 0-5 years (PfP=13%). People with the longest periods of time spent indoors (>5 hours) had the lowest prevalence (PfP=6%). At least 76% of people surveyed slept under bed-net. Multivariate analysis showed a strong evidence that people spending 5 hours outdoors had higher infection prevalence than people spending less time outdoors (OR [95%CI]=2.32 [1.62-3.33] P<0.001). Interestingly, this study also determined that people who owned bed-nets were more likely to have malaria, compared to those without (OR=2.60 [1.76 - 3.84], P=0.04). In conclusion, the findings indicate an increased risk of malaria transmission for the part of population that spends most of the evening time outdoors in Dar Es Salaam because of either behaviours or socioeconomic activities. It is therefore important to plan interventions that can offer protection to this part of the population.

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INTERNALLY DISPLACED PERSONS (IDPS) ARE AT INCREASED RISK OF MALARIA RELATIVE TO NEIGHBORING VILLAGE CONTROLS

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Globally, 26 million internally displaced persons (IDPs) have been forced to flee their homes as a result of armed conflict (yet remain within their country of nationality). Civil and international war in the Democratic Republic of Congo (DRC) has generated tens of thousands of IDPs residing within temporary housing camps, at high risk of poor mental and physical health. Malaria is the chief cause of death among children in the area, but the burden of malaria has not been well described among children within IDP camps in the DRC. We conducted 2 surveys in Walikale district in Eastern DRC to compare the point-prevalence of *P. falciparum* antigenemia among (1) children residing in an IDP camp versus children from the surrounding village, and (2) febrile children presenting for medical care at a health clinic serving both the IDP camp and surrounding village. For the first community-based survey, a random sample of 200 temporary shelters

within the Bilobilo IDP camp was visited and a child under 5 from each household was tested for malaria using HRP2-based rapid diagnostic test. As a control group, we partnered with a public vaccination drive in the surrounding village of Mubi to survey 200 households. Participants from the IDP camp reported that they had fled their homes a median of 13 (10-17) months earlier, and had suffered varying degrees of community violence including theft (50%), physical assault (9%), rape (6%), gunshot and knife injury (1 participant each). Children tested in the IDP camp and surrounding village (controls), were of a median (range) age of 2.6 (0.1-5) years (IDPs) vs 2.4 (0.7-5) years (controls). Household bednet ownership was 34% (IDPs) vs 68% (controls) [p<0.001]. Bednet use by the index child the previous night was 56% (IDP) vs 25% (controls) [p<0.001]. The point-prevalence of malaria was 19% (IDPs) vs 9.5% (controls) [relative risk 2.3 (95%CI 1.3-4.1), p=0.0095). We next examined malaria prevalence among children with acute febrile illness. We tested 100 children presenting to the clinic from Bilobilo IDP camp and 100 from Mubi village. Malaria RDT was positive in 78% (IDPs) vs 39% (controls) [relative risk 2.0 (95%CI 1.5-2.6), p<0.001]. Taken together, these data suggest that displacement is a risk factor for malaria infection, both community carriage and acute febrile illness. IDPs represent a high risk group for malaria and targeted control measures may reduce the burden of malaria in this vulnerable group.

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TEMPORAL ASSOCIATION BETWEEN FISHING ACTIVITIES AND MOSQUITO ACTIVITY PATTERNS IN RUSINGA ISLAND, WESTERN KENYA

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Fishing is a major source of livelihood for residents in Rusinga Island on the shores of Lake Victoria, western Kenya. This activity is conducted outdoors during the day but predominantly at night. Malaria is endemic in western Kenya and areas around the lake shore are associated with mosquito breeding habitats. The main objective of this study was to determine the association between fishing activities and mosquito activity patterns in a fishing beach in western Kenya. A longitudinal entomological survey on a fishing beach was conducted for a period of 5 months. Kolunga fishing beach was selected for this study as fishermen registered at this beach conduct fishing activities at night. Mosquitoes were captured using battery-powered MMX traps baited with a potent mosquito attractant. The traps were positioned approximately 10 metres inland from the shoreline. Hourly mosquito collections were conducted from 6pm till 7am the next day. The number of people at the beach who conducted fishing activities was recorded at hourly intervals. A total of 1279 mosquitoes were collected, of which 93 were malaria vectors. Of the 93 malaria vectors collected, 28 (33.7%) were *Anopheles gambiae s.l.* while 65 (66.3%) were *An. funestus*. The peak mosquito biting activity for *An. gambiae s.l.* was 9 - 10pm and 3 - 4am while *An. funestus* was recorded as 9 - 10pm and 4 - 5am. The peaks in mosquito biting times coincided with fishermen's activities at the fishing beach. In conclusion, individuals conducting fishing-related activities at the beach during the peak biting times are at high risk of receiving infectious mosquito bites.

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MALARIA PREVENTION IN VULNERABLE GROUPS NIGERIA, 2011-2012

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Malaria prevention is vital to its control. At least 80% of children less than 5 years (U5) and pregnant women (PW) sleep under long lasting insecticidal nets (LLIN) while all PW attending ante-natal clinics should receive at least two doses of Sulphadoxine-pyrimethamine for Intermittent Preventive Treatment (IPT). Even though there is gradual attempt to scale-up access to these interventions, little information is available about their currents. We analysed access to LLINs and IPT in Nigeria using secondary data.We collected national summary data on malaria preventive services from January 2011 to December 2012. Data on uptake of LLINs in U5 and PW, and uptake of IPT in PW from all reporting health facilities (HF) were extracted. Analysis was done by state using Microsoft excel. In 2011, of the 2,741,485 PW who attended ANC in 96,418 HFs, 622, 317 (22.7%) and 649, 732 (23.7%) received LLIN and IPT respectively. In 2012, of the 3, 392, 363 PW who attended ANC in the 114, 667 HFs, (9%) and (16.6%) received LLIN and IPT respectively. In 2011 and 2012 199289(3.2%) of the 6,220,672 and 301896(3.2%) of the 9,521,932 U5 seen received LLINs respectively. Despite increase in ANC attendance and reporting HF, access to LLIN and IPT remains low. In Nigeria, universal access to malaria preventive commodities and services has not yet been achieved in high risk groups. High antenatal clinic (ANC) attendance alone is not sufficient to ensure high IPT and LLIN coverage as the Roll Back Malaria target of 80% was not achieved.SP for IPT should always be made available at all HF's and health workers should be trained on the need to increase IPT coverage.

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MALARIA PREVALENCE, SPATIAL CLUSTERING AND RISK FACTORS IN A LOW ENDEMIC AREA OF EASTERN RWANDA: A CROSS SECTIONAL STUDY

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Rwanda reported significant reductions in malaria burden following scale up of control intervention from 2005 to 2010. This study sought to; measure malaria prevalence, describe spatial malaria clustering and investigate for malaria risk factors among health-centre-presumed malaria cases and their household members in Eastern Rwanda. A two-stage health centre and household-based survey was conducted in Ruhuha sector, Eastern Rwanda from April to October 2011. At the health centre, data, including malaria diagnosis and individual level malaria risk factors, was collected. At households of these Index cases, a follow-up survey, including malaria screening for all household members and collecting household level malaria risk factor data, was conducted. Malaria prevalence among health centre attendees was 22.8%. At the household level, 90 households (out of 520) had at least one malaria-infected member and the overall malaria prevalence for the 2634 household members screened was 5.1%. Among health centre attendees, the age group 5–15 years was significantly associated with an increased malaria risk and a reported ownership of ≥4 bednets was significantly associated with a reduced malaria risk. At the household level, age groups 5–15 and >15 years and being associated with a malaria positive index case were associated with an increased malaria risk, while an observed ownership of ≥4 bednets was associated with a malaria risk-protective effect. Significant spatial malaria clustering among household cases with clusters located close to water- based agro-ecosystems was observed. In conclusion, malaria prevalence was significantly higher among health centre attendees and their household members in an area with significant household spatial malaria clustering. Circle surveillance involving passive case finding at health centers and proactive case detection in households can be a powerful tool for identifying household level malaria burden, risk factors and clustering.

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CASE-CONTROL STUDY OF EFFECTIVENESS OF INSECTICIDE-TREATED BEDNETS IN CENTRAL SENEGAL

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The effectiveness of bednet (LLIN) programs in Senegal is threatened by resistance to insecticides. The aim of this study was to determine the effectiveness of LLIN use by the individual and in the local community in reducing the incidence of malaria, in central Senegal. A matched case control study was conducted in rural parts of the district of Fatick, central Senegal, in an area covered by a demographic surveillance system (DSS). All confirmed cases of uncomplicated malaria presenting at health posts in the district between October 2011 and February 2012 were included in the study. For each case, one or two controls of the same age group, selected by random sampling from the DSS listing for the district, were recruited concurrently if a malaria rapid diagnostic test was negative. For cases and controls, and members of their households and of surrounding households, bednet use and approximate age was recorded after inspecting sleeping places. Information about bednet use was also obtained for all households in the district when visited in demographic surveillance rounds from January to December 2011. Conditional logistic regression was used to estimate the rate ratio associated with use of LLIN, and with the level of local coverage of LLINs in the community grouped by quintiles, adjusted for potential confounding factors including distance to health facility, socio-economic status, and distance to mosquito breeding sites. 540 malaria case-control pairs were recruited. Sleeping under an LLIN, and local coverage of LLINs, were independently associated with reduced malaria incidence. Malaria incidence for individuals in communities with coverage in the upper quintile (>81% of persons sleep under an LLIN) was 64% (95%CI 18%, 84%) lower than in communities in the lowest quintile (<50% LLIN coverage). In conclusion, LLINs remain effective in central Senegal but high coverage is required to maximize impact. LLIN distribution programs should target communities with low coverage.

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DEFINING THE RELATIONSHIP BETWEEN *PLASMODIUM FALCIPARUM* AND *PLASMODIUM VIVAX* PARASITE RATE AND CLINICAL DISEASE

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Quantifying of the number of malaria cases that occur annually in endemic countries is essential to national control program development and evaluation. Clinical incidence of malaria has proven to be difficult to enumerate. Strategies that adjust routine case reports or model incidence values using prevalence surveys have been applied to estimates of malaria as a whole and *Plasmodium falciparum*-specific incidence, but have yet to be developed for *Plasmodium vivax*. Infection prevalence, or parasite rate, is one of the most widely available metrics of malaria endemicity and is collected using simple methodologies. To help inform the number of clinical infections of *P. falciparum* and *P. vivax* per year, it is therefore useful to quantify the relationship between prevalence and clinical incidence for each parasite. Here we present updated methods to estimate *P. falciparum* incidence and a new equivalent model for *P. vivax*, developed to accommodate the unique epidemiological features of the parasite. A systematic search for active case detection surveys was performed. Recorded incidence values were then matched to measures parasite rates. Two separate hierarchical Bayesian models, using a flexible Gaussian process prior, were fitted to the matched falciparum and vivax data. Temporal variation was modelled by using a Poisson-Gamma mixture. The fitted relationships, along with appropriate uncertainty metrics, allows for estimates of clinical incidence of known confidence to be made from wherever *P. falciparum* or *P. vivax* prevalence data are available. This will allow for updated estimates of *P. falciparum* burden as well as novel measures of the global burden of *P. vivax*.

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AN IMPROVED MATHEMATICAL MODEL OF MALARIA INCIDENCE IN COLOMBIA

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Malaria is an endemic disease in the lowlands of the tropical Americas. In Colombia, it constitutes a serious public health problem with 105,000 cases per year during 2005-2011, but showing: (1) an increasing trend during the last 50 years, and epidemic outbreaks during the occurrence of the warm phase of El Niño/Southern Oscillation (El Niño) over the tropical Pacific. Here we develop a mathematical model to represent the temporal dynamics of malaria incidence in endemic regions of the Pacific coast of Colombia, involving the complex interactions between environmental and climatic factors, the diverse developmental stages and the feeding habits of the vector (*Anopheles* albimanus), and their interaction with humans and with the parasite (*Plasmodium falciparum*). Our model overcomes diverse limitations and shortcomings of the model introduced by Ruiz et al. (2006), and provide a much-improved tool towards the development of an early warning system for malaria prevention and control in Colombia.

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MALARIOMETRIC SURVEY OF IBESHE COMMUNITY IN IKORODU, LAGOS STATE, NIGERIA: DRY SEASON

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Malariometric surveys generate data on malaria epidemiology and dynamics of transmission necessary for planning and monitoring of control activities. This study determined the prevalence of malaria and the knowledge, attitude and practice (KAP) towards malaria infection in Ibeshe, a coastal community. The study took place during the dry season in 10 villages of Ibeshe. All the participants were screened for malaria. A semi-structured questionnaire was used to capture socio-demographic data and KAP towards malaria. A total of 1489 participants with a mean age of 26.7 ±20.0 years took part in the study. Malaria prevalence was 14.7% (95% CI 13.0-16.6%) with geometric mean density of 285 parasites/µl. Over 97 % of participants were asymptomatic. Only 40(2.7%) of the participants were febrile while 227(18.1%) were anemic. Almost all the participants (95.8%) identified mosquito bite as a cause of malaria, although multiple agents were associated with the cause of malaria. The commonest symptoms associated with malaria were hot body (89.9%) and headache (84.9%). Window nets (77.0%) were preferred to LLIN (29.6%). Malaria is mesoendemic in Ibeshe during the dry season. The participants had good knowledge of symptoms of malaria, however there were a lot of misconceptions on the cause of malaria.

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

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Residents of urban areas have been shown to have lower rates of malaria infection compared to rural neighbors. There are, however, many challenges in defining urbanization. The transition from a rural settlement to one best described as urban does not follow a definite boundary. Defining the gradient between rural and urban settlements is an important factor in defining malaria prevalence patterns. In this study, we use boosted regression tree models to determine using whether (i) urbanization had a significant effect on malaria transmission and if this effect varied with urbanization definition used (ii) if population density had significant effect on malaria parasite prevalence and if so, could population density replace urban classifications in modeling malaria transmission patterns. The study focuses on 21 household surveys from the Demographic & Health Surveys (DHS) and Malaria Indicator Survey (MIS) conducted between 2006 and 2013 in 14 malaria endemic countries across Africa. Although analysis is expected to be complete by June 2014, preliminary results show that the BRT model with population density was found to perform significantly better (p-values <0.001) in predicting malaria risk when we compared the model's predictive performance to the other models with urbanization classification. However, adding urbanization to the BRT model with population density produced a better performing model implying there are aspects of the influence of urbanization on malaria prevalence that cannot be sorely explained by population density or population distribution patterns.

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THE EFFECTS OF MALARIA IN THE FIRST TRIMESTER MEASURED BY REPEATED FETAL CROWN RUMP LENGTH: AN OBSERVATIONAL STUDY

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Malaria in pregnancy causes low birth weight although the mechanism of this adverse effect is not characterized. At Shoklo Malaria Research Unit on the Thai-Myanmar border we have observed reduced fetal head diameter when women had malaria from < 14 to < 24 weeks of pregnancy. This investigation aims to determine if there is a reduction in growth with malaria infection in the first trimester (7+5 to 14 weeks) by using ultrasound confirmed fetal crown rump length measurement. Shoklo Malaria Research Unit on the Thai-Myanmar border has offered fetal biometry to all pregnant women since 2001 at their first antenatal visit to determine viability and gestation and the scan is repeated if necessary for ongoing care. The training manual and protocol for trans-abdominal CRL were from the British Medical Ultrasound society recommendations. Observed versus the expected increase in CRL measurements will be compared for women with malaria, women with fever from other causes and for women without malaria. Data collection is still in process but repeated CRL measurements are currently available for 1300 women of whom more than 100 have had malaria in the window of two CRL measurements. The final results will be available in May 2014

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UTILIZATION OF MALARIA PREVENTIVE SERVICES IN PREGNANT WOMEN ATTENDING PUBLIC HEALTH FACILITIES IN OYO STATE, SOUTHWEST NIGERIA, 2013

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Malaria is a major cause of maternal and newborn mortality globally. In areas with high malaria endemicity an estimated 50 million women become pregnant annually; and half of these women reside in Africa. Malaria in Pregnancy (MIP) is an obstetric, social, economic and medical emergency. It has been documented to be responsible for 11% of maternal deaths, 2-5% of maternal anemia, 8-15% of low birth weight infants, and 3-8% of infant deaths, in developing countries. In 2004 use of Sulphadoxine- Pyrimethamine (SP) for Intermittent Preventive treatment (IPTp), was adopted in Nigeria within the focused antenatal care package, in addition to health education, personal protection by sleeping inside Long Lasting Insecticidal Nets (LLINs), and effective case management. We determined the utilization of these preventive services and their uptake in pregnant women attending public health facilities in Oyo state. We reviewed malaria specific summary data abstracted from the Oyo state National Health Management Information Systems health facility registers of 462 facilities from January to December 2013. Descriptive analysis of MIP, and uptake of preventive strategies was done, using Microsoft excel 2007. Results: Of the 929,843 outpatient clinic attendees, 39218 (4%) were Pregnant women (PW) with fever. Of PW with fever, MIP accounted for 22,052(56%) of fever cases; 5887(27%) were clinically diagnosed and 16,165(73%) had laboratory confirmation. Of the 322,514 PW who attended ANC, 70,216(22%) and 5105(2%) received at least 2 doses of SP for IPTp and LLINs respectively. In conclusion, despite good ANC coverage, there was low utilization of laboratory diagnosis, low uptake of SP for IPTp, and poor ownership of LLINs, all less than the State's 80% target for each indicator. We recommend consistent health education to PW for prevention of MIP and adherence to guidelines for effective management of MIP by health workers, with the aim of reducing malaria burden in PW, thereby improving pregnancy outcomes and making pregnancy safer.

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AN ELITE INFECTION-CONTROL PHENOTYPE WITH IMMUNOLOGICAL CORRELATES IN A TANZANIAN BIRTH-COHORT EXPOSED TO INTENSE MALARIA TRANSMISSION

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Malaria incidence is highly heterogeneous even in hyperendemic areas, although no one has shown that innate or naturally acquired resistance can completely prevent infection. We examined immunoparasitological evidence for an elite infection-control phenotype from a birth-cohort followed 2002-2006 in Muheza, Tanzania, an area of intense transmission. Children (n=688) provided blood smears every 2 weeks during infancy and

monthly thereafter. We documented maternal and childhood demographic and clinical characteristics, cord-blood cytokine levels, and antibody responses to preerythrocytic (LISP1, SLARP, among others) and common blood stage (MSP1, AMA1) malarial antigens every 6 months. Antibody seroprevalance comparisons were assessed through generalized estimating equations, and associations of cord-blood cytokines with elite infection-controller status were estimated through logistic regression. Sixty (8.7%) children had no blood-smear positive slides over an average of two years (range: 1 to 3.5 years, ~121 person-years) and were identified as elite infection-controllers. Elite controllers were similar to non-controllers with respect to completeness of follow-up and most maternal and childhood behavioral and biomedical risk factors. Antibody seroprevalence was similar between elite and non-controllers for five of six preerythrocytic antigens, and increased with age in both groups. Elite controllers had a lower seroprevalence to MSP1 (5.6% vs. 29.3%; P<0.0001) and AMA1 (25.9% vs. 60.3%; P<0.0001) compared with non-controllers. In addition, elite controllers were over twice as likely as non-controllers to have cord levels of IL1-B(odds ratio(OR): 2.5 (1.1,5.8)), TNF-α (OR: 2.6 (1.2, 5.8)), or TNF-R1(OR: 2.2 (1.0, 4.8)) in the top third of the distribution. These data suggest that a subset of naturally exposed children is able to control malaria infection before patent parasitemia, and this control is associated with a unique immunologic profile at birth. Research on these children may identify mechanisms for highly effective naturally acquired immunity to malaria.

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MAPPING OF 'HOT SPOTS' AND SIGNIFICANCE OF REACTIVE CASE DETECTION IN DETERMINING THE MALARIA BURDEN IN AN URBAN SETTING

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Malaria is a serious public health problem causing high morbidity and mortality in India. Chennai, a metropolitan city is endemic for malaria and the transmission is perennial with a peak between July and October. *Plasmodium vivax* is the predominant parasite species accounting for 93.6 to 99.8% of the prevalence in the city. The focus of national programme is to identify and treat the individuals presenting to the health care facility. In fact, human malaria parasite reservoir consists of both symptomatic and asymptomatic infections among people in a given area. It is well known that addressing the individuals who do not seek treatment and capturing asymptomatic subjects by active surveillance is essential to move forward for malaria elimination. As a part of the study of complex malaria in an urban setting, the objective was to estimate the burden of malaria (both symptomatic and asymptomatic) adopting reactive case detection strategy and finding spatial clustering of infections. Reactive case detection activity is initiated immediately in response to positive case reported at malaria clinic involving screening of all the household members of the index case. Individuals living in close proximity (<50m radius) to passively detected case and additionally households within 100m radius are surveyed for presence of fever, also randomly selected control households without any history of fever in previous 2 weeks are screened for presence of malaria parasites by microscopy, RDT and PCR. Coordinates of all the index case and enrolled households are captured by global positioning system (GPS) to provide information on spatial distribution of infections. Preliminary data from the ongoing study indicates reactive surveillance would help to identify malaria infection which otherwise would be missed by routine passive case detection. This strategy will help to identify the malaria reservoir which includes the asymptomatic individuals harbouring malarial parasites in the community and serve as vital information on the actual magnitude of the problem for the programme to tackle and undertake appropriate intervention measures to interrupt transmission.

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ACTIVE CASE DETECTION IN MALARIA ELIMINATION SETTINGS: TIMELINESS AND COMPLETENESS OF MALARIA CASE NOTIFICATION IN ZANZIBAR

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Zanzibar Malaria Elimination Programme (ZAMEP) introduced malaria early epidemic detection system (MEEDS) in 2008 and malaria case notification (MCN), an active malaria surveillance system, in August 2012. MCN is an active surveillance system whereby individual malaria cases are transmitted in real time through the use of mobile phones and android tablets to a central database. We evaluated the timeliness of case notification after diagnosis and timeliness of case follow-up after notification. For MCN reporting, a massage for each case is sent from Health Facility to the server and the server generates an alert to the District Malaria Surveillance Officer's (DMSO) mobile phone and tablet. DMSO follows up notified cases to their households. All case-household members are tested for malaria using rapid diagnostic tests (mRDT). People with positive malaria test results are treated with artemisinine-based combination therapy (ACT). A total of 2,603 index malaria cases were notified in 2013 of which 75.8% were followed-up. Of the 1,974 cases followed-up notification after diagnosis was done within 24 hours in 50.6%, between 24 to72 hours in 25.8%, while 23.8% of cases were notified after 72 hours. The median time from diagnosis to case notification was 1.2 days (interquartile range [IQR]=0.5 to 2.6). Household follow-up was undertaken within 48 hours in 54.2%, between 48 hours and 1 week in in 21.5%, while 23.4% of cases were followed after more than 1 week. The median time to household follow-up was 3 days (IQR=1 to 8). A total of 1,812 (70% of those notified) had the household follow-up completed. Active case detection and treatment alongside other preventative interventions is likely to reduce malaria transmission and enhance malaria elimination efforts in Zanzibar. Following successful implementation of malaria case notification, ZAMEP has set performance standards for malaria case notification and case follow-up with a target of 90% of cases to be notified with 24 hours of diagnosis, 100% follow-up of all notified cases, and 90% of cases to be followed up within 48 hours of notification. Therefore more resources will be required to enable Zanzibar achieve these standards in order to make MCN more effective.

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COMPARISON OF PFHRP2-BASED RDTS AND PCR IN AN AREA OF DECLINING MALARIA TRANSMISSION IN SOUTHERN ZAMBIA

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Histidine-rich protein-2 (HRP-2)-based malaria rapid diagnostic tests (RDTs) are valuable in detecting *Plasmodium falciparum* infection; however, various factors can affect test validity. The test misses low level parasitaemia and will not detect non-*P. falciparum* species. More sensitive and species-specific tools such as PCR will become necessary to identify parasite reservoirs in elimination settings. To address this, HRP2-based RDTs, pan *Plasmodium* species nested PCR and species-specific q-PCR were compared over a five-year period in a region of declining malaria transmission in southern Zambia. Cross-sectional surveys were conducted in Choma District, Zambia from 2008 to 2012. A total of 3,292 individuals were enrolled and tested with RDT and samples collected for microscopy and dried blood spots (DBS) with latter used for pan *Plasmodium* nested PCR. Species-specific q-PCR was performed on samples that tested positive

either by microscopy, RDT or nested PCR. There were 12 individuals positive by microscopy (0.3%), 42 positive by RDT (1.3%) and 57 positive by nested PCR (1.7%). Thirty percent of 57 samples positive by nested PCR were also RDT positive. Eighty seven samples positive by either microscopy, RDT or nested PCR were also tested for the presence of Pfhrp gene. Of the 87, sixty one were positive by q-PCR for *P. falciparum* (53% of which were also RDT positive) and 7 were positive by q-PCR for P. malariae. Of the 7 positive for P. malariae, five represented co-infection with *P. falciparum*, two of which were also RDT positive. Mean copy number of Pfhrp for RDT-positive and negative samples was 1152 and 160, respectively and the difference was not statistically significant [p=0.126] indicating that the RDT-negative, q-PCR-positive samples results were likely due to low parasite density and not Pfhrp gene deletions. Findings illustrate that individuals with low-level parasitaemia and non-falciparum malaria will not be identified by HRP-2 RDTs and the importance of more sensitive and specific diagnostics to achieve malaria elimination.

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MALARIA VECTOR ABUNDANCE AND MALARIA PREVALENCE AFTER IMPLEMENTATION OF INSECTICIDE TREATED NETS IN A RURAL MALARIA ENDEMIC DISTRICT IN WESTERN KENYA

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Data on morbidity due to malaria in Webuye Health and Demographic Surveillance System (HDSS) area, Bungoma East district suggests control efforts have not had expected impact in spite of insecticide treated nets (ITN) ownership exceeding 70% and availability of Affordable Medicine Facility-malaria(AMFm).The current study was designed to determine malaria transmission and malaria prevalence after implementation of ITNs and AMFm. Malaria vectors were captured in sentinel villages and households in Webuye HDSS using pyrethrum spray catch (PSC), CDC light traps, exit traps (ET), and light traps with rotating cups. Captured mosquitoes were primarily identified, gonotrophic stages of each malaria vector recorded and the mosquitoes preserved for further analyses. Parasitological surveys are done quarterly using malaria rapid diagnostics test (RDT) kits on all household members in the same households where mosquitoes are captured by PSC and ET and their immediate neighbors. Our preliminary data show a three-fold majority (70.9%; 567/799) of unfed malaria vectors were captured resting or leaving huts compared to other gonotrophic stages (23.5%; 188/799). There were more gravid malaria vector components (57.1%; 8/14) caught in exit traps than other gonotrophic stages. PCR analysis on a small proportion (no=81) of *An gambiae* group showed *An. gambiae* ss comprised 64% while *An arabiensis* consisted of 20.3%, but 15.6% were unidentified. The sporozoite rate was low (1.4%; 1/69). 370 participants were tested for malaria; 21.6% were positive with the highest prevalence of 41% reported among children 5-10 years. From these preliminary data, we show that high number of gravid malaria vectors were captured in exit traps and the large number of the vectors caught resting indoors by other techniques confirm endophily among these vectors in spite of increased ITN coverage. However, malaria prevalence declined compared to high prevalence previously reported. Data on molecular analysis of *An gambiae* group and malaria transmission efficiency, presence of KDR mutation, and malaria prevalence; *An gambiae* characteristics will be compared to malaria prevalence and incidence at the household and neighborhoods after one year of data collection.

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MALARIA OUTBREAK IN THE INTERNALLY DISPLACED PERSONS ALONG THE CHINA-MYANMAR BORDER

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Internally displaced persons (IDPs) represent special populations with special public health concerns, particularly infectious diseases such as malaria. In such settings, infectious disease incidence rate is usually high, but innovative strategies for accessing vulnerable populations and delivering basic public health interventions may ultimately reduce transmission of infectious diseases. Contrary to common belief and results of previous studies, we report lower malaria incidence rates in IDPs compared to local residents. Malaria passive case and active case surveillances were conducted in two IDP camps and two local villages along the China-Myanmar border in the Laiza area of Myanmar from April 2011 to December 2013. Malaria vector populations were monitored using CDC light traps. The use of malaria preventive measures was investigated. Information on aid agencies and their activities during the study period was obtained through questionnaire surveys. Malaria was confirmed in 1,321 patients. Of these cases, 83.4% were *Plasmodium vivax* malaria. Seasonal malaria outbreaks were observed both in the IDP camps and in the local villages in 2013; malaria incidence in local villages peaked 4 weeks before the peaks in IDP camps. The malaria annual incidence rates were 56.5 and 164.7 cases/1,000 population in IDP camps and local villages, respectively. Older children of 5-14 yrs had the highest incidence rate in IDP camps regardless of gender and study community, while male adults had significantly higher incidence rates than females in local villages. The parasite prevalence rate was in general <5%. Over 99% of households in both communities owned bed nets, and bed net usage rates were 76.4% and 66.3% in local residents and IDPs, respectively. *Anopheles* vector density was nine-fold higher in local villages than in IDP camps. There were more active aid agencies in the IDP camps than in local villages. Temperatures in 2012 and early 2013 were 1-3°C above normal in the study area. Refugee/IDP camps are usually located in underdeveloped areas. If management practices are effective and facilities are relatively adequate, infectious diseases can be significantly reduced in IDP camps; however, under certain environmental conditions, disease outbreaks may be unavoidable. The long-term impact of malaria control strategies must be closely monitored.

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DO THE DYNAMICS OF CLASSICAL MALARIA EPIDEMICS DEMONSTRATE *PLASMODIUM FALCIPARUM'S* SURVIVAL STRATEGY?

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Areas of marginal transmission can generate enormous lethal malaria epidemics when factors favouring the parasite shift only slightly. Although usually described in terms of vectorial capacity, medical scientists working in India in the early 20th century came to the conclusion that "an epidemic of relapses" was the key triggering event of malaria epidemics. This explanation has been largely discarded as the biology of *Plasmodium falciparum* recrudescence was differentiated from *P vivax* relapse. Using detailed data from the 1908 Punjab malaria epidemic investigated by Rickard Christophers, the genesis of epidemics has been re-examined in order to inform current control efforts. Several aspects of the 1908 epidemic were well documented. It was highly focused geographically depending on recent rainfall and canal overflow. The epidemic arose very suddenly and simultaneously in several places. Malaria spleen surveys indicated very little recent malaria transmission and blood smears showed

very few gametocytes just prior to the epidemic. Population stress as indicated by high grain prices due to a poor harvest caused by drought the previous year was a key risk factor for malaria epidemics. Although increased female anopheline survival due to increased humidity played an important part in the magnification of the epidemic, it does not explain its genesis. Current studies using large blood volume molecular methods in SE Asia indicate that a large number of asymptomatic individuals exist with very low falciparum parasitemia. Human population stress triggering a shift towards gametocytogenesis is hypothesized as the key initiation factor for malaria epidemics. Its evolutionary significance may be that it allows the parasite to match the tropical agricultural cycle. Public health programs will have to eliminate all parasites in asymptomatic persons probably by mass drug administration in order to eliminate the risk of malaria epidemics during war and natural disaster.

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SCHOOL-BASED COUNTRYWIDE SEROPREVALENCE SURVEY INDICATES SPATIAL HETEROGENEITY IN MALARIA TRANSMISSION IN THE GAMBIA

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As the geographical distribution of malaria transmission becomes progressively clustered, identifying such residual pockets of transmission is important for research and for targeting interventions. Malarial antibody-based surveillance is increasingly recognised as a valuable complement to classic methods for the detection of infection foci. The study presents serological evidence of transmission heterogeneity among school children in The Gambia, measured during the dry season. Healthy primary school children were randomly selected from 30 schools across the country and screened for malaria infection (microscopy) and antimalarial antibodies ($MSP1_{19}$). Antibody distribution was modelled using finite mixture models with the Akaike's Information Criterion (AIC) used to determine best model fit. Factors associated with a positive serological status were identified in a univariate model and then combined in a multilevel mixed-effects logistic regression model, simultaneously adjusting for variations between individuals and school. A total of 4140 children, 1897 (46%) boys, were enrolled, with mean age 10.2 years (SD 2.6, range 4- 20 years). Microscopy results available for 3640 (87.9%) children showed that 1.9% (69) were positive for *Plasmodium falciparum* infections, most of them (97.1%, 67/69) asymptomatic. The overall sero-prevalence was 17.4% (720/4140) with values for the schools ranging from 0.6% to 57.5%. Age (OR 1.54, 95% CI 1.26- 1.89, P <0.001) and malaria infection (OR 4.95, 95% CI 2.76- 8.87, P <0.001) were strongly associated with seropositivity. Serological tests could identify individuals who were or had been infected, and clusters of residual transmission. Field-adapted antibody tests could guide mass screening and treatment campaigns.

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MOLECULAR EPIDEMIOLOGY OF *PLASMODIUM FALCIPARUM* IN AREAS OF DIFFERENT MALARIA ENDEMICITY - TANZANIA

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Malaria endemicity in sub Saharan Africa continues to decline in recent years as a result of disease intervention strategies. While symptomatic malaria is recognized and treated, asymptomatic infections become increasingly important for interrupting transmission as they contribute substantially the infective reservoir to mosquitoes. To determine the asymptomatic carriage in urban and rural settings in Tanzania, crossectional community surveys were conducted using highly sensitive molecular tools. A total of 440 finger prick blood samples were collected in Dar es Salaam (DSM, urban malaria with low transmission), and 506 samples from coastal Rufiji, (high malaria transmission). *Plasmodium falciparum* (Pf) was detected by mRDT (Pan/Pf), light microscopy (LM) and quantitative PCR (qPCR) or qRT-PCR targeting the S-type 18S rRNA genes or transcripts of A-type 18s rRNA respectively. Pf gametocytes were detected by light microscopy and qRT-PCR targeting transcripts of gametocyte specific marker pfs25. Pf Prevalence was 73%, 49.4%, 47.43% and 25.3% by qRT-PCR, qPCR, RDT and LM in the coastal area (high transmission); while in DSM (low transmission) prevalence was 10.4% 5.23%, 1.59 and 3.41%, respectively. Comparing the two endemic areas, we observed up to 10-fold differences in prevalence rates by the three detection methods. Gametocyte prevalence was 0.23% by LM and 0.9% by qRT-PCR in DSM. In high transmission gametocyte prevalence was 10 fold higher than in low transmission (2.57% by LM and 43.7 % by qRT-PCR). The huge difference in prevalence observed in rural Tanzania argues for reliable molecular tools in malaria surveillance, since 50% of infections would remain undetected by microscopy diagnostics. Such precise molecular data on asymptomatic carriage seems instrumental for planning of malaria interventions. Gametocyte prevalence was about 50% of asexual prevalence at high endemic settings and mirrored asexual prevalence rates. Thus, the difficult and costly molecular detection of gametocytes seems dispensable from surveillance.

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A MULTI-PATHOGEN SPATIO-TEMPORAL MODEL TO PREDICT INCIDENCE OF RARER DISEASES

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The quantification of spatial and temporal patterns of disease risk is often hampered by substantial noise associated with small population sizes, particularly for rarer diseases. Here, we propose a novel Hierarchical Bayesian model that relies on spatial and temporal correlation, as well as between-pathogen correlation, to improve the characterization of disease risk. We illustrate this model with malaria incidence data from the Brazilian Amazon collected from 2004 to 2008 by the government surveillance system, totaling an area of 3.6 million km2 and 2.4 million malaria cases. In this region, *Plasmodium falciparum* is much rarer than *P. vivax*, making it hard to estimate *P. falciparum* disease risk. However, there is greater public health concern regarding *P. falciparum* because it tends to result in more adverse health outcomes. Preliminary findings reveal striking geographical differences in disease risk for both pathogens, which seem to be strongly influenced by forest cover. Our model also reveals a strong correlation between *P. vivax* and *P. falciparum* malaria incidence, which suggests that *P. vivax* data can help in inferring *P. falciparum* risk in a way that is above and beyond the information contained in our covariates. We anticipate conducting a 10-fold cross-validation exercise by August 2014 to better assess the relative importance of temporal, spatial, and between-pathogen correlation in improving predictions of *P. falciparum* disease risk. The modeling framework proposed here can be valuable for inferring disease risk of a rarer (but more severe) disease based on a more common disease. Finally, although geospatial models have been extensively used to create interpolated surfaces of disease risk, our preliminary malaria results suggest that the uncertainty associated with these surfaces is large and that relatively few areas have significantly high disease risk (hotspots). This finding is important from a public health perspective because it reveals the critical role of uncertainty in determining priority areas for malaria interventions.

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A COUNTRYWIDE CROSS-SECTIONAL SURVEY OF THE PREVALENCE OF ASYMPTOMATIC *PLASMODIUM FALCIPARUM* INFECTION IN THE GAMBIA

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Malaria indicators have decreased substantially in The Gambia with pre-elimination status targeted for 2015. Achieving this goal requires in-depth understanding of the burden of asymptomatic *Plasmodium falciparum* parasitaemia (APFP) as asymptomatic carriers can maintain or re-initiate transmission. A cross-sectional survey was conducted to determine the prevalence of APFP in the Gambia, describe its heterogeneity and its associated risk factors. A cross-sectional survey was conducted in 36 villages in six geographical locations in November 2012 during the peak of malaria transmission. 350 persons across all age groups were randomly selected in each village (whole populations for villages <350). Finger-prick blood sample were collected for microscopy, species-specific PCR and haemoglobin estimation. Information on bed net use and previous antimalarial treatment were also collected. Data were analysed for the prevalence of APFP by village; summary statistics and factors associated with APFP are presented. Of the 9094 participants enrolled; 59.6% females and 27.2 % were aged <5 years. The median (IQR) age and mean (±SD) haemoglobin were 12 (5, 28) years and 11.7 (±2.3) g/dl, respectively. The prevalence of APFP was 16.01% with marked heterogeneity between villages ranging from 1.6% to 49.1%. The mean parasite density was 4329.7/ul. 95% of participants owned bed nets, 78.8% reported indoor residual spraying (IRS). The odds of APFP were higher in children aged 5-15 years (OR1.93; 95% CI: 1.63 -2.30) and adults (OR1.62; 95% CI: 1.35 -1.95) relative to those aged < 5 years. Participants with severe anemia had twice the odds of APFP (OR 2.76; 95% CI: 2.01 - 3.79). Other predictors associated with increased odds of APFP were; axillary temperature, sleeping on verandas at night and IRS. APFP is substantially high in the villages in the eastern region of the country despite high coverage of bed nets and use of IRS. Any intervention aiming at interrupting transmission should target asymptomatic carriers as they represent a substantial reservoir of infection.

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GENETIC INVESTIGATION OF VAR2CSA VARIANTS ASSOCIATED WITH LOW BIRTH WEIGHT IN WOMEN WITH PLACENTAL MALARIA IN MALAWI

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Each year 125 million pregnant women worldwide and 32 million pregnant women in sub-Saharan Africa are at risk of falciparum malaria infection during the course of their pregnancy. Malaria in pregnancy contributes to approximately one million cases of infant low birth weight (LBW) and of maternal anemia, with an estimated 10,000 maternal and 70,000 to 200,000 infant deaths worldwide. The effect of *Plasmodium falciparum* on infants is largely due to placental malaria, in which infected erythrocytes sequester in the placenta owing to the interaction between VAR2CSA proteins on the erythrocyte surface and placental chondroitin sulfate A (CSA). The association between specific VAR2CSA protein domains and adverse birth outcomes, such as LBW, has not been well characterized due the size and diversity of the protein. Recent studies have implicated the ID1-DBL2X-ID2 region of var2csa (~2.7 kb) as critical to placental pathogenesis because it binds to the CSA with same avidity as the whole protein. Using placental samples from 135 *P. falciparum*-infected pregnant women enrolled in a Malaria In Pregnancy Consortium (MIPC) prevention trial in Malawi, a molecular epidemiology study is being conducted to characterize the diversity of the var2csa ID1-DBL2X-ID2 region using next generation deep sequencing methods and to evaluate associations of specific ID1-DBL2X-ID2 haplotypes with LBW. A pooled case-control study design will be employed wherein parasite DNA samples will be pooled from women with and without LBW babies and deep-sequenced across the targeted domain using PacBio Circular Consensus Sequencing (CCS) to determine the frequency of each domain variant in the pool. After haplotype reconstruction from these pooled sequencing steps, associations with LBW will be examined by comparing frequencies of var2csa variants between the two groups. The results from this study will allow us to characterize the diversity of var2csa for the first time using high resolution data. It will help identify pathogenic variants of var2csa and inform efforts currently underway to develop a vaccine for pregnant women living in falciparum malaria endemic areas.

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HOUSEHOLD HEALTH CARE-SEEKING COSTS: EXPERIENCES FROM A RANDOMIZED, CONTROLLED TRIAL OF COMMUNITY-BASED MALARIA AND PNEUMONIA TREATMENT AMONG UNDER-FIVES IN EASTERN UGANDA

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Home and community-based combined treatment of malaria and pneumonia has been promoted in Uganda since mid 2011. The combined treatment is justified given the considerable overlap between the symptoms of malaria and pneumonia among infants. There is limited evidence about the extent to which community-based care reduces health care-seeking costs at the household level in rural and urban settings. This paper assesses the rural-urban differences in direct and indirect costs of seeking care from formal health facilities compared to community medicine distributors (CMDs). Exit interviews were conducted for 282 (159 rural and 123 urban) caregivers of children below five years who had received treatment for fever-related illnesses at selected health centers in Iganga and Mayuge districts. Data on the direct and indirect costs incurred while seeking care at the health center visited were obtained. Using another tool, household level direct and indirect costs of seeking care from CMDs were collected from a total of 470 caregivers (304 rural and 166 urban). Costs incurred at health facilities were then compared with costs of seeking care from CMDs. Household direct costs of seeking care from health facilities were significantly higher for urban-based caregivers than the rural (median cost = US$0.42 for urban and zero for rural; p<0.0001). The same is true for seeking care from CMDs (p=0.0038). Overall, caregivers travelled for an average of 75 min to reach health centers and spent an average of 80 min at the health center while receiving treatment. However, households in rural areas travelled for a significantly longer time (, p<0.001 to reach health care facilities than the urban-based caregivers. Besides travelling longer distances, rural caregivers spent 150 min seeking care from health facilities compared to 30 min from CMDs. In conclusion**,** time and monetary savings for seeking care from CMDs are significantly larger for rural than urban households. Thus, home and community-based treatment of child febrile illnesses is much more cost-saving for rural poor communities, who would spend more time travelling to health facilities - which time could be re-directed to productive and income-generating activities

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HIGH GENETIC DIVERSITY IN *PLASMODIUM* POPULATIONS IN AREAS OF LOW TRANSMISSION: A COMPARATIVE STUDY OF COLOMBIAN POPULATIONS

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The success of malaria programs is measured by the spatial scales at which disease burden is reduced or eliminated. Consequently, spatial patterns of transmission are of particular interest. In this study, we describe changes in the population structure of *Plasmodium vivax* (Pv) and *P. falciparum* (Pf) among endemic areas of Colombia with different transmission intensities. In 2012, some representative areas like Buenaventura (Valle) reported 979 cases in 2012, whereas Tumaco (Nariño) and Tierralta (Cordoba) reported 1,475 and 7,482 respectively. These areas display differences in the relative importance of Pf and Pv (e.g. Valle Pv 90%, Tierralta Pv 92%, and Tumaco Pv 6.9%). We analyzed a total of 550 samples, including both Pv (358) and Pf (192) from these areas using a set of unlinked microsatellite loci. We found different pattern of population structure in each parasite. Using Structure v2.3.3, two clusters were identified for Pf and 9 for Pv. We estimated a total of 37 haplotypes for Pf (Tierralta: 6, Tumaco: 33), sharing only 2 between populations, indicating limited gene flow between these areas. The heterozygosity for Pf was higher in Tumaco (He: 0.871) than in Tierralta (He: 0.453). In contrast, the genetic diversity was very similar and high in the three Pv populations (Buenaventura He: 0.976, Tierralta He: 0.985 and Tumaco He: 0.979). A total of 195 haplotypes were found in Pv and all were private haplotypes (Buenaventura: 20; Tierralta: 131 and Tumaco: 44) indicating strong population structure. Overall these set of microsatellites allowed us the identification of locally related, but geographically distinctive genotypes that potentially can be tracked in time and space. These characteristics makes possible their use in molecular surveillance (e.g. separating local cases from migrants) in the context of control and elimination.

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PLASMODIUM VIVAX MOLECULAR PREVALENCE IN THE SAHEL REGION OF MALI

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The prevalence of *Plasmodium vivax* infection is traditionally reported as low in West and Central Africa because of the high prevalence of the Duffy-negative antigen [Fy(a- b-)]. Recent studies have found cases of *P. vivax* infection in Duffy negative people, and sporozoites of *P. vivax* have also been found in the salivary glands of *Anopheles* mosquitoes in endemic areas inhabited by a predominantly Duffy-negative population. In these regions, a Duffy-positive minority may serve as a reservoir of *P. vivax*. Infection of Duffy-negative individuals may occur, with selection of parasites capable of invading erythrocytes lacking the Duffy antigen. The potential presence of *P. vivax* infection in a Duffy-negative population in Africa may be a significant public health problem in the era of malaria elimination/eradication, where strategies are mainly directed against falciparum malaria. We hypothesized that there is endemic circulation of *P. vivax* in our study population in Bandiagara, Mali and that the prevalence of vivax malaria is underestimated. Blood samples were collected from 300 children aged 0-6 years during quarterly surveys in Bandiagara, Mali. Polymerase chain reaction diagnosis of *P. vivax* was carried out using DNA samples extracted from filter paper. We will report the prevalence of *P. vivax* infection at five quarterly time points from 2009 to 2010, with confirmatory testing with microsatellites. In addition, we will characterize the Duffy antigen status in each child that was *P. vivax* positive, and we will assess the presence of *P. vivax* sporozoites in mosquitoes caught in the study areas.

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SEROLOGICAL DYNAMICS OF MULTIPLE *PLASMODIUM* SPECIES CO-INFECTIONS IN A HUMAN POPULATION

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Malaria is an important parasitic disease posing major public health challenges in developing countries. Infection with malaria parasites elicits immune responses, and after drug treatment antibodies to *Plasmodium*-specific antigens are often detectable in serum. The dynamics of antibody responses to *Plasmodium* spp. co-infection in populations from Zimbabwe where malaria is endemic were determined by ELISA. Antibody (Ab) responses to the C-terminal region of merozoite surface protein 1 (MSP 1_{19}) were determined for all four human *Plasmodium* species. A panel of other antigens, derived from MSPs of *P. falciparum*, were used to determine the serological complexity of each individual's recent *P. falciparum* exposure. Preliminary analysis of this data shows that IgG responses to MSP 1_{19} of *P. falciparum* (PfMSP 1_{19}) were the most frequently observed, followed by responses to *P. malariae* and *P. ovale*, with responses to *P. vivax* MSP 1_{19} rarely detected. Interestingly, all sera positive for *P. malariae* MSP 1_{19} were also positive for PfMSP 1_{19}, indicating that co-infection with these two species is a common occurrence, and that monospecies *P. malariae* infections are rare. Competition ELISA showed that Abs to PfMSP 1_{19} and/or PmMSP 1_{19} did not cross-react with each other, indicating Ab specificity. Serum samples with antibody reactivities to PfMSP 1_{19} often also showed reactivity to PfMSP2A and PfMSP2B derived antigens, but the patterns of reactivity with these polymorphic antigens varied markedly between individuals. This study reinforces the importance of diagnosing not only the presence of *Plasmodium* parasites, but also the species of *Plasmodium* infecting a patient for optimal treatment of malaria co-infections. Further understanding of the dynamics of multiple *Plasmodium* species infection is important, as co-infections may exacerbate the pathology of the disease or alternately decrease the severity of clinical episodes. Measurement of species-specific antibody responses may provide new insights into the extent (or indeed existence) of cross-species immunity that exists in individuals infected with multiple malaria parasite species.

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NATURAL IMMUNE RESPONSE IN ACUTE *PLASMODIUM VIVAX* INFECTED PATIENTS LIVING IN A SOLE *P. VIVAX* INFECTION COHORT IN CENTRAL CHINA

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Plasmodium vivax infection possesses a characteristic of relapsing fever indicating the re-infection by previously hidden parasites in the host. The

relapsed infection can lead to activation of memory T cells pool which might bring up protective immunity. This study aims to characterize natural immune responses in acute *P. vivax* infected patients living in a sole *P. vivax* infection cohort in Central China. We conducted the cross-sectional immune-phenotypic analysis from three recruitments: patients infected with *P. vivax*, malaria-immune and malaria-naive controls. Using flow cytometry, we showed memory T cells were elevated in blood during acute infection. The level of γΔ T cells was two fold higher than that of naïve controls. This suggested that two populations, memory and γΔ T cells, responded specifically to the *P. vivax* parasites. On contrary, B, NK and NKT cells were decreased during acute infection. In addition, regulatory T cells were reduced, suggesting the non-immune suppressive role of *P. vivax* parasites. Interestingly, *P. falciparum* antigens cross-stimulated T cells obtained from these *P. vivax*-infected patients. These results provided a further insight into interaction between *P. vivax* parasites and host cell-mediated immunity in the exclusive *P. vivax* endemic area that could be important for future development of a successful vaccine designation.

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IMPACT OF AGE OF FIRST EXPOSURE TO *PLASMODIUM FALCIPARUM* ON ANTIBODY RESPONSES TO MALARIA IN CHILDREN: A RANDOMIZED, CONTROLLED TRIAL IN MOZAMBIQUE

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Background: Malaria continues to be an important cause of morbidity and death among children younger than 5 years old. Nevertheless, The impact of the age of first *Plasmodium falciparum* infection on the rate of acquisition of immunity to malaria and on the immune correlates of protection has proven difficult to elucidate. Previous studies have explored in-depth the contribution of age and exposure in the development of protective immune response. Such that their findings suggested that more a mature immune systems like those of older children should benefit the development of protective immune systems. Moreover, previous work hypothesized a key age period where exposure to malaria parasite should occur in a way that would exalt the development of naturally acquired immune (NAI) response without jeopardize the heath of infants from endemic areas. The notable need of discerning the exact period for adequate development of NAI was the rational for the present study Methods: Participants (n=349) were enrolled at birth to one of three groups: late exposure, early exposure and control group, and were followed up for malaria morbidity and immunological analyses at birth, 2.5, 5.5, 10.5, 15 and 24 months of age. Total IgG, IgG subclasses and IgM responses to MSP-119, AMA-1, and EBA-175 were measured by ELISA, and IgG against variant antigens on the surface of infected erythrocytes by flow cytometry. Factors affecting antibody responses in relation to chemoprophylaxis and malaria incidence were evaluated. Results: Generally, antibody responses did not vary significantly between exposure groups except for levels of IgM to EBA-175, and seropositivity of IgG1 and IgG3 to MSP-119. Previous and current malaria infections were strongly associated with increased IgG against MSP-119, EBA-175 and AMA-1 ($p<0.0001$). After adjusting for exposure, only higher levels of anti-EBA-175 IgG were significantly associated with reduced clinical malaria incidence (IRR 0.67, $p=0.0178$). Conclusions:Overall, the age of first *P. falciparum* infection did not influence the magnitude and breadth of IgG responses, but previous exposure was critical for antibody acquisition. IgG responses to EBA-175 were the strongest correlate of protection against clinical malaria

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IDENTIFICATION OF NOVEL HLA CLASS I-RESTRICTED T CELL EPITOPES IN THE *PLASMODIUM FALCIPARUM* VACCINE CANDIDATE ANTIGENS CSP AND AMA1 THAT INDUCE IFN-RESPONSES IN A NATURALLY EXPOSED POPULATION

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Malaria eradication requires a concerted approach involving all available control tools and an effective vaccine would complement these efforts. For T cell multi-epitope vaccines, immunodominant epitope selection could be based on the identification of protected individuals in subunit/whole sporozoite vaccine studies with naïve persons or on the identification of persons who show resistance in endemic areas. While the former may help identify protection-associated epitopes in a few antigens, the latter is representative of a field challenge with multiple malaria antigens and may identify immunodominant epitopes within these antigens. We therefore investigated whether naturally exposed individuals had HLA-restricted T cells against *Plasmodium falciparum* CSP and AMA1 epitopes. Eleven volunteers between 18 and 49 years were recruited from an urban community of Ghana in 2011. Volunteer PBMCs were tested against 12 overlapping 15mer peptide pools spanning the entire AMA1sequence and 9 such CSP pools by *ex vivo* Elispot. Criteria for IFN-γ positivity was a stimulation index > 2 and a spot difference > 10, both with respect to spot forming cells per million PBMCs for corresponding unstimulated cells. Volunteers were HLA-typed and their PBMCs subsequently tested against mixtures of previously identified HLA Class I-restricted epitopes within AMA1 (HLA A01, A02, A03, A24, B07 and B44) and CSP (HLA A01, A02, A03, A24 and B15). These were selected because they contained at least one epitope from the positive 15mer pools or that they contained epitopes that matched volunteer HLA types. For AMA1, 10 of the 11 volunteers responded to some 15mer peptide pools while all volunteers responded to some HLA-restricted epitope mixtures. For CSP, all volunteers responded to some peptide pools whilst 10 volunteers responded to some HLA-restricted epitope mixtures. The data demonstrates the potential of T cells from naturally exposed individuals to make parasite-specific IFN-γ responses and point to the need to include field assessment of HLA-restricted epitopes in vaccine design strategies.

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OPSONIZING ANTIBODIES TO *PLASMODIUM FALCIPARUM* MEROZOITES ASSOCIATED WITH IMMUNITY TO CLINICAL MALARIA IN PAPUA NEW GUINEA

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Naturally acquired humoral immunity to the malarial parasite *Plasmodium falciparum* can protect against disease, although the precise mechanisms remain unclear. Although antibody levels can be measured by ELISA, few studies have investigated functional antibody assays in relation to clinical outcomes. We have developed a flow cytometry based functional assay to measure antibody-mediated phagocytosis of merozoites, and have applied this to a longitudinal cohort study conducted in a malaria endemic region of Papua New Guinea (PNG). Opsonising antibody responses to 3D7 merozoites were found to: i) increase with age, ii) be enhanced by concurrent infection, and iii) correlate with protection from clinical episodes and high-density parasitemia. Stronger protective associations were observed in individuals with no detectable parasitemia at baseline. When opsonising antibody responses were measured against merozoites from various isolates, stronger or weaker associations with protection from clinical disease were observed. This study presents the first evidence for

merozoite phagocytosis as a correlate of acquired immunity and clinical protection against *P. falciparum* malaria, and demonstrates that using a geographically diverse parasite strains can significantly influence the statistical associations detected.

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DENDRITIC CELLS SUBSETS MEDIATED IMMUNE RESPONSE DURING *PLASMODIUM BERGHEI* ANKA AND *P. YOELII* INFECTION

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Immune response responsible for the induction and regulation of this response are poorly understood during malaria infection. As immunity is initiated by dendritic cells (DCs), we compared their phenotype and function during *Plasmodium berghei* ANKA (PbA) and *P. yoelii* 17NXL (P.yoelii) infection in Swiss mice individually. PbA infected mice developed a greater number of myeloid and mature DCs on 8 dpi spleen, which were fully functional and capable of secreting IL-12. In contrast, nonlethal *P. yoelii* infected mice produced more plasmacytoid and less mature DCs, resulting in higher levels of IL-10. The mice infected with PbA resulted in an increase in expression of stimulatory (MHCII) and co-stimulatory (CD80 CD86 and CD40) molecules, whereas opposite result was observed during *P. yoelii* infection. Correlating with expression of splenic DCs subsets from PbA and *P. yoelii* infected spleen, our immunoblot assays confirmed the difference in expression of FoxP3, IL-17, TGF-β and IL-6. Thus, results from this study indicate that the subset, the phenotype and the type of inflammatory and anti-inflammatory signals of splenic DCs are critical factors responsible for the irregularity in the ability to induce or regulate Th1 immune responses on host pathogenesis during malaria infection.

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PROTECTIVE IMMUNITY AGAINST BLOOD-STAGE MALARIA INDUCED BY VACCINATION WITH *PLASMODIUM* MIF

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A major obstacle to a malaria vaccine is the inability of the host to establish an effective memory T cell response. All *Plasmodium* species encode a close ortholog of the innate cytokine macrophage migration inhibitory factor, termed PMIF, which regulates the IL-12/IFNγ response during blood-stage infection to promote the differentiation and exhaustion of effector T cells and reduce the development of memory precursor CD4 T cells. We hypothesized that PMIF may be an attractive vaccine target since its neutralization may reduce deleterious cytokine expression and increase protective memory T cell responses. Using a novel, non-viral self-amplifying RNA vaccine, mice (BALB/c) immunized with PMIF and infected with blood-stage *P. berghei* showed reduced parasitemia and a 37% prolongation in mean survival time when compared to a group vaccinated with control antigen (p=0.0016). This effect was associated with a greater number of antigen-experienced CD4 T cells and an 80% increase in $CD62L^{+}IL7R\alpha^{+}$ memory T cells (Tmem). Notably, PMIF-immunized mice that were infected, cured with chloroquine, and re-challenged with *P. berghei* showed 50% expansion of their *Plasmodium*-responsive Tmem populations and complete protection from re-infection when compared to a control vaccine group. Protection in this model of malaria infection also was associated with a 50% increase in plasmablasts ($CD19^{lo}B220^{lo}CD138^{hi}IgD^{-}$) and a 15-fold higher titer of an anti-*Plasmodium* antibody response (IgG2a and IgM). An enhancement in both protective humoral and cellular immunity could be demonstrated by PMIF immunization. Passive transfer of immune IgG from infected and cured mice conferred 33% (p<0.0001) protection in lethal cerebral malaria (*P.berghei* infected C57BL/6 mice) and adoptive transfer of CD4 T cells from PMIF-vaccinated mice into naive congenic BALB/c mice conferred complete protection (p<0.0001) against subsequent infection. These data suggest that targeting an active mechanism by which *Plasmodia* interfere with immunologic memory holds promise for a novel vaccine approach.

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DIFFERENT LATE STAGES OF *PLASMODIUM FALCIPARUM* STIMULATE IFN-Γ PRODUCTION IN CULTURED PBMCS FROM SYMPTOMATIC AND ASYMPTOMATIC INDIVIDUALS BY ELISPOT

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Plasmodium infections trigger complex immune reactions from their hosts against several life stages of the parasite. These immune responses are highly variable, depending on age, genetics, and the condition of the disease in the host as well as species and strain of parasite. Interferon (IFN)-gamma ELISPOT was used to monitor the magnitude of cellular immune response to *P. falciparum* antigens. Production of IFN-γ from cryopreserved PBMCs collected by leukapheresis procedure from Asymptomatic (Asym), Symptomatic (Sym) individuals infected with *P. falciparum* and Endemic Controls (C) was measured against different antigens (Ag): Hemozoin (Hz) and DNAse-treated Hz and synchronized-purified parasites at different stages (mature trophozoites, schizonts in developing and mature) from referential (ITG) and native strain (F06) of *P. falciparum* parasites cultured *in vitro*. In the Sym group we observed an increased number of Spot Forming Cells (SFC) when we stimulate with stages of developing and mature schizonts; being this response more increased with the reference strain compared to the native strain. A lower response was observed when PBMCs from these individuals was stimulated by mature trophozoite. The Asym individuals showed increased response of IFN-γ when stimulated with the native strain of *P. falciparum*, and this response was higher when PBMCs were exposed to mature stage parasites (trophozoites or schizonts). The responses in the three groups were greater when the PBMCs were stimulated with any stage of the parasite in compare to the response of the stimulation with the hemozoin. The response with this last antigen (Hz) was high in the purified hemozoin of referential strain and such response was greater in the Sym group compared to the Asym and control groups. DNAse-treated Hz presented a lower IFN-γ SFC in all groups. In conclusion; based on these results and experimental conditions, it seems that PBMCs could be stimulated by different developing stages of schizonts of *P. falciparum* in ex-vivo experiments, however; in Asym individuals the response seems to be higher when PBMCs are exposed to parasites from native strain in terms of IFN-γ response.

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EPITOPE MAPPING OF *PLASMODIUM FALCIPARUM* APICAL MEMBRANE ANTIGEN 1 USING AN *E. COLI* AUTOTRANSPORTER SYSTEM ON HIGH-THROUGHPUT PROTEIN MICROARRAYS

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Despite extreme diversity, apical membrane antigen 1 (AMA1) is a leading blood stage vaccine candidate antigen in part because anti-AMA1 antibodies inhibit erythrocyte invasion *in vitro*. Although the protein is known to be immunogenic, there is limited information on which epitopes of AMA1 are associated with cross-protective antibody responses. Autotransporters are virulence factors of gram negative bacilli that can be modified to become bacterial expression systems with advantages over other systems such as phage display: autotransporters are easily manipulated in the laboratory using standard cloning techniques, and they allow the presentation of relatively large epitopes. To identify cross-reactive and cross-protective AMA1 epitopes, we engineered EspP, an *E. coli* autotransporter, to display regions of AMA1 and variants of those regions. Sera from rabbits immunized with 3D7- and FVO- AMA1 ectodomains were reactive with the resulting recombinant AMA1-autotransporters. When p-distance and Grantham variation scores were calculated by comparing rabbit immunogen/AMA1-autotransporter sequence pairs, we found that sera were cross-reactive with heterologous haplotype constructs when p-distance and Grantham variation scores were smaller. We optimized expression and purification of AMA1-autotransporters for use in high throughput protein microarrays. We will present results of protein microarray reactivity with human sera from children and adults pre- and post- malaria season and pre- and post- receipt of AMA1 vaccine FMP2.1/AS02A. Our hypothesis is that seroreactivity to AMA1-autotransporters will be higher in adults than in children and increase in magnitude and breadth over the course of a malaria season, reflecting acquisition of immunity to AMA1 epitopes and ultimately allowing for the identification of cross protective epitopes. A protein expression system that can readily express conformational peptides in a variety of sizes will be helpful in determining AMA1 epitopes important to a broadly protective AMA1-based malaria vaccine.

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PLASMODIUM FALCIPARUM, BUT NOT *P. VIVAX*, CAN INDUCE ERYTHROCYTIC APOPTOSIS

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Apoptosis can occur in red blood cells (RBC) and this process could play a role in the pathogenesis of many diseases. In malaria it is well known the participation of parasitized RBC in anemia and thrombotic processes. However, non-parasitized RBC (npRBC) apoptosis could amplify the hematologic disorders associated to malaria. In fact, in experimental malaria, increased levels of apoptosis were observed in npRBC during *Plasmodium yoelii* infection, but in human malaria erythrocytic apoptosis has never been studied. The present study was performed to investigate if npRBC apoptosis also occurs in *P. vivax* and *P. falciparum* infections. Apoptosis of npRBC was evaluated in blood samples of *P. vivax* malaria patients and clinical health individuals living in Manaus, Brazil, both *ex vivo* and after incubation of RBC for 24h. Additionally, it was tested the capacity of *P. vivax* or *P. falciparum* plasma patients to induce *in vitro* apoptosis of normal RBC from a clinical health individual living in Rio de Janeiro, a non-endemic malaria region. Apoptosis was detected by flow cytometry using annexin V staining. Contrasting with experimental malaria that significantly increase the levels of apoptotic npRBC both ex-vivo and after 24h of incubation, no significant alteration on apoptotic npRBC rates was detected in *P. vivax* infected patients, when compared with non-infected control individuals. Similar results were observed when plasma of these *P. vivax* patients was incubated with normal RBC. Conversely, plasma from *P. falciparum*-infected subjects induced significant apoptosis of these cells. It was concluded that apoptosis of normal RBC can be induced by plasma from individuals with *P. falciparum* (but not with *P. vivax*) malaria. This finding could reflect the existence of erythrocytic apoptosis during the course of falciparum malaria that could contribute to the pathogenesis of hematological and vascular complications associated to *P. falciparum* malaria.

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STUDY ON ASSOCIATION BETWEEN GENETIC POLYMORPHIMS OF TUMOR NECROSIS FACTOR-A, INTERLEUKIN-10, INTERFERON-Γ, AND MALARIA VIVAX IN BRAZIL

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Malaria is a major cause of morbidity and mortality in many tropical and subtropical countries. In brazil, the *Plasmodium vivax* has been the most prevalent species, accounting for approximately 83% of malaria cases in the brazilian amazon region. Despite of the clinical descriptions of the disease caused by *P. vivax* is well described, standards regarding humoral and cellular immune response, as well as the pattern of cytokines are scarce and not fully understood. Polymorphisms in genes cd28, ifnγ, tnfα and il10, that are encoder molecules that interact in modulating pathways of the cellular and humoral immune response, may influence the resistance or susceptibility to malaria. We had analyzed 90 blood samples from patients with vivax malaria diagnosed by molecular and non-molecular techniques and 51 from non-malarial and all from goianésia of pará city, pará state, brazil. Polymorphisms in genes IL10 (-819 c>t, -592 c>a), ifnγ (-183 g>t), tnfα (-238g>a), and cd28 (-372g>a, +17t>c) were analyzed by PCR-RFLP. All subjects were genotyped with 48 ancestry informative insertion-deletion polymorphisms to determine the proportion of African, European and Amerindian ancestry to avoid bias due to differences in ancestry contributions in malaria and non-malaria groups. We used the fisher exact test to measure association between genotypes and malaria infection. All polymorphisms tested were in hardy-weinberg equilibrium. The African, European and native American admixture did not differ among cases and controls. No significant association was found between the polymorphisms tested and vivax malaria and non-malarial individuals. P-values in co-dominant, dominant and recessive models were also calculated and no significant association was found. These findings make us to believe that the analyzed polymorphisms are not associated with susceptibility or resistance with vivax malaria in the studied population.the results will be finalized by june 2016.

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CONCURRENT MALARIA PARASITES AND CHIKUNGUNYA VIRUS CO-INFECTION ABATES NEUROPATHOLOGY OF EXPERIMENTAL CEREBRAL MALARIA

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Malaria and Chikungunya fever (CHIKF) are arthropod borne diseases that share the same geographical distributions and recent reports in human shows that co-infections do occur. With the increase in the spread of Chikungunya virus (CHIKV) in the tropics, the potential risk of co-infection in malaria patients is heightened. However, little is known if malaria-associated pathologies are modified during co-infections. In this project, we studied the effect of the viral infection on the pathologies in non-lethal and experimental cerebral malaria (ECM) using *Plasmodium yoelii* cl1.7x (Py17x) and *Plasmodium berghei* ANKA (PbA) respectively. No effect was observed in sequential or concurrent co-infection of CHIKV and Py17x. Interestingly, although no effect was seen in sequential co-infection of CHIKV and PbA, protective effects were observed during concurrent co-infection. Concurrent co-infection reduced ECM mortality with little effects on the parasitemia. We found that the reduction of ECM were associated with reduced parasite load in the brain, improved blood-brain-barrier integrity, reduced pathogenic T cells in the brain and reduced cross-presentation of pathogenic epitopes by brain endothelial cells. Leukocytes profiling in the spleen early in the infection suggests that the lack of pathogenic T cells in the brain is not a result of T cells induction but of migration. Taken together, this study demonstrates that host pathogenic immune response towards *Plasmodium* parasites was altered during CHIKV co-infection leading to reduced neuropathology.

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IL4 GENE POLYMORPHISMS ARE NOT ASSOCIATED WITH *PLASMODIUM VIVAX* MALARIA IN BRAZIL

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Interleukin 4 (IL-4) is an anti-inflammatory cytokine, which regulates balance between Th1 and Th2 immune response, immunoglobulin class switching and humoral immunity. The present study investigated the influence of polymorphisms in IL-4 gene related to the immune system in patients with malaria caused by *Plasmodium vivax* in Brazilian endemic area. A total of 83 individuals infected by *P. vivax* were genotyped by pcr/rflp for two (-590 c/t, -33 c/t) single nucleotide polymorphisms (snps) and the intron 3 vntr polymorphism pcr method in IL4 gene. The density of parasitemia in the infected individuals was recorded and expressed as the number of asexual *P. vivax* per microliter of blood assuming a count of 100 fields per slide. The serum levels of IL-4 were detected by milliplex map kit (human cytokine/chemokine magnetic bead panel-hcytomag-60k) using magpix/luminex®. Analyses were performed using r version 2.8.1 statistical software. For the polymorphism at position - 590 in the il4 gene, the c/t genotype had the highest frequency (55.4%). For the polymorphism at position -33, the c/t genotype had the highest frequency (51.8%) for the polymorphism at vntr the b1b2 genotype had the highest frequency (50.6%). The genotype frequencies were according to the Hardy-Weinberg equilibrium. The il-4 serum level ranged from 0,61 to 9,32 pg/ml. The parasitemia on the thick blood films ranged from 5 to 15.000 parasites/mm3. There were no statistically significant differences either in parasitaemia, serum il4 level among individuals with different genotypes and haplotypes. Our findings suggest that il4 gene polymorphisms were not associated with serum cytokine and peripheral *P. vivax* parasitaemia in brazilian amazon region. The present findings reinforce and increase our understanding about the role of the immune system on the clinical course of the no severe malaria vivax.

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POTENTIAL IMPACT OF INTERMITTENT PREVENTIVE TREATMENT (IPT) ON THE ACQUISITION OF ANTIBODIES TO MALARIA ANTIGENS GLURP-R0 AND AMA-1 IN SENEGALESE CHILDREN

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Intermittent preventive treatment of infants and children (IPTi/IPTc), currently implemented in several sub-Saharan countries with endemic malaria, may disturb natural acquisition of malaria immunity in infants and children. This study aimed to determine the possible impact of IPTi/IPTc on IgG responses to Glutamate-rich Protein-R0 (GLURP-R0) and Apical Membrane Antigen 1 (AMA1) antigens, as markers of acquisition of malaria immunity in Senegalese children living in Saraya district, where IPTi/c has been implemented since 2007 (IPT+), and in Tambacounda district without IPTi/c (IPT-). Sera from 372 randomly selected blood samples (186 from IPT+ and 186 from IPT- communities) blotted onto filter paper, were obtained tree years after IPT was introduced in IPT+ communities and tested by ELISA for IgG response to *AMA1* and *GLURP-R0* antigens. Malaria prevalence by microscopy was 7.5% (176/2353) and 10.1% (81/804) in the IPT+ and IPT- districts respectively. For both antigens, total IgG prevalence and concentrations were higher in IPT- than in the IPT+ group. Among *P. falciparum* negatives samples; the mean antibody response (OD) against *GLURP-R0* was significantly higher (0.089 vs. 0.025 OD) in the IPT- as compared to IPT+ group. Likewise, for the *Plasmodium falciparum* positives samples, the IgG response was significantly higher in IPT- group (*GLURP-R0*=0.258; *AMA-1*=0.089) compared to IPT+ group (*GLURP-R0*=0.128; *AMA-1*=0.043). The low level of IgG antibody response to AMA-1 and GLURP-R0 antigens observed in the IPT+ group indicate that the IPT strategy could delay acquisition of antimalarial immunity. However, other factors could also explain these findings such as marginal differences in the intensity of malaria transmission in the two localities and/or genetic differences between the two populations in their response to the antigens.

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REGULATORY T CELLS AND IL-10 KINETICS DURING ACUTE UNCOMPLICATED MALARIA AND CONVALESCENCE IN MALAWIAN CHILDREN RESIDING IN HIGH AND LOW MALARIA TRANSMISSION AREAS

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Malaria still infects most Malawian children causing mortality in about 5% of those infected living in both high and low transmission areas. Several factors contribute towards why some children develop the more severe form of malaria which causes death one of which is the host's own immune response to the infection. Regulatory T cells (Tregs) are known to play some role in negating immune-related pathology but also

favour multiplication and maturation of malaria parasites. High levels of Tregs during convalescence would essentially predispose the infected children to more infections in subsequent months and also increase the chance of them developing the more severe, and often lethal, clinical form of the disease. In a prospective cohort study, we recruited children aged 6-60 months presenting to hospital with uncomplicated malaria (UCM) at the point confirmed parasitemia and scores 5 on Blantyre Coma Score (BCS) in Blantyre (low malaria transmission area) and Chikwawa districts (high malaria transmission area). Children were followed after a month and three months during convalescence. A total of 5ml venous blood was collected prior to treatment with LA and we determined the proportion of Tregs CD4+ T cells:CD4+CD25intCD127lowFoxP3+ by immuno-phenotying (IPT), and quantified inflammatory cytokines : IFN-γ, TNF-α and anti-inflammatory cytokine: IL-10, TGFβ using ELISA. Children presenting with UCM had mild lymphopenia and spleenomegaly which normalised in convalescence. Children with UCM had higher levels of Tregs, IL-10 and IFN-γ which also normalised in convalescence. Tregs levels were associated with concentrations of IL-10 but not of TGF-β. As high as 15% of the children recruited when presenting with UCM were still *P. falciparum* parasiteamic during convalescence but none of the participants developed any form of symptomatic malaria during the three months follow-up period. In conclusion, recovery from UCM does not translate to total parasiteamia clearance in infected children in both low and high transmission areas. Therefore, children recovering from UCM should be strongly advised to sleep under ITNs and should be screened for parasiteamia during convalescence and referred for antimalarial prophylaxis where possible.

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POLYMORPHISM ANALYSIS OF THE CTLA-4 GENE IN *PLASMODIUM VIVAX* MALARIA PATIENTS FROM BRAZILIAN AMAZON REGION

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Plasmodium vivax has been the most common cause of the human malaria parasite in the brazilian amazon region. Cell-mediated immunity requires costimulatory activity to initiate or inhibit antigen-specific t-cell responses. CTLA-4 is an inhibitory receptor expressed by activated and regulatory t cells. The aim of this study was to analyze two snps located on promotor region of CTLA4 gene in *P. vivax* patients and correlated it with parasitaemia and IL-4 levels. A total of 188 *P. vivax* malaria patients were enrolled in the study. Dna was extracted from blood samples using dna-easy kit (invitrogen). A PCR-RFLP protocol was used to analyze the genotype and allele frequencies of these polymorphisms. The density of parasitemia in the infected individuals was recorded and expressed as the number of asexual *P. vivax* per microliter of blood assuming a count of 100 microscopy fields and estimated before treatment. The serum levels of IL-4 were detected by milliplex map kit using magpix/luminex®. Analyses were performed using r version 2.8.1 statistical software. For the polymorphism at position -1577 g>a, the g/a genotype had the highest frequency (49.4%), followed by the g/g genotype (41%) and the a/a genotype (9.6%).for the polymorphism at position -1722 t>c, the t/t genotype had the highest frequency (86.7%), followed by the T/C genotype (12.3%) with the least frequent being the C/C genotype (1%). The IL-4 plasma level ranged from 0,61 to 9,32 pg/ml. There were no statistically significant differences either in parasitaemia and plasma il4 levels among individuals with different genotypes. This study showed that there was no association between the CTLA-4 snps with the development of malaria vivax, serum cytokine and peripheral *P. vivax* parasitaemia in brazilian amazon region. The CTLA -4 snps may be associated with malaria vivax in other endemic areas, but it appears to have no such effect in this studied population. The study also highlights the importance of conducting genetic association studies in different ethnic populations.

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ACQUIRED IMMUNITY AND RISK OF RECURRENCE FOLLOWING RADICAL TREATMENT AGAINST *PLASMODIUM VIVAX*

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Plasmodium vivax recurrence after supervised Primaquine/Choloquine (CQ/PQ) radical cure is due to interactions between parasite and host factors controlling recrudescence, relapse or re-infection. Since immunity to symptomatic malaria is primarily mediated by antibodies, we hypothesized that high antibody titers against merozoite surface proteins (MSP) during acute malaria infection would have decreased risk of recurrence following radical cure. A clinical trial aimed at assessing the efficacy of three different primaquine regimes in the Peruvian Amazon enrolled 540 acute *P. vivax* monoinfections between 2006-8. Patients were followed for 210 days after treatment and 90 recurrent cases were identified while 395 individuals did not experienced malaria recurrence. Antibodies against selected merozoite proteins were measured using ELISA assays and six putatively neutral microsatellite markers was genotyped to distinguish homologues vs. heterologous recurrence. The relationship between age-dependent antibody responses during acute infection and the risk of recurrence in this population was analyzed using survival analysis. The median titers of anti-*P. vivax* MSP-1 IgG antibodies (PvMSP-1 IgG) was 3.5 times higher among recurrent cases versus recurrence-free individuals ($p<0.001$). Only 2% (4/194) of subjects in the two upper antibody level quintiles experienced recurrence while 36% (69/194) and 17% (17/97) had recurrences in the second/third and lower quintile, respectively. This effect was confirmed by Cox hazard regression analysis and was found to be independent of patient age, parasitemia, PQ dose and other covariates likely to influence recurrence rate. Our findings suggest that the intensity of anti PvMSP-1 antibodies during acute infection is strongly associated with symptomatic recurrence independently of the dose of primaquine used in the radical cure treatment.

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ACUTE *PLASMODIUM FALCIPARUM* MALARIA EXPANDS T FOLLICULAR HELPER CELLS IN MALIAN CHILDREN

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Immunity to clinical malaria is only acquired after years of repeated *Plasmodium falciparum* infections in children living in malaria endemic areas. Antibodies have been shown to be protective against clinical malaria, but the acquisition and maintenance of long-lived plasma cells and memory B cells in response to *P. falciparum* appear inefficient in response to natural infection. The generation of long-lived plasma cells and memory B cells requires the function of follicular helper $CD4^+$ T (T_{FH}) cells in germinal center reactions. To better understand the cellular basis of the inefficient acquisition of protective antibody immunity in malaria, we conducted a longitudinal study to determine whether T_{FH} cells are generated in response to natural *P. falciparum* infections in children. We found that T_{FH} cells as defined as $CD4^+$, $CD45RO^{+/-}$, $CXCR5^+$, $PD1^+$ and $CXCR3^{+/-}$, increase in the peripheral circulation of children during acute *P. falciparum* malaria compared to the children's pre-infection baselines. ICOS, a costimulator that is essential for T_{FH} development and function, was markedly up-regulated during infection suggesting that these T_{FH} may

be highly functional. Work is in progress to directly measure the capacity of these T_{FH} cells to provide help to memory B cells *in vitro*. In addition we are examining whether the magnitude and quality of malaria-induced T_{FH} correlate with the magnitude and quality of *P. falciparum*-specific antibody responses and clinical malaria outcomes.

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A CROSS-SECTIONAL SURVEY OF ANTIBODY RESPONSES TO *PLASMODIUM FALCIPARUM* ANTIGENS IN A REGION OF DECLINING MALARIA TRANSMISSION IN SOUTHERN ZAMBIA

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Malaria transmission has declined in southern Zambia during the past decade. With reduced transmission, malaria reservoirs become increasing difficult to identify using conventional diagnostic methods such as rapid diagnostic test (RDT). Serology could be a useful tool to detect areas with ongoing transmission. Households were randomly selected using satellite imagery and participants were enrolled into a cross-sectional survey in 2012. A blood sample was collected by finger prick for RDT and dried blood spots (DBS) following written, informed consent. The DBS were collected on Whatman, Protein Saver card 903 and dried overnight. The DBS were later assayed for immunoglobulin G (IgG) antibodies to *P. falciparum* whole asexual stage lysate using an enzyme immunoassay (EIA). The threshold for seropositivity was determined using serum from US residents never exposed to malaria (which was defined as three standard deviations from ten samples). In 2012, 686 DBS were collected and analysed. The parasite prevalence by RDT was 0.2%. The mean EIA optical density (OD) values were 0.65, 0.71, 0.83, 0.90 and 1.1 for age less than 5 years, 5-10 years, 10-15 years, 15-20 years and older than 20 years, respectively. Similarly, seropositivity increased with age and was 12%, 22%, 45%, 57% and 75% for age less than 5 years, 5-10 years, 10-15 years, 15-20 years and older than 20 years, respectively. The low seropositivity and mean EIA OD values in younger age groups are consistent with a reduction in exposure to *P. falciparum*. The household locations of seropositive children younger than five years of age were mapped using ArcGIS to identify areas of malaria transmission. Serology can complement information obtained from RDTs to identify hot spots of recent malaria transmission.

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ELEVATED LEVELS OF IL17 AND ITS ASSOCIATION WITH LOW HAEMOGLOBIN IN SEMI-IMMUNE MICE INFECTED WITH *PLASMODIUM BERGHEI* ANKA

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Malaria anaemia is still a major public health problem and its pathogenesis still unclear. Interestingly, the progression of anaemia is at relatively low parasitaemia with some mortality in the semi-immune individuals in the endemic areas. A recent study has observed variation in IL17 levels in different mice strains, thus implicating autoimmune mechanism in RBC loss due to *Plasmodium* infection. The objective of this study was to evaluate IL17 levels in anaemic condition and to determine if genetic factors are involved. To address this objective, we crossed two mice strains (Balb/c of low parasitaemia) and CBA (moderately high parasitaemic) to get the progeny, called the F1. Balb/c (8), CBA (8) and F1 (12) were taken through 6-7 cycles of infection (with *Plasmodium berghei* ANKA) and treatment (with chloroquine/pyremethamine) to generate semi-immune status. Parasitaemia and haematological parameters were monitored. Kinetics of antibody production, cytokine levels (in serum and cultured supernatant of stimulated spleen cells) and CD4+CD25+T regulatory cells were evaluated by ELISA, bead-based multiplex assay kit and FACs respectively; at days 0, 16, 28 for Balb/c and F1, and days 0, 8 and 12 for CBA. Similar survival (>70%), mean %Hb loss (45%) and mean parasitaemia (5%) was observed in Balb/c and F1, while 0% survival, mean %Hb loss (80%) and mean parasitaemia of 15% was observed in CBA. IgG subtypes were two times higher in Balb/c and F1 than CBA. While IL1a, IL4, IL10, IL12a, IFNγ and TNFα were similar in the three mice strains, IL17 was 3 times higher in Balb/c and F1 than CBA. Maximum cytokine level was observed at D16 (point at which recovery from parasitaemia occurs, with lowest Hb) in the Balb/c and F1. CD4+CD25+ Treg cells in CBA were lower than those of Balb/c and F1. In conclusion, autoimmune is implicated in Hb loss due to high IL17 levels and may be controlled by a genetic factor. Further studies of F2 between the F1 and Balb/c will be informative to evaluate if these genes are segregated or further apart

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THE MALARIA VACCINE CANDIDATE GMZ2 ELICITS FUNCTIONAL ANTIBODIES IN INDIVIDUALS FROM MALARIA-ENDEMIC AND NON-ENDEMIC AREAS

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GMZ2 is a hybrid protein consisting of the N-terminal region of the glutamate-rich protein fused in frame to the C-terminal region of merozoite surface protein 3 (MSP3). GMZ2 formulated in Al(OH)3 has been tested in 3 published phase 1 clinical trials. The GMZ2/alum formulation showed good safety, tolerability, and immunogenicity, but whether antibodies elicited by vaccination are functional is not known. Serum samples prior to vaccination and 4 weeks after the last vaccination from the 3 clinical trials were used to perform a comparative assessment of biological activity against *Plasmodium falciparum*. We showed that the maximum level of immunoglobulin G (IgG) antibodies obtained by GMZ2 vaccination is independent of ethnicity, time under malaria-exposure, and vaccine dose and that GMZ2 elicits high levels of functionally active IgG antibodies. Both, malaria-naive adults and malaria-exposed preschool children elicit vaccine-specific antibodies with broad inhibitory activity against geographically diverse *P. falciparum* isolates. Peptide-mapping studies of IgG subclass responses identified IgG3 against a peptide derived from MSP3 as the strongest predictor of antibody-dependent cellular inhibition. These findings suggest that GMZ2 adjuvanted in Al(OH)3 elicits high levels of specific and functional antibodies with the capacity to control parasite multiplication.

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FREQUENCY AND PHENOTYPE OF ΓΔ T CELL SUBSETS IN AN AREA OF HIGH MALARIA TRANSMISSION INTENSITY

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Plasmodium falciparum infection is thought to induce potent immunoregulatory responses, but the precise mechanisms in humans are unclear. Prior studies have shown that a subset of γΔ lymphocytes, VΔ2$^+$ T cells, become rapidly activated upon stimulation with *P. falciparum* antigens, suggestive of an innate-like immune response. We have found that repeated exposure to malaria is associated with loss and dysfunction of VΔ2$^+$ T cells in 4-year-old children living in a high endemicity setting. To test the hypothesis that repeated exposure to malaria also leads to increased expression of immunoregulatory markers on this γΔ subset,

we measured the frequency and phenotype of γΔ T cell subsets in children and their caregivers (n=171) enrolled in a longitudinal cohort study in Nagongera, a rural area in Eastern Uganda with high perennial transmission intensity (EIR 315). We measured frequencies of VΔ1$^+$ and VΔ2$^+$ T cells and evaluated cell surface expression of Tim-3, PD1, ILT2, CD57, CD56, CD16, and NKG2A by multiparameter flow cytometry using freshly obtained peripheral blood mononuclear cells. The mean age of children in the cohort was 6.6 yrs (range 10 months-11 years, n=128), and was 37 yrs in their caregivers (range 20.2-67.7 years, n=43). The mean incidence of malaria was 4 episodes ppy in children (range 0-11 ppy) and 1 episode ppy in their caregivers (range 0-5 ppy). In multivariate linear regression, we observed a significant inverse association between the frequency of VΔ2$^+$ T cells and the prior incidence of malaria (P<0.001), independent of age. We further found significantly increased expression of Tim-3, CD57, NKG2A, and CD16 on VΔ2$^+$ T cells with increasing age. Our findings are consistent with the hypothesis that, in high endemicity settings, cumulative exposure to malaria may be associated with increased expression of regulatory markers on VΔ2$^+$ T cells. These findings have important implications in understanding the immunopathogenesis of malaria in childhood.

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THE ASSOCIATION BETWEEN MATERNAL HOOKWORM INFECTION IN PREGNANCY AND ANTIMALARIAL ANTIBODY RESPONSES IN THE OFFSPRING

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Several studies have shown that helminth infections have adverse effects on malaria. However, the immunological mechanism by which helminths modify the host response to malaria is uncertain. In a trial of anthelminthics in 2, 507 pregnant women [ISRCTN32849447], maternal hookworm infection was associated with an increased risk of clinical and asymptomatic malaria in their offsprings. We hypothesised that helminth infection in pregnancy modifies the fetus' initial response to malaria and, consequently, their antimalarial immune responses in early childhood. Stored blood samples from children of study women were tested for IgG antibody concentrations (µg/ml) to Apical Membrane Antigen-1 (AMA1) and Merozoite Surface Protein-1 (MSP1) by enzyme immunoassay (EIA). Overall, antibody concentrations to anti-AMA1 and anti-MSP1 were significantly higher (p<0.001) among children with asymptomatic parasitaemia compared to children without parasitaemia. However, at the age of one year, parasitaemic children of hookworm infected mothers had a significantly lower concentration of anti-AMA1 antibody (mean $\log_{10}$ (95% CI): 3.51 (3.03-4.07)) compared with parasitaemic children of uninfected mothers (4.07 (3.58-4.63)), (p for interaction = 0.003). Similarly at five years of age, parasitaemic children of hookworm infected mothers had a significantly lower concentration of anti-AMA1 antibody (4.48 (3.67-5.48)) compared with parasitaemic children of uninfected mothers (5.84 (5.01-6.80)), (p for interaction = 0.01). Maternal hookworm infection did not modify anti-MSP1 responses in children. These results highlight the importance of controlling for helminth co-infections in the assessment of malaria vaccine efficacy. Further immunological studies to understand the role of helminth co-infections on antimalarial immune responses are essential, and could subsequently lead to improved results for the malaria vaccine effort.

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PHAGOCYTOSIS AND OPSONIZATION OF *PLASMODIUM FALCIPARUM* GAMETOCYTES

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During natural infection malaria parasites are injected into the bloodstream of a human host by the bite of an infected female *Anopheles* mosquito. Immune responses are generated against various life cycle stages of the parasite that have important roles in resistance to malaria and in reducing natural transmission. A better understanding of the early immune response against mature gametocyte stage of the parasite will inform transmission blocking vaccine strategies. The asexual and sexual erythrocytic life cycle forms of *Plasmodium* are stages that pass through humans into *Anopheles* mosquitoes during a blood meal. The sexual stages develop into competent parasites in the mosquitoes and are later injected back into a human host to complete a transmission cycle. There is a fairly good understanding of the adaptive cellular and humoral immune responses, but little is clearly known about the innate immune response. Several published studies suggest that phagocytosis and opsonization of asexual, immature sexual and free merozoite stages occur. We hypothesize that circulating mature gametocytes are phagocytosed directly or after opsonization with specific antibodies present in immune sera from people with previous exposure to malaria. We evaluated phagocytosis of various developmental forms of erythrocytic stage parasites in the absence of immune sera, and opsonization in the presence of immune sera. Results focusing on the uptake of mature gametocytes by phagocytic cells in order to differentiate the opsonic and phagocytic responses will be presented. Identification of phagocytic receptors and targets of opsonizing antibodies will enhance understanding of the role of phagocytosis in the clearance of circulating mature gametocytes, and the link to humoral arm of adaptive immunity. Phagocytosis and opsonization mediated immune activation together with presentation of gametocyte specific antigens will contribute to natural transmission blocking immunity. Specific strategies may also be developed to induce similar antibodies by vaccines aimed at overall transmission reduction.

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ASYMPTOMATIC MULTICLONAL *PLASMODIUM FALCIPARUM* INFECTIONS CARRIED THROUGH THE DRY SEASON PREDICT PROTECTION AGAINST CLINICAL MALARIA IN THE FOLLOWING HIGH TRANSMISSION SEASON

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Immunity to the antigenically diverse *Plasmodium falciparum* parasite is acquired gradually after repeated exposure. Infections often consist of several concurrent parasite clones and the diversity of infections in asymptomatic individuals has predicted a reduced risk of malaria during follow up in high transmission settings. In areas of continuous transmission, the number of clones might be a marker of exposure and it is difficult to distinguish between persistent and transient infections. Therefore, we assessed the genetic diversity of *P. falciparum* infections in a longitudinally followed cohort in an area of highly demarcated seasonal malaria transmission in Mali. During the one-year study period, cases of febrile malaria were detected by passive surveillance among 225 individuals aged 2-25 years of age.. Genotyping of the highly polymorphic merozoite surface protein 2 gene (MSP2) was performed on blood samples collected at five cross-sectional surveys. The genetic diversity was highest in children 5-10 years old and increased during the peak of transmission in all age groups. Detection of multiclonal infections before the rainy season

conferred a decreased risk of malaria, compared to being parasite negative or having one clone detected, when assessing time to first malaria episode The results suggest that persistent multiclonal infections carried through the dry season protect against subsequent clinical malaria, probably by maintaining otherwise waning protective immune responses.

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IDENTIFYING MRNA TARGETS OF *PLASMODIUM FALCIPARUM* RNA BINDING PROTEINS USING DNA MICROARRAY

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Although resistance has rendered the once standard antimalarial Pyrimethamine ineffective against *Plasmodium falciparum* across the world, it is hope that new compounds can be developed to combat the pyrimethamine resistant strains of *P. falciparum*. Although much is known about the drug-resistant variant enzymes in terms of their structures and biochemical characteristics, little is known of how the DHFR-TS enzyme is regulated in the parasite cell. Through greater understanding of the cellular regulation, antifolates could be employed in a more effective manner, e.g. avoiding the known phenomenon of drug-induced expression of DHFR-TS. Intringuingly, DHFR-TS has nucleic acid binding activity *in vitro*, and is thought to be involved with post-transcriptional regulation of gene expression, including itself. In an attempt to characterize its putative mRNA targets and identify conserved regulatory elements amongst the targets, we use the recombinant DHFR-TS to separate its targets from total parasite RNA. The selected RNAs are then interrogated by DNA microarray analysis and identified en masse. In order to validate the approach, the RNA targets of the *P. falciparum* Bruno-like putative RNA binding proteins will be characterized initially and compared by bioinformatics with known Bruno-like mRNA targets in other species.

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RAPID GENE REPLACEMENTS IN *PLASMODIUM FALCIPARUM* USING A NEW APPLICATION OF ATTP X ATTB TECHNOLOGY

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Allelic exchange transfection in *Plasmodium falciparum* is an arduous, low efficiency process that often takes 6 - 12 months; many experiments do not work and are presumed to fail because the desired changes may be lethal to the haploid blood-stage parasite. We have found that Bxb1 integrase mediated attP x attB technology could be successfully used to overcome limitations in manipulating the endogenous copy of clag3, recently shown to be essential for parasite nutrient acquisition. The attB sequence was successfully introduced into a native clag3 intron by conventional allelic exchange transfection. Introduction of this sequence did not adversely affect intron splicing and yielded an intact, unmodified protein. Subsequent transfection with attP-containing plasmid that carries desired downstream changes in the clag3 gene yielded rapid introduction of desired site-specific integrants that came up as early as 3 weeks in culture. The major limitation of this strategy of introducing attB into an intronic sequence is that nearly half of the genes in the *Plasmodium* genome are devoid of introns To overcome this limitation, we are adapting this strategy to study the function of endogenous copies of nonintronic genes such as Dynamin-2 (PfDyn2), Cardiolipin synthase (PfCLS) and MSP7. We are introducing an intron that contains the attB sequence, into the coding (exon) regions of PfDyn2, PfCLS and MSP7. For integration into the ORF of PfDyn2, we have made a construct that comprises a 2 Kb gene fragment from the 3' end of PfDyn2, which incorporates a 200bp intron that includes a central 40bp attB site. The distal 800bp of the 2 kb construct has been re-coded (using E.coli codon frequencies) to ensure integration occurs upstream of the intron. A similar approach has been followed for making the PfCLS construct. Transfection studies are underway, and upon successful introduction of introns with the attB sequence into the coding regions, we will be able to rapidly manipulate the PfDyn2, PfCLS and MSP7 loci by Bxb1 integrase mediated, rapid site specific recombination of attP-containing constructs into the attB sequence. This technology will help us manipulate endogenous copies of nonintronic genes and is highly cost-effective. It has the potential to open up new avenues for investigations of malaria parasite molecular biology.

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GENETIC CHARACTERISTICS OF PARASITES IN PREGNANCY-ASSOCIATED MALARIA IN COLOMBIA

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This study wanted to analyze the genetic diversity and frequency of resistant-sensitive genotypes of *Plasmodium falciparum* and *P. vivax* isolated from peripheral and placental blood of pregnant women, and non-pregnant population of Colombia, as well as to explore associations between the plasmodial infection in peripheral and placental blood with placental histology, maternal anemia and low birth weight. Study populations: a) 96 pregnant women at delivery, independently of malaria symptoms; b) 130 patients with acute malaria (about half per each species), corresponding to 50 pregnant women attending antenatal care, 40 non pregnant women and 40 men. Plasmodial infection was detected by thick smear and quantitative real time PCR (qPCR). Parasite genotypes in compartments (periphery vs. placenta) and populations (pregnant vs. non pregnant) were compared using five molecular markers per each species, which were analyzed by nested PCR and capillary electrophoresis. Point mutations in the genes dhps, dhfr and mdr1 of both species were analyzed by direct sequencing. In pregnant women at delivery, qPCR detected significantly more infections (130/288) than microscopy (20/288); 65% of women had at least one positive compartment and the placenta exhibited the highest frequency of infection (57%). *P. falciparum* was detected in 63% (82/130) of infections at delivery and most of them (65%; 84/130) had <2 DNA copies/µL. Both *P. vivax* and *P. falciparum* microscopic and submicroscopic placental infections were associated with villitis and intervillitis. Placental malaria infections were not associated with maternal anemia or low birth weight. *P. falciparum* placental isolates had significant genetic differentiation compared to peripheral isolates from pregnant and non-pregnant patients with acute malaria. On the contrary, all the *P. vivax* isolates were genetically similar independently of the population or compartment. More than 90% of the analyzed isolates of both species had the triple mutation genotype in genes dhfr-dhps associated with sulfadoxine/pyrimethamine resistance. All *P. falciparum* isolates had the mutation 86Y in Pfmdr1, while only one *P. vivax* isolate had the double mutation 976F-1076L in Pvmdr1, associated with chloroquine resistance.

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ANTI-PLASMODIAL PROPERTIES OF SOME SELECTED GHANAIAN MEDICINAL PLANTS; IDENTIFICATION OF NOVEL ACTIVE COMPOUNDS AGAINST *PLASMODIUM FALCIPARUM*

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Malaria, caused by *Plasmodium* spp has been considered as a major disease of public health importance affecting multitudes of people worldwide, particularly in the tropics and sub-tropics. Due to increasing drug resistant parasites to existing drugs, development of new anti-malarial drugs are eagerly awaited. In Africa, there is extensive use of traditional medicinal plants for treatment of various diseases. Many research studies into medicinal plants have shown their significant potentials for anti-plasmodial properties but a few of the active ingredients have been studied.. The present study aimed at screening selected medicinal plants used in Ghana for activity against *Plasmodium falciparum* to determine their active compounds. A high-throughput 96 wells flow-cytometry screening system was established using the method reported by Smilkstein and others, with modification. 50% Et-OH crude extracts of medicinal plants were prepared and applied to synchronized *P. falciparum* (3D7 strains) culture and FACS analysis was carried out to determine the IC_{50} of the extracts. Although screening is still ongoing, one active crude extract, JJNC008L, was found possessing activities against *P. falciparum* and identified two novel compounds from this extract, compound #1 and #2. These compounds have significantly high anti- *P. falciparum* activities with IC_{50} values of 3.89 µM and 1.52 µM respectively. Novel compounds identified in this study could be candidates to develop new chemotherapy for malaria. Furthermore, our high-throughput FACS screening system could be useful tool for malaria drug assay.

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PRODUCTION AND CHARACTERIZATION OF A LIBRARY OF FULL-LENGTH *PLASMODIUM VIVAX* MEROZOITE PROTEINS

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A vaccine targeting the illness-inducing blood stage of parasite development is an essential component of any worldwide malaria eradication campaign, but major gaps in our understanding of *Plasmodium vivax* biology, including the protein-protein interactions that mediate erythrocyte invasion, hinder the search for an effective vaccine. Only a single parasite ligand-host receptor interaction is presently known, that between *P. vivax* Duffy Binding Protein (PvDBP) and Duffy Antigen Receptor for Chemokines (DARC), and strain-specific immune responses to PvDBP make this antigen a challenging vaccine target. We are carrying out a comprehensive study of *P. vivax* proteins that mediate erythrocyte binding and invasion in order to identify additional vaccine candidates. As a first step, we produced a library of 39 full-length recombinant *P. vivax* proteins to test for erythrocyte binding and immunoreactivity. To our knowledge, this represents the largest full-length *P. vivax* antigen set ever assembled. Candidates were selected based on predicted localization to the merozoite surface or invasive secretory organelles, and on homology to *P. falciparum* vaccine candidates. 37/39 *P. vivax* recombinant proteins were expressed in the HEK293E cell system, which has been successfully used for expression of full-length *P. falciparum* invasion ligands such as PfRH5. Known or predicted functions, such as the interaction between merozoite surface proteins Pv12 and Pv41, were confirmed and several novel parasite protein-protein interactions were identified. Pilot immunoreactivity screens using sera from Cambodian patients with *P. vivax* malaria showed that IgG variously recognize the majority of antigens tested. The large-scale initial screenings of this library will be presented through protein expression, protein interaction and seroreactivity data, as well as immuno-epidemiological studies.

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GENETIC VARIABILITY AND POPULATION STRUCTURE OF KENYAN *PLASMODIUM FALCIPARUM* ISOLATES

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Malaria parasite genetic variability and population structure varies among areas of differing endemicity and are key factors in malaria control strategies. Unlike previously used antigen-encoding loci under strong selection, *Plasmodium falciparum* microsatellite markers are an attractive target for population structure studies. In Kenya, molecular studies on drug resistant malaria and antigenic molecules have been inadequate in providing accurate genetic profiles of parasite populations in the country. Understanding the genetic structure of malaria parasites is essential to predict how fast phenotypes of interest, such as novel antigenic variants or drug resistant alleles, originate and spread in populations. Twelve polymorphic *P. falciparum* microsatellite loci were genotyped in 250 parasite isolates from five locations in Kenya using capillary electrophoresis. Analysis of the generated fragments was performed to determine proportions of mixed genotype infections, genotype diversity among isolates, multilocus standardized index of association, and inter-population differentiation. The data revealed dramatic differences in parasite population structure in different geographical locations. An 80% multiplicity of infection was detected in parasites circulating in Kenya. Samples from Kisumu, a high malaria endemic region had a high diversity (He _ 0.73) revealing genetic crossing. Results have shown a *P. falciparum* population structure as well as significant linkage disequilibrium in Kenyan parasites. Regional diversity was observed in range of population structures. These results could be related to geographic difference and low flow of parasites between sites. These data reveal a range of population structures within a single pathogen species and suggest intimate links between patterns of epidemiology and genetic structure.

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GENETIC DIVERSITY OF *PLASMODIUM VIVAX* IN AREAS OF HIGH RISK OF MALARIA IN CÓRDOBA-COLOMBIA

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The aim of this work was to study the genetic diversity of natural populations of *Plasmodium vivax* in areas of high malaria risk in Córdoba-Colombia. Molecular confirmation of infection by *P. vivax* DNA extraction using Chelex-100 was made, thereafter a nested PCR protocol was used to amplify the gene encoding 18S ribosomal small subunit (ssrARN) *P. vivax* and *P. falciparum*. A sample confirmed as *P. vivax* underwent a PCR -RFLP for Pvmsp - 3α gene, restriction enzymes used were Alu I and Hha I, which generated an electrophoretic profile that identified different haplotypes for Pvmsp - 3α gene.Of the 125 samples analyzed by nested PCR for Pvmsp - 3α gene, 116 successfully amplified. The size of the PCR products allowed to demonstrate the movement of three different genotypes for the Pvmsp - 3α gene: A (1900 bp), type B (1500 bp) and type C (1100 bp), being the most frequent genotype A (88%). 97.4 % (113 /116) of the samples showed simple infections and 2,6% (3/116) polyclonal infections, two by types A and B and one by types A and C. Besides finding a band is reported in the electrophoretic profile of the amplification products Pvmsp -3α gene with an approximate size of 800 bp which does not correspond with the sizes reported to date, which may represent a new allele of Pvmsp-3α gene however, these results should be interpreted cautiously, pending the results of sequencing. Digestion of PCR products obtained for the Pvmsp - 3α gene with enzyme Alu I showed ten different restriction patterns while the with the enzyme Hha I produced nine. The results of

the restriction enzyme digestion of the 113 samples tested revealed that 40/113 (35.3%) and 47 /113 (41.6%) of these samples showed polyclonal infections when they are digested with the enzyme Alu I and Hha I, respectively. There is a high degree of genetic variability in Pvmsp-3α gene of *P. vivax* parasite population circulating in the department of Córdoba.

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THE ROLE OF TEMPORALLY REGULATED RNA-BINDING PROTEINS IN *PLASMODIUM* INFECTION

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Translational repression of specific mRNAs allows the temporal modulation of gene expression by *Plasmodium* species. This mechanism enables regulated preparation for the vector/host and host/vector transmission events and perhaps the infection of a new red blood cell. To further our understanding of this mechanism, we have employed genetic manipulation of *P. yoelii*, a rodent-infectious parasite, to investigate RNA-binding proteins that may play critical roles in this process. Our previous studies have shown that Puf2, a member of the Pumilio-FBF family of RNA-binding proteins, plays an integral role in maintaining the infectivity of salivary gland sporozoites, as well as regulating RNA homeostasis and translational repression. In the absence of Puf2, salivary gland sporozoites become less infectious with prolonged salivary gland residence time, and undergo premature developmental changes, resembling liver stage parasites while still in the salivary gland. In the absence of Puf2, the transcript abundance of ALBA4 increases by approximately 120-fold. Proteins containing the ALBA domain (acetylation lowers binding affinity) are functionally important in several parasite species, including *Leishmania, Trypanosoma*, and *Plasmodium*, where it has been shown to bind DNA and RNA. Because of their ability to bind RNA, we are interested in the potential role of ALBA proteins in translational repression. In the human-infectious species *Plasmodium falciparum*¸ ALBA4 protein localization changes from perinuclear to cytoplasmic during blood stage progression. In *P. yoelii*, we also have evidence for cytoplasmic localization of ALBA4 in blood stage schizonts as seen by live fluorescence microscopy. To fully understand the implications of this finding, we are extending these studies by genetically deleting ALBA4 to determine its essentiality, and examining ALBA4's expression profile in other life cycle stages to determine its function and importance.

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GENETIC VARIATIONS OF *PLASMODIUM VIVAX* IN THE PERUVIAN AMAZON: DIVERSITY ASSOCIATED WITH POPULATION GENETICS AND VACCINE CANDIDATES GENES AGAINST MALARIA

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Knowledge of both genetic variability of *Plasmodium vivax* and polymorphism of its main vaccine candidate genes is of great importance to complement the strategies of control, treatment and development of vaccines against this parasite. The current study had two main objectives, first, to determine the genetic variability of *P. vivax* in the Peruvian Amazon among years 2006-2009 using the merozoite surface protein gene (PvMSP-3α) as a molecular marker, and second, to determine the genetic polymorphism of both the Circumsporozoite protein (PvCSP) and major transmission blocking vaccine candidates, Pvs25 and Pvs28. A total of 1780 samples were obtained from the communities of Santo Tomas, San Jose de Lupuna, Padrecocha and Mazan. By PCR, we found 3 variants of PvMSP-3α gene named type A of 1.9 kb (93.4%), type B of 1.5 kb (6.1%) and type C of 1.2 kb (0.3%); besides, a mixture of two types of amplified fragments (A/B or A/C) were showed in 0.2% of the samples. Also by PCR-RFLP, we identified 11 different alleles of PvMSP-3α. To know the Circumsporozoite protein genetic polymorphism, 42 samples were analyzed; we found 11 types of PvCSP which were composed of 5 different nonapeptides, all belonging to VK210 type. On the other hand, the genetic polymorphism of Pvs25 and Pvs28 were studied by sequencing 51 and 49 samples, respectively; each result were compared to reference strain Sal I. For Pvs25, a non synonymous mutation (Q87K[cag/aac] was identified giving two haplotypes. For Pvs28, four non synonymous mutation were reported (M52L[atg/ctg], D87N[gat/aat], N110Y[aat/tat] y T140S[acc/agc]) resulting on four new haplotypes. All Pvs25 haplotypes and just one Pvs 28 haplotype were reported for all the studied Peruvian Amazon Communities. Our results confirm there are different genotypes of P vivax in the Peruvian Amazon and that polymorphism of major transmission blocking vaccine candidates, Pvs25 and Pvs28, is more limited than polymorphism of PvCSP; the implications of these genetic diversity in vaccine development should be studied.

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INHIBITION OF RETICULOCYTE MATURATION TO INCREASE *PLASMODIUM VIVAX* PROLIFERATION *IN VITRO*

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Plasmodium vivax is restricted to the reticulocyte for both growth and invasion. One of the major obstacles to successful continuous *in vitro* culture of *P. vivax* is that reticulocytes rapidly mature to erythrocytes *in vitro*. This steady maturation effectively dilutes the culture, as constant replenishment of reticulocytes is essential for sustained parasite propagation. We hypothesize that inhibition of reticulocyte maturation would increase the number of viable cells for invasion and would enhance *P. vivax* proliferation overall. Reticulocyte maturation involves hallmark cellular processes: (i) membrane remodeling and (ii) autophagy of organelles, which can be targeted by small molecules. We initially undertook a small-scale screen to show that reticulocyte maturation can be inhibited and the inhibited cells can support parasite invasion and growth. On going research includes a large-scale screen with a panel of bioactive small compounds and a genetic approach using RNAi with cultured reticulocytes from hematopoietic stem cells.

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PLASMODIUM VIVAX: A GENOTYPIC INTERPRETATIONAL PROBLEM

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It is conventionally assumed that in the absence of either reinfection, or recrudescence following persistence of merozoites in the bloodstream, the hypnozoite stage (this term was coined by me 3.5 decades ago) is the source of recurrent *Plasmodium vivax* malaria, including recurrences caused by parasites that are genetically similar to those which were responsible for the initial clinical manifestations. How frequently (if ever) this is the case, though, is uncertain, partly because hypnozoites are never, as far as is known, generated by the prior blood-stage infection. Consequently, the source of homologous recurrences is on genetic grounds conceptually not easily ascribable to hypnozoites. This parasite form seems to be directly sporozoite-derived. If so, it has yet to be shown that genotypically homologous sporozoites inoculated by the mosquito can behave in two different ways, i.e. involving some sporozoites initiating early hepatic schizogony but others becoming dormant as hypnozoites. This might indeed happen, of course. However, there are

also other reasons (partly resulting from recent research findings) for asking the question: "Could it normally or sometimes be non-hepatic, non-bloodstream, quiescent parasites (as opposed to hypnozoites) that give rise to recurrent homologous *P. vivax* malarial episodes?" Attention will be drawn via the poster to both new and hitherto overlooked, indirect evidence for this possibility (if not probability). That aside, uncertainty concerning whether *P. vivax* recurrences have a dual origin (sometimes hypnozoites and other times non-hypnozoites) could complicate molecular identification of drug-resistant parasites, for reasons that are perhaps not readily apparent.

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DNA DOUBLE STRAND BREAK AND REPAIR IN ANTI-MALARIAL ACTION OF ARTESUNATE

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Artemisinin-based combination therapy (ACT) is the recommended first line treatment for *Plasmodium falciparum* malaria. Mechanisms involved in the anti-malarial action of artemisinin are poorly understood, and it has been suggested that the cytotoxic effect of artemisinin is mediated by free radicals followed by alkylation of malarial proteins1-3. The endoperoxide bridge, the active moiety of artemisinin derivatives (ART), is cleaved in the presence of ferrous iron generating reactive oxygen species (ROS) and superoxide anions4,5. However, the emergence of resistance to artemisinin in *P. falciparum* underscores the need for new insights into the molecular mechanisms of anti-malarial activity of artemisinin. Here we show that artesunate (ART) functions by causing DNA double strand breaks in malaria parasites in a pharmacologically relevant dose and time-dependent manner. We found that DNA damage induced by ART was accompanied by an increase in intracellular ROS in the parasites. Presence of mannitol, a ROS scavenger, during the first 60 minutes reversed the cytotoxic effect of ART and reduced DNA damage with minimal effect on parasite growth. Accumulation of DNA damage resulting from ROS was accompanied by an anti-parasite effect, suggesting a causal relationship between ROS, DNA damage and parasite death. Furthermore, we show that modulating glutathione (GSH) levels impacts ROS and DNA damage induced by ART. Finally, we also show that ART-induced ROS production involves a potential role for NADPH oxidase, an enzyme involved in the production of superoxide anions. Our results provide novel insights into the molecular mechanisms underlying the anti-malarial activity of artemisinin. These studies will provide enhanced understanding of the molecular mechanism involved in resistance to artemisinin and help in the design of new anti-malarials against the most virulent *Plasmodium* species.

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HEME-INDUCED ENDOTHELIAL CELL APOPTOSIS IS MEDIATED BY MULTIPLE SIGNALING PATHWAYS

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Recent studies in our laboratory and by others demonstrate the increasing importance of elevated serum concentrations of free heme, a by-product of parasite proliferation, as a key inducer of inflammation and damage to the host microvascular endothelium as well as the mortality associated with fatal cerebral malaria (CM). Previous results indicate that heme induced human brain vascular endothelial cells (HBVEC) apoptosis in a dose dependent fashion. Since brain microvascular endothelial cells are key components of the BBB, which are severely disrupted during CM pathogensis, we hypothesized that heme (at physiologically relevant levels) induced inflammation and apoptosis in HBVEC are mediated by independent pro-inflammatory and pro-apoptotic signaling pathways. Our goal was to investigate the mechanism underlying the apoptotic and inflammatory effects of heme in HBVEC *in vitro*. We used real time RT^2 Profile polymerase chain reaction (PCR) array for apoptosis to analyze apoptotic gene expression profiles of HBVEC cells 24h after heme treatment. ***2)*** The involvement of selected pro-apoptotic, anti-apoptotic and inflammatory genes was further analyzed by quantitative PCR (qPCR). Heme causes cell death in HBVEC by increasing apoptotic rate ***2)*** The results of real time RT^2 Profile PCR array analysis are expressed as the fold changes in expression obtained by comparing HBVEC treated with heme or with vehicle as control. The up-regulated genes (with fold-change greater than 2) include ABL1, BAG1, BCL2, BCL2L10, BIRC3, BNIP3, BNIP3L, CASP3, CASP4, CASP5, CD40LG, CD70, CIDEA, CYCS, FASLG, MCL1, TNFRSF9, TNFSF10, TP73, and TRAF3. The down regulated genes consist of BAD, BAK1, CASP2, CASP6, CD27, CRADD, GADD45A, LTA, TNFRSF1A, BAG3 and BCL2A1. ***3)*** Several genes with prominent pro-apoptotic function were up-regulated in HBVEC cells after treatment with heme and confirmed by validation of qPCR, including BCL2L10, CD40LG, CD70, CIDEA, FASLG, TNFRSF9, TNFSF10 and TP73. Some genes for anti-apoptosis were down-regulated, such as BIRC3, BAG3 and BCL2A1. In conclusion, heme induces endothelial cell apoptosis through multiple signaling pathways. This indicates that reducing or inhibiting production of excess free heme may potentially reduce the adverse outcomes associated with CM and other hemolytic diseases.

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QUANTITATIVE EXPRESSION OF MCHERRY-LUCIFERASE IN ALL LIFE CYCLE STAGES OF *PLASMODIUM FALCIPARUM*

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Live cell imaging and sensitive quantitative assays are valuable tools in the study of *Plasmodium falciparum* life cycle, especially for stages in the mosquito vector and in the liver. The objective of the project is to generate a stable *P. falciparum* line with high levels of luciferase and mCherry expression for drug discovery in liver stages or other life cycle stages. Since the emergence of GFP several fluorescent proteins have been employed for live imaging of *Plasmodium* parasites. Relative to GFP mCherry has improved photostability and less background fluorescence, resulting in better signal-to-noise ratios. In contrast, luciferase provides a more sensitive measure in quantitative assays and as consequence has been widely used for high throughput screening (HTS) in drug discovery. To create stable transgenic parasites expressing mCherry-luciferase we used the TTAA-specific transposon *piggy*Bac genetic engineering of *P. falciparum*. Luciferase and PbDHFR3' UTR gene was amplified from existing expression cassette and inserted it into a mCherry-hDHFR *piggyBac* vector pL-BacII-bEDMH to obtain pL-BACII-bEDMH-Luc plasmid vector. The vector was designed to express both mCherry and luciferase driven by *P. berghei* EF1α, which is a constitutive promoter active in all developmental stages. Promega Luciferase Reporter System was used to detect luciferase activity and mCherry was checked by live cell imaging. Seven clones carrying integrated transposon reporter cassettes were confirmed. Theses integrated clones express high level of luciferase and mCherry signals through all stage of parasite blood cycle. The clone PfKF7G4 was confirmed to infect mosquito, form sporozoites and express both luciferase and mCherry in mosquito stage. The luciferase-mCherry expressing cassette was successfully integrated in the genome of *P. falciparum* KF7 by *piggyBac* transposon system. In blood cycle and mosquito stage, a stable high level expressing luciferase and mCherry reporter gene *P. falciparum* parasite line-PfKF7G4 has been developed. PfK7FG4 can be used for parasite visualization and quantitative analysis. It may offer more efficient drug assays for vector and liver stage studies of *P. falciparum*.

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INTRAGENIC PROMOTER HAPLOTYPES OF IL-10 AND TNF-α INFLUENCE SUSCEPTIBILITY TO *PLASMODIUM FALCIPARUM*-INDUCED SEVERE MALARIAL ANEMIA

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Plasmodium falciparum associated severe malarial anemia [SMA, hemoglobin (Hb)<5.0g/dL] is a leading cause of morbidity and mortality in African children. Examination of the host immune response and underlying genotypic traits that condition SMA can offer an improved understanding of malaria pathogenesis. Pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α) serve as early response signaling molecules that activate the innate immune response. Discordantly, anti-inflammatory mediators, such as interleukin-10 (IL-10) act as secondary modulators to regulate and balance the pro-inflammatory response. Dysregulation of both TNF-α and IL-10 have been shown to influence clinical outcomes in human malarial infections. To investigate the role of polymorphic variation in the *IL-10* and *TNF*-α genes on susceptibility to SMA, haplotypes were constructed using six promoter variants [IL-10 -627A/C, IL-10 -854C/T, IL-10 -1117A/G, TNF-α -238G/A, TNF-α -308G/A and TNF-α -1031T/C]. Selection of variants was based on minor allelic distribution of >10% in African populations and potential transcription factor binding elements. Acutely infected children (n=1220, aged 3-36 mos.) from western Kenya were investigated in this study. Logistic regression was performed [controlling for age, gender, nutritional status, co-infections (HIV-1, bacteremia), G6PD deficiency, alpha-thalasemmia and HbS]. These analyses revealed that presence of the IL-10-627AC/-854CT genotypes were associated with protection against SMA (average OR, 0.68; 95% CI, 0.47-0.91; *P<0.05*), whereas the TNF-α -308GG genotype enhanced susceptibility to SMA (OR, 1.23; 95% CI, 1.08-1.54; *P<0.05*). Similarly, several extended haplotypes between the two promoters (i.e., list) were associated with enhanced susceptibility to SMA (average OR 1.39; 95% CI 1.02-1.94; *P<0.05*), while others (list) showed protection against SMA (average OR 0.88; 95% CI 0.35-0.97; *P<0.05*). Taken together, these results demonstrate that promoter variation in *IL-10* and *TNF*-α are important in conditioning susceptibility to SMA.

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SCHOOL-BASED TYPHOID VACCINATION PROGRAM IN KARACHI: POLICY IMPLICATIONS FOR TYPHOID CONTROL

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Population-based surveillance conducted from 2002-2003 as part of the Diseases of the Most Impoverished (DOMI) Program indicated high burden of typhoid fever in the slums of Karachi-Pakistan. Incidence of typhoid fever was 573 per 100,000 persons per year in children aged 2-4 years and 423 per 100,000 in children aged 5-15 years. Adding to the available evidence providence for information for decision making, all parties involved in the typhoid control including International Vaccine Institute, Aga Khan University and Trust for Vaccines & Immunization as part of Vi-Vaccines for Asia (ViVA) Initiative piloted a program of Vi-Vaccine in schools and madrasahs of two Towns of Karachi. The goal of the program was to introduce typhoid Vi-polysaccharide vaccine through school-based campaigns and demonstrate to policymakers that a school-based typhoid vaccination campaign is feasible and desired by the population. Prior to vaccination campaigns, social mobilization activities were conducted to create awareness of typhoid fever and its prevention. The main focus of social mobilization was to build and enhance awareness of the risks, signs, symptoms and preventive measures of typhoid fever. Schools were provided with multiple informational tools on typhoid fever and vaccination campaign. In order to ensure quality, clarity and relevance of messages, formative research was also conducted prior to social mobilization activities. Vaccination campaigns were conducted according to international guidelines. Approximately 120,000 school and madrasah going children were given typhoid vaccine. AEFI cases were reported and documented among children who were vaccinated accounting for about 0.28% of children vaccinated. 30 % increase in vaccination coverage was found in Jamshed Town as compared to Gulshan Town as result of implementation of modified communication strategy. The initial phase of the project has sensitized the population for Typhoid prevention. The collaborative efforts of the use of typhoid vaccine in the schools of Karachi added to existing information on the safety and feasibility of injectable typhoid vaccine. A routine vaccination program in schools will increase the use of the vaccine for prevention of typhoid. Policy advocacy is need of hour for inclusion of Typhoid vaccine in routine EPI program.

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CHOLERA OUTBREAK IN BAKURA LOCAL GOVERNMENT AREA, ZAMFARA STATE-NIGERIA. SEPTEMBER-OCTOBER 2013

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On 22nd October 2013, 150 cases of watery diarrhea were reported to the Federal Ministry of health from Bakura, Zamfara state. We investigated to identify the causative organism, infection source, implement public health interventions and propose recommendations. We defined a case as any person aged 1 year or more with acute watery diarrhea with or without vomiting, living in Bakura between 22nd September to 22nd October 2013. Twelve stool specimens were sent for laboratory testing. Cases were selected using the case definition and confirmed using laboratory tests. A descriptive study and a case control study were conducted to identify the source of infection. We conducted additional investigations to assess the sanitation practices and water source in the area. We identified 274 cases that met the case definition among the 100,125 residents of Bakura (Overall attack rate: 0.3%). There were 4 deaths (Case fatality rate: 1.5%). Males were more affected than females (67.9% versus 32.1%). The first case-patient developed an illness during the third week of September, 2013. The median age of cases: 17 years (range: 2- 42 years), while median age of controls: 31 years (range: 5- 43 years). Compared with 28 controls, the 28 cases did not differ in terms of residential location and environmental exposure.Cases were more likely to use well as a source of drinking water (OR: 1.15; 95% CI: 0.35-3.76), pit latrine as a means of sewage disposal (OR: 67.50; 95% CI: 7.3-15.74), practice poor hand washing (OR:10.8; 95% CI: 2.52-50.62)and hence develop disease. Of the 12 stool specimens from case-patients tested for Vibrio cholerae ,12(100 %) were positive for serotypes 01 and 0139. 2(100%)Water samples from communal wells also yielded growth of Vibrio cholerae. The outbreak in Bakura was confirmed to be cholera infection. The plausible risk factors associated with cholera infection during the outbreak were: Poor hand washing practices, use of contaminated wells as water source and use of pit latrines. We provided health education using community health teams, drugs, intravenous fluid therapy, disinfection packs and treated water wells. We recommended continued health education by health workers for community members within the affected communities on the importance of person hygiene and environmental sanitation.Findings from the outbreak investigation were forwarded to the state and Federal ministry of health.

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GUT HOMING MUCOSAL RESPONSE TO ORAL VACCINES IN INFANTS: A COMPARISON BETWEEN UNDERNOURISHED AND WELL NOURISHED

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Oral polio vaccine (OPV) and rotavirus vaccines are less effective in children in the developing world. The reasons for this poor response to these oral vaccines are not well understood. In the present study we investigated the association of malnutrition with the mucosal response to Polio and Rotavirus vaccine in infants from an urban slum of Kolkata, India. An ELISPOT assay was utilized to measure Polio and Rotavirus vaccines induced immune response. The assay was used for simultaneous detection of mucosal derived gut homing IgA producing antibody secreting cells (ASCs) expressing mucosal integrin $\alpha4\beta7$ against Polio Virus 1, 2 and 3 and Rotavirus. Systemic responses to Poliovirus vaccines were also measured by positive selection of ASCs expressing HLA-DR and CD19 antigen. HLA-Dr and CD19 markers are expressed during maturation and development of B-cells. In infants 4 fold rise in gut mucosal ASC count were observed to Polio vaccine. The percentage of subject with 4 fold rise in ASC count to Polio type1, 2 and 3 were 50%, 30% and 50% respectively. In the case of Rotavirus vaccine the 4 fold rise in gut mucosal ASC count was observed in approximately 26% of subjects. Infants with moderate to severe malnutrition (HAZ<–2) compared to better-nourished infants had significantly lower polio type 1 (Number of Positive respondents in HAZ -2, P=0.01) and type 3 (Number of Positive respondents in HAZ -2, P=0.03) ASCs response. However, in case of polio type 2 (Number of Positive respondents in HAZ -2, P=0.25) and rotavirus vaccine (Number of Positive respondents in HAZ <-2versus HAZ> -2, P=0.47), no association of vaccine response and malnutrition was observed. The ELISPOT data suggested a poor mucosal response to oral vaccines in infants. The mucosal immune response to Polio vaccine was affected by malnutrition. However, there was no effect of malnutrition on Rotavirus vaccination in this population at the measured time points. This study has also demonstrated the role of ELISPOT in measuring mucosal response to oral vaccines. However, this data needs to be correlated with other vaccine measures to determine whether these blood ASC responses could provide proxy markers of the mucosal immune response to oral vaccine.

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DIARRHEAGENIC *ESCHERICHIA COLI* IN MOTHER-CHILD PAIRS IN ILE-IFE, SOUTHWESTERN NIGERIA

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Diarrhoeagenic *Escherichia coli* are among the most common bacterial causes of morbidity and mortality particularly in young children. These pathogens are not sought routinely and capacity in detection is still grossly limited in developing countries. We investigated the distribution and dissemination of diarrhoeagenic *E. coli* (DEC) in children paired with their mothers in a Nigerian community. A total of 252 stool samples obtained from 126 children with diarrhea paired with their mothers at a state hospital in Ile-Ife, Nigeria were screened for DEC by multiplex polymerase chain reaction. DEC were identified in specimens from 35.7 % of individuals. The most common DEC pathotype in children with diarrhoea as well as their mothers was shiga toxin producing *E. coli* (STEC) (42, 16.7%). Identical pathotypes were found in 13 (10.3%) of the mother-child pairs. These consisted of enterotoxigenic *E. coli* (ETEC) (5, 4.0%), enteroaggregative *E. coli* (EAEC) (5, 4.0%) and STEC (3, 2.4%). The DEC isolates were commonly resistant to ampicillin (121, 96.8%), sulphonamide (118, 94.4%), tetracycline (119, 95.2%), streptomycin (115, 92.2%) and trimethoprim (113, 90.4%), but less commonly resistant to ciprofloxacin (9, 7.2%). This study suggests that healthy mothers may be asymptomatic reservoirs of multiply resistant strains of *E. coli* that are pathogenic in their children.

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POPULATION-BASED INCIDENCE OF ROTAVIRUS AMONG CHILDREN AGED < 5 YEARS IN THE CATCHMENT AREAS OF TWO DIARRHEA SURVEILLANCE HOSPITALS IN DHAKA, BANGLADESH

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Rotavirus is the most common cause of severe diarrhea in children worldwide. It is a vaccine-preventable disease. However, introduction of a vaccine in a resource constrained country like Bangladesh depends on its cost-effectiveness which, in turn, depends on incidence. icddr,b conducts diarrhea surveillance in its Dhaka and Mirpur hospital, but hospital-based incidence underestimates the real burden because many patients seek treatment elsewhere. We estimated the incidence of rotavirus among children aged < 5 years in the catchment area of Dhaka and Mirpur hospital by adjusting the hospital-based incidence by the proportion of severe diarrheal children in the hospital catchment areas who were admitted to surveillance hospitals. In Dhaka Hospital every 50th admitted patient and in Mirpur Hospital every 10th admitted patient was enrolled in surveillance and tested for rotavirus using a stool enzyme immunoassay. We extrapolated the total number of rotavirus cases in surveillance hospitals among all children admitted from the hospital catchment areas. We defined the catchment areas of the surveillance hospitals as those neighborhoods where more than two-thirds of admitted patients resided. To estimate the proportion of severe diarrhea cases among children aged < 5 years in the hospital catchment area who were admitted to surveillance hospitals, we conducted a house-to-house survey in randomly selected areas. We considered diarrhea in the preceding 12 months to be severe if a child with diarrhea was admitted to a healthcare facility, or received intravenous rehydration, or died following frequent loose or watery stools. Among the surveillance enrolled children aged < 5 years from January 2011 through December 2013, 1,247 (42%) in Dhaka Hospital and 626 (33%) in Mirpur Hospital were positive for rotavirus. According to survey in hospital catchment areas, the proportion of severe diarrheal children who were admitted to surveillance hospitals was 0.69 (95% CI: 0.62-0.75) in Dhaka Hospital and 0.77 (0.70-0.83) in Mirpur Hospital catchment areas. The population-based incidence of rotavirus among children aged < 5 years was estimated to be 28 (95% CI: 25-31) in Dhaka Hospital and 38 (95% CI: 35-42) in Mirpur Hospital catchment areas per 1,000 population. This study provides a credible estimate of rotavirus incidence in Dhaka, which can be used to assess the cost effectiveness of rotavirus prevention activities including vaccination.

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DETECTION AND CHARACTERIZATION OF ESBL AND PLASMIDIC AMPC BETALACTAMASES IN *SALMONELLA* SPP. FOODBORNE ISOLATES FROM LIMA, PERU

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The role of food as carrier of pathogens is a major public health problem being *Salmonella enterica* infections, mostly isolated in chickens or derived products, the leading cause of foodborne human infections in many countries. The problem is exacerbated by the increasing levels of antibacterial resistance, including resistance to cephalosporins by the presence of extended-spectrum beta-lactamases (ESBL) and AmpC beta-lactamases. The aim of this study was to analyse the presence of ESBL and AmpC, the general susceptibility and the mechanisms of resistance to beta-lactams in *Salmonella* spp. isolated from meat samples from Peru. Twenty-one strains of *Salmonella* spp. isolated from meat from 5 markets of Lima were serotyped by microarray. Antimicrobial susceptibility was tested by disk diffusion. The presence of ESBL was determined by double synergy test and the presence of AmpC was assessed by disk antagonism. The presence of blaSHV, blaTEM, blaCARB, blaOXA1-like, blaOXA2-3-like, blaOXA5-7-like, blaCTX-M, blaCTX-M-9-group and class I integron was analysed by PCR and sequencing. The serotyping resulted in 17 *S. enterica* serovar Infantis, 2 *S. enterica* serovar Enteritidis, 1 *S. enterica* serovar Kentucky and 1 *S. enterica* serovar Anatum. The isolates were completely or highly resistant to rifampicin, ampicillin, cotrimoxazole, amoxicillin and nalidixic acid amongst others. The ESBL phenotype was found in 3 isolates, identified as blaCTX-M-65. The presence of AmpC beta-lactamases was confirmed in 7 isolates. Class I integron carrying the aadA1 gene was found in 18 isolates, conferring resistance to streptomycin. The most prevalent serotype found was *S.* Infantis. The presence of these mechanisms, and the multiresistance shown, involves a high risk of transmission to humans since it has already been reported outbreaks caused by *S.* Infantis ESBL-producers. Moreover it is necessary to further characterize the AmpC beta-lactamases found. All this shows the high importance of adequate sanitary control in animal production to prevent foodborne human infections and complications derived from antimicrobial resistance.

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INCIDENCE AND SEVERITY OF TRAVELERS' DIARRHEA AMONG STUDENTS AT A SPANISH LANGUAGE SCHOOL IN CUSCO, PERU

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Travelers' diarrhea (TD) is the most common illness among travelers to low and middle income countries, yet there are few prospective incidence studies evaluating the full spectrum of disease. To evaluate the incidence and causes of TD, we conducted a prospective cohort study among travelers in Cuzco, Peru from June 2012 to January 2014. Participants provided 1 stool sample at enrollment, then weekly, and when sick with diarrhea. Two definitions of diarrhea were used: 1) the WHO definition of 3 or more loose stools in a 24 hour period and 2) any self-reported diarrhea by participants. Subjects reporting diarrhea were evaluated daily to record signs and symptoms. In total, 249 participants were enrolled accounting for 663 observed patient-weeks. Participants were 65% female with a mean age of 28.1 years (SD 11.4 years) and represented 22 different countries principally from North America and Europe. Of the 711 samples collected, 168 (24%) represent diarrheal episodes and 543 (76%) represent asymptomatic baseline or follow-up samples. We recorded 174 episodes of diarrhea representing 131 (53%) participants and an incidence rate of 1.14 episodes per patient per month, or a 26% chance of having diarrhea each week while in Cusco. Seventy two percent of diarrhea episodes met the WHO definition, however 28% still self-reported problems with diarrhea. Those meeting the WHO definition lasted on average, 1.18 days longer (95% CI 0.53-1.82) than those not meeting the definition. However, subjects in both groups lost a workday (45% WHO; 37% non-WHO) and experienced abdominal pain (6% WHO; 8% non-WHO), excessive thirst (43% WHO; 40% non-WHO), decreased urination (18% WHO; 15% non-WHO) and dark urine (19% WHO; 20% non-WHO) each observation day. Non-WHO cases were more likely to report nausea (OR: 2.54; 95% CI: 1.29, 5.02) and bloating (OR: 2.02; 95% CI: 1.03, 3.96) per observation day. In conclusion, the WHO definition misses ¼ of TD episodes which still have similar symptoms and impact among travelers. A better diarrhea definition for travelers should be considered.

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DIVERSITY OF ANTIBIOTIC RESISTANCE GENES AND STAPHYLOCOCCAL CASSETTE CHROMOSOME *MEC* ELEMENTS IN FAECAL ISOLATES OF COAGULASE-NEGATIVE STAPHYLOCOCCI FROM NIGERIA

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Coagulase-negative staphylococci (CoNS) are opportunistic pathogens found as colonisers of the human gut. This study was carried out to examine the genetic drug resistance mechanisms in faecal isolates of CoNS. The study investigated 53 non-duplicate CoNS isolates obtained from the fresh stool samples of apparently health subjects in the community of Ile-Ife, South-Western Nigeria. Phenotypic antibiotic susceptibility testing was assessed by the disc diffusion test while antibiotic resistance genes were analysed by PCR. Isolates with *mecA* were subjected to Staphylococcal Chromosome Cassette *mec* and cassette chromosome recombinase *ccr* complex type determinations. Resistance genes were detected only in isolates that showed resistance by phenotypic screening. The *aac(6')-aph(2")* gene was detected in all the three isolates resistant to gentamicin. Four out of five erythromycin resistant isolates were positive to *ermC,* the remaining carried the *msrA*. The *tetK* gene was detected in 6 of the 7 tetracycline resistant isolates while 4 possessed the *tetM* gene. Three of the isolates (*S. haemolyticus*, *S. xylosus* and *S. capitis*) had both genes. SCC*mec* types found were: SCC*mec* I- *ccrAB*β2-α2 (4 isolates: 3 *S. epidermidis*, 1 *S. warneri*), SCC*mec*IVb- *ccrAB*β2-α3 (1 isolate: *S. epidermidis*), SCC*mec*IVd- *ccrAB*β2-α3 (8 isolates: 3 *S. epidermidis*, 2 *S. xylosus*, 1 *S. saprophyticus*, 1 *S. warneri*, 1 *S. capitis*), and untypable (2 isolates: *S. epidermidis*). This genetic background could be a reservoir for interspecies gene transfer among CoNS and *S. aureus* in the intestinal tract.

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PERSISTENT TYPHOID FEVER EPIDEMICS IN INTERNALLY DISPLACED PERSONS CAMPS IN UGANDA

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Typhoid fever has continued to cause recurrent epidemics in some districts in Uganda from 2009 to date. According to the epidemiological data from the Ugandan Ministry of Health (MOH) from 2009 to 2013, there has been an increase in the number reported cases of typhoid fever particularly from districts with people living in internally displaced peoples camps (IDPs) where some patients are even admitted with complications such as intestinal perforations. The factors leading to this increase have not yet been established and worse still, is the fact that the reported typhoid fever cases were diagnosed using the Widal test which is non-specific

and may lead to over diagnosis of the disease. The study therefore was to attempt to establish factors leading to the increase of typhoid fever cases and to confirm all such reported cases with culture methods. The specific objectives were to study the socio-demographic profiles of the residents of the four most affected districts, their environmental sanitation situations as well as the water sources used. Both Widal and culture methods were used to establish the typhoid fever causative organisms, establish the carrier status of recovered cases, and establish antibiotic sensitivity of the salmonella organisms isolated. A survey was conducted to study the situations of residents of four camps in the four districts. Follow-up interviews and Focus group discussions were carried out. Observations of the homesteads and latrines for sanitation and hygiene were also conducted. Water samples from sources and households were analysed for faecal contamination. Similarly blood samples obtained from admitted patients and previously confirmed cases who had recovered from typhoid fever were cultured and the isolated salmonella typhi organisms, tested for antibiotic sensitivity. The main findings why typhoid fever is recurrent in four camps in the four districts included: poor hygienic practices, poor sanitation. Low latrine coverage (40%), unsafe water sources, contaminated with faeces (both *E. coli* and *Salmonella typhi* organisms were isolated. *Salmonella typhi* was isolated from 27/81(33%) patients by blood culture, while 58/81(71.6%) were positive with the Widal test. 76% of the cultured *S.typhi* organisms were resistant to amp, sulf, tetr and cotri. which are first line drugs in Uganda, but susceptible to chloral, na. and cipro. carrier status was 36%.

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SPATIOTEMPORAL TRENDS AND IMPORTANT LOCAL INFLUENCES ON HOSPITALIZED PEDIATRIC DIARRHEA IN HO CHI MINH CITY, VIETNAM

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Diarrheal disease in Ho Chi Minh City (HCMC), Vietnam remains a significant cause of morbidity in young children. Distinct seasonal patterns are present in diarrheal disease reporting to hospital in this setting, with large peaks occurring in the colder and drier months. The reasons for these trends are unclear. In order to explain these observed patterns, we analyzed a set of hospitalized pediatric diarrheal admissions from three large hospitals in HCMC from 2005-2010. We developed a mixed effects model to explore important influences in reported diarrhea, examining time series trends and associations with a variety of environmental covariates including flood level, temperature and rainfall. Additionally, we used Empirical Bayesian Kriging to map smoothed rates of reported diarrhea stratified by season and age-group to investigate the spatiotemporal distribution of reported diarrhea cases across HCMC. Our results highlight the continuing burden of pediatric diarrheal disease in this setting: of ~480,000 hospital admissions, gastroenteritis was the second most common reason for hospitalization in children under 16, accounting for 12% of all admissions. From our mixed effects model, we found a strong epidemic component that was spatially synchronized across HCMC, suggesting citywide epidemics, probably due to viral pathogens. We speculate that these patterns are due to the entrance of new seasonal variants or related to the seasonal birthrate across the city. We additionally identified a strong positive association with temperature, a relationship which strengthened significantly with distance from the city center. Furthermore, an evaluation of pairwise correlations between the time series of the 24 districts of HCMC and the distance between district centroids identified a spatial dependence radius of only 5km, suggesting the presence of both city-wide and local effects. Finally, visualization of smoothed rates of reported diarrhea highlight substantial differences in diarrheal hotspots by season and age, suggesting varying pathogens are present at different times in different areas amongst different ages. Although several crucial limitations are present in such a dataset, the results still provide insight for public health practitioners and clinicians working in HCMC, Vietnam and may also reflect trends present in other industrializing, densely populated settings.

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HOST GENE POLYMORPHISMS AND SUSCEPTIBILITY TO BACTERIAL ENTEROPATHOGENS IN CHILDREN LIVING IN GAMBIA AND KENYA

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The Global Enteric Multicenter Study (GEMS) is a prospective collaborative study to determine the pathogen specific, diarrhea associated attributable disease burden of diarrhea in Africa and Asia in children less than 5 years of age. Using samples from children that participated in this case-control study and were seen at in the GEMS clinics in The Gambia and Kenya, we conducted a host gene candidate study that examined the association of the host DNA single nucleotide polymorphisms (SNPs) and specific bacterial enteropathogens causing diarrhea. Cases of diarrhea were matched with healthy controls from the same area. Stool was collected and examined comprehensively for the presence of enteropathogens and host DNA polymorphisms. We studied de-identified fecal DNA for the presence of SNPs (N=144) in 26 genes that code for host proteins involved in pathogen attachment, inflammation, innate and acquired immune responses to enteropathogens. We analyzed the distribution of host genotypes and compared cases vs. controls according to enteropathogens identified. Comparisons were made using SNPSTATs software following an additive model. A mixed logistic regression model was used to adjust for potential confounding factors including site, age, weight for height and weight for age scores. Microbiological and genotype data were available in 1,164 subjects. Diarrhea was associated with 6 SNPs located in *SELPLG, CD55, LPLUNC, IL12 B*, and *CORO1C*. In The Gambia, diarrhea due to *Shigella* was associated with a SNP in *SELPLG*, diarrhea from enterotoxigenic *E. coli* was associated with SNPs in *DAF (CD55), LPLUNC, SELPLG*, diarrhea from enteroaggregative *E. coli* was associated with SNPs in CORO1C. In Kenya, diarrhea from enteroaggregative *E.coli* was associated with SNPs in *IL12B* and *CORO1C* Distinct SNPs were associated with pathogen specific diarrhea in children under 5 years of age living in two African countries.

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GUT MICROBIAL SUCCESSION AFTER ENTEROTOXIGENIC *ESCHERICHIA COLI* INFECTION IN BANGLADESH

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Enterotoxigenic *Escherichia coli* (ETEC) is a common cause of bacterial enteritis in the developing world. Recent human and animal studies

suggest that alterations in commensal flora influence recovery from enteric infections. We examined serial rectal swabs from patients after ETEC infection to observe microbial succession during the recovery period. DNA was extracted from rectal swabs and analyzed using 16S ribosomal RNA gene sequencing. Over a three-month follow-up period, 75 samples were analyzed from 18 individuals in Dhaka, Bangladesh. To simplify the analysis of these complex communities, we investigated bacterial dynamics at the genus level. The bacterial family including E. coli species was most dominant on the day of patient enrollment when ETEC stool culture was positive. The day after antibiotic treatment for ETEC, bacteria including skin flora such as Gram positive organisms were most dominant, with a decrease in bacteria from the family including E. coli species. Compared to day 10 of follow up, the skin flora group was predominant on Day 2 (Day 2, 7/16 44% versus Day 10, 0/17, P=0.003 by Fisher's exact test). On day 10 of follow up, anaerobic flora such as Prevotella species and other common enteric anaerobes were predominant (Day 10 12/17 71%, Day 2 4/16 25%, P=0.011 by Fisher's exact test). The predominant bacterial populations on Day 10 and Day 30 were similar to gut microbiome studies in healthy controls in the developing world. The gut microbiome following ETEC infection rapidly recovers to baseline after a dramatic perturbation and progression through distinct phases of recovery. Understanding the pattern of microbial succession in recovery from enteric disease is a foundation for studying factors of susceptibility and protection from disease and could potentially lead to interventions to hasten recovery from infection.

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MEMORY B CELL RESPONSES TO *VIBRIO CHOLERAE* 01 O-SPECIFIC POLYSACCHARIDE (OSP) IN PATIENTS WITH CHOLERA IN DHAKA, BANGLADESH

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Cholera is a severe, dehydrating diarrheal illness that in the last two decades has been caused largely by *Vibrio cholerae* O1. There is a growing body of evidence that protective immunity against cholera targets lipopolysaccharide (LPS), especially the O-specific polysaccharide (OSP) component of LPS. Immunity following wild-type cholera persists for at least 3 to 10 years, even in young children. Immunity following oral cholera vaccination is of lesser magnitude and shorter duration, especially in young children under the age of five years. Following wild-type cholera, protection against cholera persists despite decreases in effector immune responses, suggesting that memory responses may play a critical role in mediating long-term protection against cholera. We took advantage of the recently described ability to purify *V. cholerae* O1 OSP, and assessed memory B cell responses targeting OSP in patients recovering from wild type cholera in Bangladesh. The Ogawa serotype was the primary circulating serotype at the time of this study, and we assessed immune responses out to 6 months after presentation with illness. We found that serum OSP IgA and IgG responses were evident within 7 days of presentation, and that these values decreased toward baseline over the follow-up period. We found that IgA and IgG memory B cell responses targeting OSP were also present within 30 days of presentation, and persisted through the follow-up period. When we analyzed the data by age of patients, we found IgG and IgA memory responses targeting OSP were detectable in adults (>18 years of age, n=15), older children (6-17 years of age, n=20) and children (2-5 years of age, n=11). Our results suggest that memory B cell responses following wild-type cholera are induced, that they persist in time, and that these responses are comparable among adults, older children, and younger children in this cholera endemic area. These results suggest that memory B cell responses targeting OSP could play a role in mediating long-term immunity against cholera.

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ASSESSING SERUM LEPTIN LEVELS IN CHILDREN HOSPITALIZED WITH CHOLERA IN BANGLADESH

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Cholera is a severe watery diarrhea caused by the noninvasive gram-negative bacterium *Vibrio cholerae*. Leptin is a critical hormone in human metabolism that plays a role in developing adequate immune responses. Malnourished children with low leptin levels have reduced T cell responses. In order to evaluate the potential role of leptin during cholera, we measured leptin levels on day 2, 7 and 30 in cholera patients 5 years of age and younger at the International Centre for Diarrhoeal Disease Research in Dhaka, Bangladesh. We compared these levels with respect to age, gender and nutritionally-matched healthy controls, as well as day 180 levels within the cholera patients themselves. We found that patients at the acute stage of cholera had significantly lower serum leptin levels than matched healthy controls, and that these acute-phase leptin levels rose through convalescence. We also evaluated the association between leptin on day 2 and immunological responses that developed at later time points to cholera toxin B subunit (CtxB), a T cell-dependent antigen, and *V. cholerae* lipopolysaccharide (LPS), a T cell-independent antigen. We found a significant association of acute phase leptin levels to immune responses to CtxB. Our results suggest that leptin may play a role during cholera, especially in maturation of immune responses to T cell-dependent antigens. Our results also suggest that malnourished children that have low leptin levels may be impaired in their ability to develop such responses.

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CAMPYLOBACTER JEJUNI CAPSULAR TYPE DISTRIBUTION IN THE AMAZON AND COASTAL REGIONS IN PERU

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The enteric pathogen *Campylobacter jejuni* constitutes one of the major causatives agents of diarrhea worldwide. *C. jejuni* expresses 47 different capsule polysaccharides (CPS) and constitutes a major factor in *C. jejuni*-mediated pathogenesis. Recent published findings demonstrate a CPS-mutant diphtheria toxin conjugate vaccine as protective against CPS-homologous *C. jejuni* infection in an *Aotus nancymaae* non-human primate diarrheal model. World-wide surveillance describing CPS type distribution is necessity to determine regional vaccine efficacy of CPS-specific vaccines against *C. jejuni*. To this end, two regionally distinct pediatric prospective community based studies were considered to evaluate CPS distribution in Peru. Santa Clara, a rural community located in the Peruvian Amazon was compared to Pampas de San Juan, a shanty town located in the peripheral area of Lima. Both populations were analyzed by a multiplex PCR capable to detect the 47 CPS types of *C. jejuni*. Santa Clara study included 131 symptomatic and asymptomatic individuals of which 201 *Campylobacter jejuni* isolates were acquired from stool samples. The Pampas de San Juan study included 95 samples obtained from 51 symptomatic and asymptomatic children. Only 7% of the isolates from Santa Clara were untypable by this technique. The most prevalent CPS types detected from Santa Clara were HS8/HS17 (15%), HS15 (13%) and HS3 complex (12%). In Pampas de San Juan, a higher percentage of isolates, 25%, were untypable by this new technique while HS15 (9%), HS4 complex A (7%) and HS41 (6%) were the most

prevalent CPS types. In addition, tracking of CPS types in individuals over time demonstrate different CPS types present from *C. jejuni* re-infection suggesting a possible acquired immunity to distinct CPS. The results herein describe differential CPS type distribution in two distinct regions of Peru, with HS8/HS17 (15%) and HS15 (9%) being the most prevalent in Santa Clara and Pampas de San Juan, respectively.

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A HIGH PREVALENCE OF HYDATIDOSIS IN SLAUGHTERED GOATS AT A SOUTHWESTERN ABATTOIR, NIGERIA: IMPLICATIONS ON LIVESTOCK PRODUCTION AND HUMAN HEALTH

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Hydatid disease is an important zoonosis globally particularly in developing countries like Nigeria where backyard slaughter is rampart and carcass wastes are easily accessible to scavenging dogs. Adult goats raised on extensive system of management slaughtered at a tropical abattoir in south-western Nigeria were inspected for the presence of hydatid cysts. In all, a total of 2,140 goats were slaughtered from which 214 were randomly selected and inspected over the study period. The results show a prevalence of 53.27% with 50.88%, 38.6%, 6.14% and 4.39%, respectively located in the lungs, liver, heart and intestines. In addition, 33.33% of the hydatid cysts were fertile with a significantly higher proportion (51.72%) from the lungs. Our findings reveal a high prevalence of hydatidosis in slaughtered goats in south-western Nigeria and this portends serious economic implications on livestock production through reduced productivity and even death. The prevalence of fertile cysts obtained in this study suggests a high potential for human infection with hydatidosis through contacts with exposed dogs or consuming water contaminated with infected dogs faeces. The current results suggest that a thorough investigation that leads to a disease control strategy is required to reduce the economic and public health consequences of hydatidosis.

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KAP (KNOWLEDGE, ATTITUDE AND PRACTICE) OF ECHINOCOCCOSIS - FIRST REPORT FROM CENTRAL SUDAN

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In Sudan, Echinoccosis is a chronic neglected disease caused by *Echinoccocus granulosus* (EC). It affects animals and humans can be accidentally infected through direct contact with dogs or ingestion of contaminated water or food. Studies have shown infection rate as follows: Dogs 50-70% , camels 35% , sheep,goats and cattle10-11%,where as humans in central Sudan 0.3-1.0%. Hydatid cyst in humans might lead to serious complications if left untreated. Treatment is expensive and sometime is cumbersome where advanced surgery can be needed. The study area is known by its large livestock with camels prepondance and where previous surveys in dogs, camels and human has been performed. Screening in humans has led to successful medical treatment only, using portable ultrasound in all asymptomatic cases. In Sudan regarding Human EC genotyping, G6 (camel strain) is the cause for almost all human samples collected. Therefore, the objective of this study is to explore knowledge, attitude and practice (KAP) of people living in three villages in Tambool area, central of Sudan. A cross sectional study using close ended structured questionnaire covering the thematic areas of Hydatid KAP was administered to 312 household (full coverage). The data was entered and analyzed by SPSS. Results showed that participants mean age was 37.5 ±16.3 years. Substantial number of the respondents (73.7%) never heard about the disease. Only 26% heard about it, however they reflect low knowledge about cause, mode of transmission, prevention and control of Echinococcosis. In terms of attitude, half of the participants who heard about the disease believe Hydatid cyst patients should be isolated. This might indicate potential stigma. Participants practice showed some behavioral risk as 81.7% slaughter animals inside home without veterinary supervision and 70 % throw the offal outside home where stray dogs eat it. In conclusion, the study mapped the baseline for KAP of the community of the study area. It revealed that the population had poor knowledge and attitude. Their practices might help circulating the parasite in the area. Consequently health education is highly needed to prevent and control the parasite and disease.

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OVALBUMIN-INDUCED AIRWAY INFLAMMATION IS REDUCED BY *ECHINOCOCCUS GRANULOSUS* INFECTION WHICH DOWN-REGULATED IL17 IN MICE

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Cystic echinococcosis (CE) is a cosmopolitan zoonosis caused by the larval stage of the dog tapeworm *Echinococcus granulosus*. The infection induces a polarized T-helper type 2 (Th2) response in its intermediate hosts. Here, we examined the effects of *E. granulosus* infection on mouse ovalbumin (OVA)-induced asthma. BALB/c mice were intraperitoneally transplanted with 50 small Eg cysts cultured *in vitro* 3 months prior to being sensitized and challenged with OVA. The mice with cysts transplanted harbored an average of 34.8 cysts per mouse, with a mean diameter of 4.4 mm. Histological staining of lung tissues showed that *E. granulosus* infection significantly reduced the severity of OVA-induced airway inflammation including reduction of eosinophil cell accumulation and mucus production. Airway hyperresponsiveness of OVA-challenged mice after *E. granulosus* infection was significantly suppressed as compared to the OVA-only challenged mice. The infection significantly increased both Th1 and Th2 cytokine expression in PBMC and also in lung tissue, but significantly down-regulated IL-17a expression. When the mice were challenged with OVA, the expressions of IFN-γ, IL-2, IL-4 and IL-5 in lung were down-regulated,whereas, IL-2 and IFN-γ were expressed in high level. In conclusion, *E. granulosus* infection markedly reduced the severity of OVA-induced airway inflammation. Down-regulation of Th17 responses in lung tissue may be a potential therapeutic treatment against allergic asthma.

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RIEC: THE FIRST REGISTRY OF CYSTIC ECHINOCOCCOSIS, FROM ITALIAN TO EUROPEAN

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Cystic Echinococcosis (CE) is endemic in Eastern and Southern Europe, Italy included. However, its real burden is largely unknown due to the lack of efficient reporting systems designed to take into account the peculiar features of this disease. In Italy, only a yearly summary of regional data is required by the health authorities and the prevalence and incidence of human CE are hugely underestimated because only hospitalized cases are registered, while the majority of CE cases are diagnosed and managed on an outpatient basis. Furthermore, no official data are transmitted to European authorities. The neglect of CE also results in general lack of knowledge on its diagnosis and clinical management outside referral centres, with consequent heterogeneity in clinical practices and often unnecessary procedures with associated risks and costs. In 2012 the Istituto Superiore di Sanità (ISS - Italian National Health Institute - Rome) in collaboration with the University of Pavia, WHO Collaborative Centre for the Clinical management of Cystic Echinococcosis, implemented the Italian Registry of Cystic Echinococcosis (RIEC). This is a prospective multicenter registry of CE patients visited from January 2012 in Italian health centres that adhered voluntarily. RIEC is accessible on the website of ISS since October 2012 with the aims of: indicating the burden of CE in Italy; bringing the importance of this infection to the attention of health authorities; encouraging public health policies geared toward its control; stimulating research on CE. Moreover, it provides an useful tool for patients follow-up and evaluation of therapeutic interventions. As of February 2014, 346 patients were enrolled in 11 centres, figures largely outnumbering the national reports of many endemic European countries. We will discuss updated results and challenges of RIEC, that is the template for the European Registry of CE, to be implemented within the FP7-HERACLES project.

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IDENTIFICATION OF FUNCTIONAL MITOGEN-ACTIVATED PROTEIN KINASE KINASES (MAPKK) HOMOLOGUES FROM *ECHINOCOCCUS GRANULOSUS*: PROTOSCOLICIDAL ACTIVITY OF MAPK SIGNALING PATHWAY INHIBITORS

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Cystic echinococcosis (CE) treatment urgently needs a new drug. Understanding molecular regulation is essential for identification of drug targets. In the present study, we identified two new members of the MAPKKs, MEK3/6 and MEK1/2 homologues (termed as EgMKK1 and EgMKK2, respectively) from *Echinococcus granulosus*, the causative agent from CE. Both EgMKK1 and EgMKK2 were co-expressed in the larval stages. EgMKK1 encoded protein was localized in the subtegumental and tegumental layer and EgMKK2 was localized in the tegumental and sucker of the parasite. As shown by yeast two-hybrid and Co-immunoprecipitation analysis, EgMKK1 strongly interacted with the p38-like MAP kinase Egp38. EgMKK2, on the other hand, not only interacted with a member of the parasite's 14-3-3 protein family, but also with the Erk-like MAP kinase EgERK. Recombinant EgMKK1 and EgMKK2 displayed kinase activity towards myelin basic protein substrate. When Sorafenib Tosylate (Bay 43-9006), an inhibitor of MAPKKK (Raf-1/B-Raf), and U0126 and PD184352, inhibitors of MAPKK (MEK1/2), were added to the medium for *in vitro* cultivation of *E. granulosus* protoscoleces, these inhibitors resulted in a marked dephosphorylation of EgERK for 20 and 30 days, then had actually an inhibitory and cytolytic effect on the larval stage of the parasite. In addition, Sorafenib Tosylate killed protoscoleces more effectively. *In vitro* culture of protoscoleces in the presence of 25 μM Sorafenib Tosylate or 100 μM U0126 for a period of 30 days was parasiticidal, as determined by murine bioassays, while treatment with 100 μM PD184352 was not. Our study indicates that small inhibitors of MAPK signaling pathway may be potential drugs for the treatment of CE.

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TRANSCRIPTIONAL PROFILES OF PROTOSCOLECES OF *ECHINOCOCCUS GRANULOSUS* IN RESPONSE TO TGF-Β REVEAL INCREASED EXPRESSION OF GENES INVOLVED IN GROWTH OR DEVELOPMENT

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TGF-β is a crucial cytokine participate in the interplay between the intermediate host and helminthes. TGF-β receptors were discovered in many cestode, and could bind the human TGF-β. However, the function of host TGF-β on the *Echinococcus* is still not elucidated, and this paper aim to explore the question at transcription level. Microarray analysis was used to investigate differential expression genes in protoscolices of *Echinococcus granulosus* cultured in the presence or absence of human TGF-β at different time points (4h, 8h and 24h) *in vitro*. A total of 523 genes were up- or down-regulated in response to TGF-β, compared with control group, 390 genes were up-regulated and 47 genes were down-regulated at 8h, and 376 genes were up-regulated and 19 genes were down-regulated at 12h, including 310 differential genes were regulated at both time point. Gene ontology (GO) analysis showed that the biological process of the up-regulated genes in protoscolex were predominantly involved in DNA packaging, nucleosome assembly, chromatin assembly, etc. And the cellular component gene were located in cell nucleus. TGF-β appeared to promote growth or development of the protoscolex by up-regulated the gene related with mitosis. In addition, the study also indicated that TGF-β has a multiple influence on the protoscolex, as reflected in the increased stimulation of gene expression of the ErbB signaling pathway, MAPK signaling pathway, Notch signaling pathway and VEGF signaling pathway.

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IDENTIFICATION OF FUNCTIONAL SMAD8 AND SMAD4 HOMOLOGUES FROM *ECHINOCOCCUS GRANULOSUS*: PROTOSCOLICIDAL ACTIVITY OF TGF-Β/BMP SIGNALING PATHWAY INHIBITORS

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Cystic echinococcosis (CE) treatment urgently needs a new drug. Understanding molecular regulation is essential for identification of drug targets. In the present study, we identified two new members of the Smad proteins, Smad8 and Smad4 homologues (termed as EgSmad8 and EgSmad4, respectively) from *Echinococcus granulosus*, the causative

agent from CE. Both EgSmad8 and EgSmad4 were co-expressed in the larval stages and their encoded proteins were localized in the subtegumental and tegumental layer of the parasite. As shown by yeast two-hybrid and pull-down analysis, EgSmad8 displayed a positive binding interaction with EgSmad4 and a previously identified EgTβR I. In addition, EgSmad8 localized in the nuclei of Mv1Lu cells (mink lung epithelial cells) upon treatment with human TGF-β1 or human BMP2, indicating that translocation of EgSmad8 into nuclei depends upon signals initiated by the ligands of human origin. When LDN193189, an inhibitor of TGF-β/BMP type receptors, and SIS3, an inhibitor of R-Smads, were added to the medium for *in vitro* cultivation of *E. granulosus* protoscoleces, these inhibitors had actually an inhibitory and cytolytic effect on the larval stage of the parasite. Our study indicates that small inhibitors of TGF/BMP signaling pathway may be potential drugs for the treatment of CE.

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NEW ULTRASONOGRAPHIC CLASSIFICATION OF HEPATIC ALVEOLAR ECHINOCOCCOSIS

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Ultrasonography provides one of the most important diagnostic tools in suspected alveolar echinococcosis. The aim of the study was to establish a new sonographic classification based on a large patient population with confirmed hepatic alveolar echinococcosis. In 225 patients, ultrasound morphology of liver lesions due to an alveolar echinococcosis was retrospectively examined. The findings were grouped into the new classification scheme. The following classification have been established: storm and hail pattern, pseudo hemangioma-like pattern, ossification pattern and metastasis-like pattern. The respective classification patterns are demonstrated by imaging examples. The proposed ultrasonographic classification improves the diagnosis of hepatic alveolar echinococcosis. This makes it possible to interpret different clinical courses better and helps in the context of scientific studies to improve the comparability of ultrasonographic findings.

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HYDATID DISEASE IN IRAQ: A RETROSPECTIVE STUDY OF 1,980 PATIENTS IN BAGHDAD

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Human hydatid disease caused by *Echinococcus granulosus* has been endemic in Iraq for many years. Migration of people and intermediate hosts over time can impact the distribution and epidemiology of this disease, especially in developing countries and in times of conflict. Observations made on the clinical services that care for hydatid patients in Baghdad posed the question of whether there is an age-related association with the location of cysts in various organs. Therefore to address this question we conducted a retrospective chart review of 1,980 patients with hydatid cyst admitted to three major hospitals in Baghdad, Iraq. In particular we noted the age at which the patient presented and the location of hydatid cysts diagnosed radiographically or surgically. The majority of patients with hydatid disease of the body were between 20 and 40 years old. In our series, we observed that abdominal hydatid was seen in 66.7%, liver (54%), spleen (3.2%), kidney (3.2%), peritoneum (3.2%), retroperitoneal (1.1%), pelvis (0.8%), and pancreas (0.3%). The chest was involved in 29%, lungs 28%, pleura 0.2%, pericardium 0.1%, mediastinum 0.4%, bone and spine 0.8%, soft tissue 3.1% and brain and orbit 0.5%. When we analyzed patients by age groups, we noted an apparent association of certain ages with the highest risks of particular organ involvement. Hydatid disease of the liver was most common in the 20-39 year age group, whereas lung involvement was most common in a slightly younger subset. Kidney cysts were most commonly observed in the 40-49 year age group. Other frequencies were noted for bone and spine (30-39 years). Brain and orbit disease was most common in the 5-19 year old age group. These observations suggest that several possible explanations for the majority of brain/orbit disease in young people. The age and/or immune status when hydatid disease is acquired by humans may impact the ultimate location of cysts in the body. These observations may lead to new insights into the pathogenesis, diagnosis and treatment of human hydatid disease.

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PREDICTION AND IDENTIFICATION OF ANTIGENIC EPITOPES IN EG95 OF *ECHINOCOCCUS GRANULOSUS*

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This study was undertaken 1) to predict the T and B union epitope of the Eg95 antigen of *Echinococcus granulosus* , 2) to obtain the different peptide fragment of T-B union epitope via Phage display technology, 3) toidentify and screen the peptide fragment via Western Blot and ELISA, and 4) to provide the basis for the T and B union epitope vaccine's manufacture of Echinococcosis. Online networks BCEpred and LEPS predicted the B cell epitope of Eg95 antigen. Using SYFPEITHI software predicted the MHC I nonamers T cell epitope of HLAA 0201 and H2-Db. To analyzes the secondary structure, transmembrane structure, B cell epitope, T cell epitope of humans and mice. Finally, determine the area of B cell and T cell union epitope.Using DNAman software design primers, amplified amino acid sequence of T-B cell union epitope,then restruct the gene and M13KE Phage carrier using T4 Ligase. Constructed M13KE/Eg95-1 M13KE/Eg95-2 and M13KE/Eg95-3 plasmid then transformed into E.coliER2738 respectively, identified the correct sequence using PCR. Purified the recombinant phage by PEG/NaCl way of precipitation. Identified the expression level of recombinant protein rPIII by SDS-PAGE electrophoresis analysis.Prepared the rEg95 patient serum and antiserum as antibody, confirmed the correctness of epitope peptide antigen by Western Blot. Finally,compared with epitope peptide antigen's reactivity by ELISA, screened and comfirmed better of antigen epitope peptide as candidate epitopes of epitope vaccine of Echinococcosis. Predicted the advantage Eg95 antigen epitope by line through using the bioinformatics method, and confirmed 3 T-B union epitope. To successfully cloned and constructed the prokaryotic expression plasmid M13KE/Eg95-1, M13KE/Eg95-2 and M13KE/Eg95-3, Purified the recombinant phage by PEG/NaCl way of precipitation. SDS-PAGE assay showed that Eg95-1,Eg95-2 and Eg95-3 protein III was successfully expressed. Echinococcosis patient and anti-rEg95 serum can identify the 3 T-B union epitope, but the signal intension was differences.

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HUMAN CYSTIC ECHINOCOCCOSIS IN SUDAN: A SILENT HEALTH THREAT

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Cystic echinococcosis (CE) is a zoonosis caused by cestodes of the genus Echinococcus. The adult tapeworms are intestinal parasites of dogs and some other carnivores which require a larval tissue stage (hydatid cyst) in other animal species (intermediate hosts). This larva usually grows in the form of a large, fluid-filled cyst ('cystic echinococcosis'), which can affect many organs, most often liver and lungs. In humans, CE causes significant morbidity, largely depending on the size and localization of the cyst(s). Treatment requires abdominal or pulmonary surgery or other invasive procedures (percutaneous treatment techniques) in combination with

prolonged chemotherapy, which is costly and often not available in remote areas. The economic burden of CE is caused by human mortality and disability. The transmission of the parasite to humans is facilitated by the presence of dogs and livestock in conjunction with slaughtering practices (feeding of raw offal to dogs) and poor hygiene. CE has a particular impact in developing countries and in terms of global disease burden (in DALYs), it is close to African trypanosomosis and schistosomosis, zoonoses which receive far more attention in terms of research and control. Prevalence levels of CE can be >5% in the nomadic or semi-nomadic ethnicities of northern Kenya or southern Sudan and yet not exactly determined in other parts. The access to therapy for this particular part of the population is usually sporadic, and established procedures for treatment are not always feasible under the local conditions. Preventive programs are difficult to implement and sustain over sufficiently long periods due to the lack of infrastructure and/or financial resources. It is important to have a clear picture about the disease in the country to enable its control regardless of all these difficulties, thus study aims at highlighting the prevalence, diagnosis and distribution of cystic echinococcosis in patients distributed in different parts of the Sudan since the disease was first reported in 1908.

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TH1 /TH2 AND TH17/TREG IMMUNE RESPONSE IMBALANCE IN PATIENTS WITH HEPATIC ALVEOLAR ECHINOCOCCOSIS

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Alveolar echinococcosis (AE), caused by the larval stage of *echinococcus multilocularis (E.m),* continues to be a worldwide public health problem. During the infection, *E.m* interacts with and modulate host's immune system for its successful survival. Herein, we have comprehensively studied T cell subsets immune response profile in patients with AE. Total number of 55 subjects were enrolled and divided into three groups: AE group, (n=15), AE with albendazole treatment (AE+ABZ, n=14), and healthy controls (HC, n=26). Th17 and Treg cells frequencies in peripheral blood mononuclear cells (PBMCs) were measured by flow cytometry. The mRNA expression levels of T-bet, GATA3, RORrt, and FoxP3 were measured by RT-PCR. Plasma level of IFN-r (Th1), IL-5 and IL-6 (Th2), IL-17A, IL-17F, and IL-23 (Th17); and IL-10 (Treg) were detected by ELISA. Result showed that the Th17 cell frequency significantly decreased in AE group, however it is remarkably increased in AE+ABZ group than that in HC group; T-bet mRNA expression in AE group is lower than HC group, while, higher in AE+ABZ group; GATA3 mRNA expression is slightly increased in AE group, however, it is significantly decreased in AE + ABZ group; RoRrt mRNA expression level decreased in both group than that in HC group, moreover, a slight increase showed in AE +ABZ group than AE group; Foxp3 mRNA expression level slightly increased in AE group than HC group, however, it is decreased in AE + ABZ group. Plasma level of IFN-r slightly decreased in AE, and increased in AE + ABZ group compared to HC group; Levels of plasma IL-5 and IL-6 slightly increased in AE group, whereas, it decreased in AE +ABZ group than that in HC group; IL-17A, IL-17F, and IL-23 significantly increased in both group than that in HC group, however, the levels are lower in AE + ABZ group; Plasma levels of IL-10 are increased in both AE and AE + ABZ group, however, the level was lower in AE +ABZ group. Our results demonstrated that there is Th1 / Th2, and Th17 / Treg immune response imbalance exist during the infection, this imbalance may play a potential role in parasite immune evasion and immune-pathogenesis of hepatic alveolar echinococcosis.

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EXPRESSION OF TOLL-LIKE RECEPTOR 2, 4 AND 7 IN PERIPHERAL BLOOD MONONUCLEAR CELLS IN PATIENTS WITH HEPATIC CYSTIC AND ALVEOLAR ECHINOCOCCOSIS

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This study aims at to explore expression of Toll-like receptors (TLRs) and related cytokines in patients with cystic echinococcosis (CE) and alveolar echinococcosis (AE). 55 subjects were enrolled and divided into three groups: AE group (N=15), CE group (N=14), and healthy controls (HC, N=26). The mRNA expression levels of TLR2, TLR4 and TLR7 in peripheral blood mononuclear cells (PBMCs) were measured by using real-time fluorescent quantitative reverse-transcription polymerase chain reaction (RT-PCR). Plasma levels of INF-γ, IL-5, IL-6, IL-10 and IL-23 were detected by using ELISA. Values are expressed as mean ± SD and the data were analyzed by ANOVA. If significance was found, Newman-Keuls test was performed for post-hoc analysis to detect the difference among groups. TLR2 mRNA expression was significantly increased in the CE and AE groups compared to the HC group (P<0.05). TLR4 mRNA expression was higher in the CE and AE groups and statistical significance was only shown in CE group (P<0.05). However, TLR7 mRNA expression was remarkably decreased both in CE and AE groups with statistical significance. Plasma levels of IL-10 and IL-23 in patients with CE and AE were significantly higher than those in controls (P<0.05). Levels of serum IL-5 and IL-6 CE and AE group were higher than those in HC group with no statistical significance (P>0.05), IFN-γ level was slightly decreased in AE group, oppositely, increased in CE group with no statistical significance. Expression pattern of TLR 2, 4 and 7 in PBMCs in patients with AE and CE might be involved in the cytokine modulation, which allowed the parasite to escape, which seems to be stronger in AE, host immune-surveillance and promoted chronic infection.

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NEUROCYSTICERCOSIS: AN UNKNOWN, A FORGOTTEN, OR A NEGLECTED PARASITIC ZOONOSIS IN NIGERIA?

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Epilepsy or seizure is among the most common neurological disorders all over the world, including Nigeria and the aetiology may vary. However, the International League Against Epilepsy recognizes neurocysticercosis as a growing problem in many tropical countries and as a leading cause of epilepsy and seizures. Neurocysticercosis (NCC) is an infection of the central nervous system (CNS) caused by the metacestode stage (*Cysticercus cellulosae*) of the pig tapeworm of man *Taenia solium*. It is probably the most common helminth parasite incriminated in central nervous system parasitic infections in human beings. *T. solium* taeniasis and therefore, NCC are common in many of the world's poorer countries especially where the environmental hygiene is poor and families raise free-roaming pigs that have easy access to and consume human faeces. In Nigeria, *T. solium* cysticercosis is common in pigs but there is little information on human *T. solium* taeniasis and virtually none on human *T. solium* cysticercosis (ocular- and neuro-cysticercosis). Conversely, epilepsy is the commonest neurological condition diagnosed in adults in most Teaching Hospitals in Nigeria but whether it could be attributed to neurocysticercosis is not clear. This paper examines neurocysticercosis vis a vis epilepsy and seizures and draws attention to this important zoonosis which it seems in Nigeria is an unknown, a neglected or a completely forgotten major causative agent of neurological conditions.

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RE-EVALUATION OF PORCINE CYSTICERCOSIS IN NSUKKA AREA OF ENUGU STATE, NIGERIA

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The prevalence of porcine cysticercosis in Nsukka Area of Enugu State, Nigeria was re-evaluated between February and June, 2013, using structured questionnaire and post mortem examination of slaughtered pig carcasses at Ibagwa, Orba and Nsukka slaughter slabs. Questionnaires were distributed to willing butchers and pig marketers and completed copies retrieved and analysed. The slabs were visited every other week and pig carcasses examined in accordance with standard meat inspection procedures. Briefly, carcasses were examined visually under natural light and palpated before longitudinal incisions were made on the heart, tongue, masseter, neck, intercostal, shoulder and thigh muscles and examined. Carcasses containing cysticerci were recorded as positive for *Cysticercus cellulosae*. The sex, age and breed of all animals examined were determined. Results showed cysticercosis prevalence of 3.3%, 4.3% and 0% for Ibagwa, Orba and Nsukka slaughter slabs respectively and an overall prevalence of 2.4% for the three study areas during the period. Moreover, the type of pig husbandry practiced was the most important factor influencing the prevalence of the infection. Analysis of the questionnaire responses showed that the majority of the respondents were not aware of the zoonotic implication of porcine cysticercosis irrespective of their educational background and that epilepsy in the family could be associated with the infection in man. It is concluded that *Taenia solium* cysticercosis remains endemic in Nsukka area of Enugu State, Nigeria and poses a major health hazard that must be addressed by coordinated control programs.

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EVALUATION OF TRICLABENDAZOLE AGAINST *TAENIA SOLIUM* METACESTODE IN NATURALLY INFECTED PIGS

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Taenia solium cysticercosis is an important zoonotic disease in developing countries. Many studies have been conducted to evaluate the efficacy of different antiparasitic drugs against the larval stage (cysticercus) of *T. solium*. Currently, oxfendazole (OFZ) is the best drug against porcine cysticercosis, with excellent efficacy using a single oral dose. On the other hand, triclabendazole (TCZ) is the most common drug used to treat livestock helminthiases. Therefore, the aim of this study was to evaluate the efficacy of TCZ in naturally cysticercosis-infected pigs. Eighteen pigs were divided into 3 groups of 6 individuals each. The groups were treated as follows: a first group was treated orally with TCZ at a single dose of 30 mg/kg of body weight, the second group was treated orally with OFZ at a single dose of 30 mg/kg of body weight and the third group was left untreated (control). All animals were kept under the same management conditions. The pigs were humanely killed 17 weeks post-treatment and the number of surviving cysts in muscles was assessed. Cysts in pigs treated with TCZ had a normal appearance, not different from the control group ($p>0.05$). All pigs treated with OFZ had only degenerated cysts in their carcasses ($p<0.05$ for parasiticidal efficacy compared with the control group). TCZ is not efficacious to treat porcine cysticercosis.

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TAENIA SOLIUM GENOTYPES IN A RURAL COMMUNITY: RELATIONSHIP BETWEEN PORCINE CYSTICERCOSIS AND HUMAN TAENIOSIS

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Taenia solium is a cestode that infects pigs and humans in different regions of the world and is cause of neurocysticercosis in humans. The understanding of the association between the genetic variability of the parasite with geographic origin and phenotypic characteristics like infectivity, pathogenicity and response to treatment, will be a critical tool in the efforts to prevent and control this disease. After analyzing the entire *T. solium* genome, we demonstrated that microsatellite markers are widely distributed throughout of the genome. In addition these markers have shown to have enough variation to differentiate strains. The objective of this study is to use microsatellite markers and to compare the genetic profiles between cysts recovered from naturally infected pigs and tapeworms from human hosts in a rural community in Northern Peru. We performed a cross sectional study where four tapeworms were genotyped and showed different profiles. All pigs in the community were screened for cysticercosis, using the tongue test. We identified 8 cysticercosis-tongue-positive pigs. Animals were humanely sacrificed and 30 individually cysts were recovered from the carcass of each pig. Cysts were genotyped with the same microsatellite markers and compared to the genotypes of the previously identified tapeworms. We found genetic variation among this population. Two pigs had about 30% of cysts with a genotype that exactly matched to the tapeworm corresponding to the geographically closest human host. Interestingly, one pig showed 50% of cysts with a genotype different to any of the tested tapeworms. We propose microsatellites as promising genetic markers with which it would be possible to study the genetic epidemiology of this disease, in particular the transmission from tapeworm carrier to pig and the reintroduction of foreign cysts/tapeworms into a community. We are conducting additional studies to verify the source of genetic variation of microsatellites in *T. solium* cysts.

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PURIFICATION OF VESICULAR FLUID ANTIGEN OF CYSTICERCI *TAENIA SOLIUM*, FOR HUMAN DIAGNOSIS IN THE ANDEAN TRAPEZIUM AND UNASUR COUNTRIES

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Neurocysticercosis (NCC) is a neglected parasitic disease and a leading cause of seizures and epilepsy in developing countries. Serological assays detecting *T. solium* antigens complement standard imaging techniques to establish NCC diagnosis. Crude antigens extracted from the vesicular fluid of *T. solium* cysticerci (*Tsol*.VFAg) have been used for this purpose; however, the availability of technology and cost required to purify is limited in developing countries. We compared and evaluated four purification methods to obtain *T. solium* antigens for serological assays. Cysticerci were collected from different endemic areas of Peru and *Tsol*. VFAg were purified using the following methods: i) ammonium sulphate, ii) lentil-lectin sepharose (affinity chromatography), iii) sephadex G-75, and iv) electro-elution. The analytic sensitivity and specificity were evaluated using patient serum from individuals with NCC and other parasitosis. The crude antigenic protein concentration was 3.6µg/µL which is within the range as suggested by CDC and also SDS-PAGE electrophoresis revealed seven glycoproteins (GP50, GP42-39, GP24, GP21, GP18, GP14 and GP13kDa) with affinity/lentil-lectin method. Our research identified eight

bands diagnostics with affinity/lentil-lectin method (GPs.35, 31, 24, 23, 18, 17, 14 and 13kDa.), while the other three methods detected different fractions of those proteins (ammonium sulphate: no band, electro-elution: 6 bands of 97-GP12kDa, and sephadex G-75: 17 bands of 100-GP6KDa). Western blot using purified glycoprotein (affinity/lentil-lectin method) identified 50/50 patients with NCC (100% sensitivity), 50/50 healthy control individuals (100% specificity), 20 individuals with other parasitosis (*Hymenolepis nana, Echinococcus granulosus, Fasciola hepatica*) give cross-reacted with the GP42-39Kda.protein. Affinity chromatography (lentil-lectin sepharose) is the method of choice to purify glycoproteins from *T. solium* cysticerci for the development of a reliable and affordable immunodiagnostic kit for NCC which can be applied in the Andean trapezium and UNASUR countries.

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EPILEPSY AND CYSTICERCOSIS IN THE HIGHLANDS OF MADAGASCAR: IS SEROLOGY RELEVANT?

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In sub-Saharan Africa epilepsy reflects complicated delivery and sequelae of infections during childhood. However in rural countries like Madagascar the profile of epilepsy is mostly unknown. In the same time, Madagascar is one of the hotspot of cysticercosis and its burden during epilepsy is unknown. To describe this profile and relation with cysticercosis a survey was conducted on epileptics in 40 dispensaries of the Moramanga district, a rural area and in two private hospitals in Antsirabe (a small town near the capital). Patients older than 5 years were recruited after at least two unexplained seizures. For each patient recruited in dispensaries a control subject matched on age was recruited in household randomly selected in the same village. Socioeconomic and clinical data were collected. Serology for cysticercosis was conducted using glycosylated crude proteins of the cysticercus in ELISA and western blot. A total of 247 subjects were investigated (125 epileptics and 122 controls). No type of seizure was associated with positive serology for cysticercocis. Seroprevalence was the same for epileptics and controls (49.5% / 50.4%). It was higher in rural than in urban area, and for female than for male. However epileptics have higher ELISA mean OD than controls (0.461 vs 0.26), and younger epileptics have higher OD than older ones. In univariate analysis higher prevalence of cysticercosis in epileptics was associated with the number of children in the household and the use of backyard wells for water supply, but not with the presence of pigs. In multivariate analysis age, gender, urban/rural habitat and presence of pigs were retained to explain epilepsy but not seropositivity for cysticercosis. For seropositivity only the absence of toilets and the number of children in the household were retained. Over all poor access to sanitation and the number of children in the household were the major parameters associated with cysticercosis, undoubtfully in relation with taeniasis. However in this area, serology for cysticercosis is not relevant for diagnostic.

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MONOCLONAL ANTIBODIES FOR DETECTION OF PARASITE ANTIGENS IN BODY FLUIDS FROM PATIENTS WITH NEUROCYSTICERCOSIS

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Neurocysticercosis (NCC), an infection of the brain by *Taenia solium* cysts, represents the most common cause of adult-onset epilepsy in developing countries. Serologic diagnosis has historically been based on detection of antibodies to *T. solium* in serum, but antigen (Ag) detection assays using T. saginata cross reactive monoclonal antibodies (MAbs) have been applied to serum and body fluids more recently to identify individuals with live parasites. We generated and characterized 22 murine monoclonal antibodies against *T. solium* cysts in BALB/c mice using whole cyst (WA), vesicular fluid and excretion/secretion Ags of *T. solium* cysts as immunogens. Using these MAbs in an enzyme-linked immunosorbent assay (ELISA) format, we developed an Ag capture assay for antigen detection in body fluids. Only one of 20 MAbs tested demonstrated weak cross-reactivity to *Echinococcus granulosus* Ags. Localization of the target Ags on the parasite was performed by immunofluorescence and immunohistochemistry on histological sections of muscle cysts from naturally infected pigs showing reactivity to different anatomical locations on the parasite: 1 MAb was reactive to the neck and fluid spaces, 4 to the neck alone, 9 to the cyst wall and neck, and 6 to the cyst wall, neck and fluid space. Pooled samples of serum and urine from patients with NCC and negative controls were evaluated by the Ag capture ELISA, using the MAbs as a capture antibody and a polyclonal rabbit anti-*T. solium* WA antibody for detection. ELISA results show that 7 MAbs could detect antigens in serum and 3 in urine samples. Reactivity of these MAbs expressed as normalized ratios of optical densities (OD positive control/OD negative control) show that 3 MAbs had ratios >20 and 4 between 5-20. These results suggest that these monoclonal antibodies have potential utility for the diagnosis and follow up of treatment in NCC patients. Use of MAb for detection of different parasite Ag in the same patients may provide insight into the kinetics of antigen release in relation to parasite damage during treatment in NCC.

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GENOTYPING OF *TAENIA SOLIUM*

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Human taeniasis/cysticercosis due to *Taenia solium* remains a serious public health problem particularly in developing countries. Genetic variability among *T. solium* is limited because the nature of the adult tapeworm. The objective of the present study was to develop a genetic tool to genotype tapeworm isolates. The internal transcribed spacers (ITS1 and ITS2) including the 5.8S gene of the ribosomal genes were amplified by PCR. The amplified products were then subjected to restriction enzyme analysis (REA). Restriction products were further analyzed by gel electrophoresis, different genotypes were analyzed and compared using PAUP 4 and MEGA 4 softwares. A total of 78 tapeworm isolates were analyzed, two distinct genotypes were observed when Alu I and Msp I were used, while two others genotypes were observed when Hae III and Mbo I were employed. Analysis of ribosomal spacer sequences might be an important tool to differentiate *T. solium* isolates.

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EVALUATION OF A MONOCLONAL ANTIBODY TO DETECT OF URINE ANTIGENS FOR THE DIAGNOSIS OF NEUROCYSTICERCOSIS

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Neurocysticercosis (NCC), a zoonotic disease caused by the larval stage of the parasite *Taenia solium*, is considered the main cause of acquired epilepsy around the world. Computed tomography (CT) and Magnetic resonance imaging (MRI) are the standard neuroimaging techniques for the diagnosis of NCC. Diagnosis of NCC is supported by immunological testing, through antibody detection by Enzyme-linked immunoelectrotransfer blot (EITB) assay, and recently by antigen detection using an enzyme-linked immunosorbent assay (ELISA). Detection of antigens either in serum, cerebrospinal fluid or urine may be an indicator of active infection. In this study an ELISA was developed using both monoclonal (for detection) and rabbit polyclonal antibodies (for capture) raised against a crude *T. solium* cyst extract, and evaluated for its capacity to detect antigens in urine samples of patients with NCC. Urine samples from 108 patients with diverse types of neurocysticercosis (n=61) and from individuals negative for NCC by CT scan and EITB (n=47) were processed by Ag-ELISA. Fifty three of 61 NCC positive samples were Ag-ELISA positive. All patients with subarachnoid NCC were Ag-ELISA positive. Among 8 individuals with NCC who tested negative by the Ag-ELISA, 3 patients presented only viable cysts with no inflammation (2-3 cysts). All NCC negative patients gave a negative result by the Ag-ELISA test. Overall the Ag-ELISA test demonstrated 87% sensitivity and 100% specificity. Additional modification of antibodies such as conjugation of MAbs to biotin might further improve sensitivity of the test.

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NOVEL MRI STAGING SCORE AND MONOCLONAL ANTIBODY BASED ANTIGEN DETECTION ASSAY IN SUBARACHNOID NEUROCYSTICERCOSIS: MONITORING RECURRENCE AND RESOLUTION OF DISEASE

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Increasingly physicians are faced with patients presenting with neurocysticercosis involving the subarachnoid space (SANCC). Treatment needs to be prolonged with close follow up, but endpoints are not well defined. A neuroimaging staging system and a monoclonal antibody based antigen detection assay were evaluated. A chart review of SANCC patients since 2000 was undertaken. An MRI staging score based on number of cysts in a location, cyst size and enhancement was used. Antigen levels were recorded at end of treatment. Twenty-three patients were identified. Median age was 35 (range 18-69), 73.9% males. Median time from immigration to presentation was 7 years (0-34). Patients presented with seizure (30.4%), hydrocephalus (26.1%), vascular complications (21.7%) and headache (21.7%). Exclusive involvement in the Sylvian fissures was present in 4, basilar cisterns in 7 and in both regions in 10. Two patients had only enhancement. Concurrent spinal disease in 5. Median MRI score was 15 (2-30). Median treatment length was 56 weeks (4-2000 weeks). During treatment the following events occurred: Vascular (17.4%), hydrocephalus (17.4%), clinical deterioration and increased inflammation on MRI (both, 13.0%). Those that resolved on MRI (52.2%) were more likely to have a negative antigen level at the end of treatment. Of the 8 who had a negative antigen 87.5% resolved, whereas only 28.8% resolved of the 7 with a positive antigen (p=0.041). Also, of the 7 patients with a positive antigen at the end of treatment 5 recurred (71.4%), whereas those with a negative antigen none recurred (p=0.007). Median baseline MRI score was lower among those resolved (p=0.006). It was no different among those whose disease recurred (p=0.784) or those who suffered events during treatment (p=0.396). In this study, three-quarters of patients had clinical events during treatment, and resolution occurred in about half the cases. MRI score and antigen level correlated with disease resolution. Antigen level was associated with disease recurrence. The two scores used in combination seem promising for use in treatment decisions.

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PRESENTATION AND MEDICAL TREATMENT OF SPINAL NEUROCYSTICERCOSIS

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Spinal cord involvement is noted in about 1-3% of cases of neurocysticercosis (NCC) patients. Leptomeningeal spinal NCC, evident in about 80% of spinal cases, usually presents as an extension of the subarachnoid disease of the brain (SANCC). The cysts are thought to migrate downward from the basilar cisterns eventually become fixed at one level and can be accompanied by inflammation resulting in arachnoiditis and dural thickening. Surgical treatment is most commonly reported in the literature. This is a large series of medically managed spinal NCC cases. We performed a retrospective review of spinal NCC seen in Jacobi Medical Center's Tropical Medicine Center between the years 2000 and 2014. Eight cases were identified. The median age was 35.5 (range 28-65), 7 males and 7 were born in Latin America and median time from immigration was 8 years (1-18). Six patients had concurrent SANCC of the brain, 1 had a 4th ventricular cyst and 1 was a primary spinal case. Location of lesions was: cervical (62.5%), thoracic (37.5%), lumbar (75%). Four had cysts and all had arachnoiditis and hydrocephalus at presentation. Symptoms in descending order of frequency were: headache (75%), seizures (50%), radicular pain (37.5%), extremity weakness (37.5%), and back pain (25%). The median time for treatment with albendazole and steroids was 58 weeks (12-157). Aseptic meningitis was seen in 62.5% of the patients. At the end of treatment only 1 resolved completely, the other 7 had never clumping and of these 5 had enhancement. One patient continued to have cysts and he was actively being treated. Only half improved their clinically. Spinal NCC is commonly associated with SANCC and requires prolonged medical therapy. In this series the clinical presentation was due to both brain and spinal involvement. Patients presenting with aseptic meningitis with hydrocephalus from an endemic region should be evaluated for both spinal and SANCC. Radiographic improvement was seen, but residual nerve clumping may account for the persistent clinical symptoms.

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BIOMARKER-BASED ASSOCIATION MODELS FOR PREDICTING COGNITION DEFICITS IN CASSAVA CYANOGENIC POISONING

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While risk factors for konzo are known, determinants of cognitive impairment in konzo-affected children remain unknown. We anchored cognitive performance (KABC-II scores) to serum levels of free-thyroxine (free-T4), thyroid-stimulating hormone (TSH), albumin, and motor proficiency (BOT-2 scores) in 40 children including 21 with konzo (median age: 9 years) and 19 without konzo (median age: 8 years). A multiple regression model was used to determine variables associated with changes in KABC-II scores. The results were: Age (: - 0.818, 95%CI: - 1.48, - 0.152) (p=0.018), gender (: - 5.72; 95% CI: - 9.87, -1.57 for females) (p=0.009), BOT-2 score (: 0.390; 95% CI: 0.113, 0.667) (p=0.008), and free-T4 (: 1.88; 95% CI: 0.009, 3.74) (p=0.049) explained 61.1% of variation in KABC-II scores. Subclinical hypothyroidism was not associated with poor cognition. A crude association was found between serum albumin and KABC-II scores (: 1.26; 95% CI: 0.136, 2.39) (p=0.029). On spot urinary thiocyanate reached 688 µmol/l in children without konzo and 1032 µmol/L in those with konzo**.** In conclusion, **f**emale gender and low serum albumin are risk factors common to cognitive and proportionally associated motor deficits in children exposed to cassava cyanogens. The two types of deficits may share common mechanisms.

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EASE OF USE AND SAFETY OF A CONICAL CUP BLOOD TRANSFER DEVICE FOR USE WITH RAPID DIAGNOSTIC TESTS FOR HUMAN AFRICAN TRYPANOSOMIASIS (HAT)

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Rapid diagnostic tests (RDTs) are increasingly being used for screening and diagnosis of infectious diseases in remote settings. Appropriate devices to safely transfer fixed amounts of blood from finger pricks to RDTs pose a significant challenge. Recently, a 5-µL inverted cup was evaluated and found to be the most acceptable to health workers (HWs) in terms of safety, ease of use and accuracy of volume transferred when used with malaria RDTs. The success of this device and its subsequent uptake by various manufacturers led to the development of a 23-µL conical cup for RDTs requiring higher volumes of blood. This study compares this conical cup with a plastic pipette when used for screening for Human African Trypanosomiasis (HAT) infection with the SD BIOLINE HAT RDT in Yumbe and Arua districts in Northwestern Uganda. After a half-day of training, conical cups and plastic pipettes were given to 49 HWs who used them for testing of suspect patients who voluntarily accepted to participate in the study. Each HW used the blood transfer devices with at least 10 patients in the field. Questionnaires and focused group discussions were used to gather information on ease of use, blood safety and acceptability. Preliminary results show that HWs generally preferred the conical cup over the plastic pipette. A higher rate of successful blood transfers and fewer occurrences of blood safety issues were observed when using the conical cup. Results of this study not only demonstrate the ease of use of a new blood transfer device for RDTs for HAT and other diseases such as HIV, but also raise awareness about the need for further improvements in BTDs for the use of RDTs on hands of health workers in remote areas. Additional and more detailed results will be presented during the meeting. Final results will be available by the end of April.

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THE EFFECT OF MAGNESIUM SULPHATE ON AUTONOMIC DYSREGULATION IN ENTEROVIRUS 71 RELATED HAND FOOT AND MOUTH DISEASE IN VIETNAMESE CHILDREN

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Enterovirus 71 (EV71) associated hand foot and mouth disease (HFMD) has emerged across much of Asia as a serious infection of young children during the last decade. HFMD predominantly affects children under 5 years of age, with a clinical spectrum varying from a relatively mild self-limited febrile illness with a rash, to fatal cardiopulmonary collapse. An outbreak of HFMD in 2011 in southern Vietnam resulted in over 110,000 reported cases and 116 deaths, and epidemiological studies from neighboring China report over 7 million HFMD cases between 2008 to 2012, with 2,457 deaths. Autonomic nervous system (ANS) dysregulation is considered as the main indicator of progression to severe disease, initially manifesting with hypertension, and later progressing to cardiopulmonary failure and death in a small proportion of cases. Although lacking a formal evidence base, current guidelines in many Asian countries suggest milrinone as the first choice for management of ANS related hypertension in patients with severe HFMD. Based on clinical experience and evidence from a randomized controlled trial magnesium sulphate (MgSO4) has become the mainstay of treatment for ANS dysregulation in patients with tetanus in many centres. We describe a case series involving 10 EV71 confirmed HFMD cases with ANS dysregulation managed at HTD over a 4 month period in early 2012. Magnesium sulphate was added when hypertension remained poorly controlled despite high dose milrinone (up to 0.75 ug/kg/minute), and in all cases the blood pressure reduced within 30-60 minutes and remained stable subsequently on a continuous magnesium infusion for 48-72 hours. No patient required hemofiltration, although 2 of 10 cases were ventilated because of respiratory distress. Brain MRI was performed later in 4 of these cases and in the 2 children with neurological sequelae abnormalities were found, involving the medulla in both cases, and with extensive atrophic changes in one child. These promising initial results are currently being investigated in a formal randomized controlled trial of magnesium sulphate versus placebo in Vietnamese children with severe HFMD and signs of ANS dysregulation.

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ATTRITION AND ITS ASSOCIATED FACTORS AMONG PRE-ART CLIENTS REGISTERED IN CARE AND TREATMENT CENTERS IN MOROGORO, TANZANIA 2010 -2011

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Despite significant success in scaling up care and treatment programmes in Tanzania, a majority of people living with HIV (PLHIV) do not access them. Successfully enrolled PLHIV in care and treatment clinics (CTC) are lost at every step along the continuum of care. This study aims at determining factors associated with attrition among Pre-ART adults in CTC.We

conducted a clinic based retrospective cohort study that involves review of data from Pre-ART adult clients (≥15years) register and client treatment card number 2 (CTC2 Card) at three CTCs in Morogoro from July, 2010 to July 2011. Pre ART clients who were not in care at their original sites at 1 year of follow up were traced through home based care volunteers and phone calls. Relative risk was then calculated using Epi info statistical soft ware.A total of 351 CTC clients were enrolled between July 2010 and July 2011. Most enrolled clients were ART eligible 161(45.9%) of whom 92(57.1%) were initiated on ART. Despite being ART eligible 69 (42.9%) of enrolled clients were not Initiated on ART. Mortality among those not initiated ART was 47 (17.6%) of whom 30 (63.8%) were ART eligible. Majority 259 (66.7%) of enrolled clients had no ART start Status, of the 259 clients status of 25(9.6%) could not be determined until the end of the study. 67(25.9%) were confirmed not to be in care at one year of follow up due to different reasons such as death, opt out and some were reached and promised to return to care. 67(25.95) were pre ART and still attending at their original clinics whereby 80 (30.9) were in care with other providers. Being enrolled in care at an advanced disease stage, paying for transport to go to the clinic and stigma were risk factors for Pre ART attrition.Attrition due to mortality is high among Pre ART clients who are enrolled in CTC while already in advanced disease stage. Attrition from clinic is higher than attrition from care. Self stigma and advanced disease stage were pre ART attrition risk factors. Status disclosure and sensitization to reduce stigma as well as Strengthening of CTC to improve linkage and referrals between CTC and other clinics as well as prioritizing ART initiation among clients who are ART eligible is recommended.

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COMMON CLINICAL COMPLICATIONS OF HUMAN MONKEYPOX INFECTION AT THE GENERAL HOSPITAL OF KOLE, IN DEMOCRATIC REPUBLIC OF CONGO

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Monkeypox (MPX) virus is an orthopoxvirus that is endemic in central and West Africa, causing a smallpox-like disease ranging from subclinical to severe cases. With the cessation of smallpox vaccination in 1982, rural populations in central Africa were increasingly susceptible to MPX infection due to waning herd immunity to poxviruses. We have observed an increase in severe clinical manifestations of MPX in both children and adults, leading to more frequent clinical complications. Most of these complications are moderate requiring nurse care and prolongation of the hospitalization, however, severe complications can be fatal (death, miscarriage) or lead to grave sequels (blindness), albeit rarely. To describe frequent and severe clinical complications observed in human subjects with MPX infection at the Kole General Hospital, one of the most remote Ministry of Health Hospital in the rain forest of the Sankuru District, in Democratic Republic of Congo (DRC) where the majority of the population relies on bush meat as a principal source of protein. From 2007 to 2011, we conducted an observational study of 229 cases of human MPX infection in order to describe its clinical and biological characteristic as it occurs in the DRC. Clinical signs, symptoms, and laboratory results were recorded during their hospitalization. Clinical complications observed during this study were identified and recorded. Miscarriage was the most common complication in infected pregnant women (3/4) with evidence of fetal contamination, followed by secondary dermatitis on infected skin lesions (30/229), death (4/229), abscess of cervical lymph node (6/229), keratitis (3/229), staphyloma occurring approximately 20 months later after the onset of keratitis, and caseation of eye lesions (1/229) in confluent lesions spreading in the sclera. Clinical complications in human MPX infection are common in DRC, which is likely attributable to the decline of cross-protective immunity after cessation of smallpox vaccination and the subsequent increase in the susceptible population in endemic regions. Infected pregnant women were the most likely to have complications resulting in death.. Certain complications can be treated with nursing care or minor surgery, whereas others require intensive care or plastic surgery which are not often available in remote areas where MPX most often occurs, thus leading to grave sequelae.

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INTRAVENOUS ARTUSENATE VS. QUININE IN THE TREATMENT OF SEVERE MALARIA IN LIBERIAN CHILDREN

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Liberia is a West African Country whose health care system is recovering from years of civil war. Liberia is endemic for malaria and recent reports estimate the malaria prevalence to be as high as 32% in children under 5 years of age. Recent data collected at JFK Medical Center, the national referral hospital, shows that 42% of pediatric admissions are for the treatment of severe malaria. The goal of this study was to compare the outcomes of children admitted with severe malaria and treated with either quinine or artusenate, to establish the optimal treatment regimen in this setting. We studied children age 5 and under who were admitted to JFKMC during the time period from May 2013 through October 2013 with a diagnosis of severe malaria. 92 children were treated with artusenate (2.4 mg/kg/dose IV q 12 x 4 doses) and 73 children were treated with quinine (15mg/kg/dose IV q12 x 3 doses). We measured time to symptom resolution, length of hospital stay and clearance of parasitemia for each treatment arm. Of the 165 study participants, the average age was 27 months; 43% were female, 57% were male, and 48% used bednets regularly. 39% had at least one prior episode of malaria. Children treated with artusenate cleared their symptoms sooner (2.08 days) and had shorter hospitalizations (3.56 days) than their counterparts treated with quinine (cleared symptoms in 2.32 days and hospitalized an average of 3.67 days). A larger number of children treated with artusenate had persistent parasitemia on hospital day 3 (6.5%) versus those children treated with quinine (1.3%). These results align well with prior reports of the superiority of parenteral artusenate over quinine for the treatment of severe malaria in children in Sub Saharan Africa. Future studies include following children post hospital discharge to examine clearance of parasites after completion of outpatient regimens, and screening for drug resistance mutations in this population.

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IMPLEMENTATION AND EVALUATION OF A RAPID ASSESSMENT CLINIC FOR FEBRILE RETURNED TRAVELERS IN AMBULATORY TROPICAL MEDICINE

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Fever in the returned traveler is a medical emergency warranting prompt medical attention to exclude potentially life-threatening illnesses such as malaria. However, prolonged evaluation in the Emergency Room (ER) may not be required for all patients. As a quality improvement initiative, we designed and implemented an algorithm for rapid assessment of febrile travelers (RAFT) in an ambulatory tropical medicine clinic. Criteria for referral to the RAFT clinic include: presentation to the ER, history of and/or documented fever, and travel to the tropics or sub-tropics within the past year. Exclusion criteria include a diagnosis of *Plasmodium falciparum* malaria and fulfillment of other admission criteria such as unstable vital signs or significant laboratory derangements. We performed a time series analysis pre- and post-implementation of the clinic, with primary outcome of time to definitive tropical medicine consultation. Secondary outcome measures include number of ER visits averted for repeat malaria testing

and number of ER visits averted for definitive management of an infectious illness. Interim analysis 2-weeks post-implementation indicate a mean time to RAFT clinic assessment of 1.29 days (range 0_2 days) compared to 5.44 days (range 0_26 days) prior to implementation (p=0.15). A total of 7 patients were referred to the RAFT clinic over 2 weeks, thus averting 7 repeat ER visits for follow-up malaria screening. Of 7 RAFT patients, 4 (57%) had an infectious illness that required specific therapy, thus, 4 "call-backs" to the ER over 2 weeks were averted. Extrapolated over 1 year, our interim results suggest that implementation of a RAFT clinic can avert 182 repeat ER visits for follow-up malaria screening, and 104 ER "call-backs" for management of a positive infectious work-up. In addition to provision of more timely care to ambulatory febrile returned travelers, we have demonstrated at our interim analysis that implementation of a RAFT clinic in an urban tertiary care setting can reduce ER visits by 5.5 per week.

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COST EFFECTIVENESS OF A POINT OF CARE TEST FOR SEPSIS AMONG PATIENTS WITH FEBRILE ILLNESS IN LOW RESOURCE SETTINGS

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Bacterial sepsis is an important cause of mortality in low- and middle-income countries, yet distinguishing patients with sepsis from others remains a challenge. Currently management decisions are based on clinical assessment using algorithms such as Integrated Management of Adolescent and Adult Illness (IMAI). Efforts to develop and evaluate point-of-care (POC) diagnostic tests for sepsis to guide decisions on the use of antimicrobials are underway. To establish the minimum performance characteristics of such a test, we varied the characteristics of a hypothetical POC test for sepsis required for it to be cost effective. We applied a decision tree model to a population of febrile patients presenting at the district hospital level in a low-resource setting. We compared existing clinical assessment algorithms against the POC test. Patient survival was the outcome of interest. Costs and performance characteristics for the POC test were benchmarked against existing malaria rapid diagnostic tests. Prevalence of bacterial sepsis among febrile patients and outcome data were informed by the literature. We used a case fatality probability of 20% for appropriately treated sepsis and of 50% for inappropriately treated sepsis. A 13.4% prevalence of sepsis among febrile patients seen at the district hospital was assumed. Based on a clinical assessment for sepsis with the established sensitivity of 83% and specificity of 62%, we found a POC test for sepsis with a specificity of 94% and a sensitivity of 83% was cost effective, resulting in parity or equivalence in survival but costing US$2.05 less per live saved than clinical assessment. A POC test with sensitivity and specificity of 100%, equivalent to the best malaria rapid diagnostic tests, yielded an incremental cost effectiveness ratio that showed strong dominance, that is being both cheaper and more effective. Our results establish performance targets for POC tests for sepsis in low-resource areas.

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HIGH INCIDENCE OF SNAKE BITES IN THE UPPER EAST REGION OF GHANA: A CLARION CALL FOR IMPROVEMENT IN SNAKE ENVENOMING OUTCOMES

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Snake bites and snake envenoming are a common emergency problem in many rural health centres in sub-Saharan Africa. Although this phenomenon is not peculiar to sub-Saharan countries due to the presence of poisonous snakes on every continent and in almost every country, the incidence of snake bites is higher in the inter-tropical world than the temperate regions. Records on snake bites are minimal and scanty and the statistics may be misleading. This study sought to estimate the incidence of snake bites and mortality rates in a savannah farming area in Ghana. A review of records on snake bites in the Regional Hospital, a referral facility of the Upper East Region of Ghana for the year 2013. The Region has a population of approximately, 1,084,621. A total of 146 persons (male: 68/146 (46.6%); female: 78/146 (53.4%)) with snake bites were referred to the hospital out of a total of 94,707 OPD cases for year 2013. The overall snake bites incidence in the region for the year was 13.4 per 100,000 persons. A mortality rate of 7.5% (11/146) was reported (54.5% (6/11) females; 45.5% (5/11) males). 83.2% (129/146) of the snake bites were recorded in the peak farming season of March-August, which is also the major raining season. This study shows a high incidence of snake bites and relatively high mortality. This calls for efficient health care delivery for snake bite victims to improve snake envenoming outcomes.

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TRAVEL MEDICINE SPECIALIZATION REDUCES MEDICAL ERRORS: CHLOROQUINE PRESCRIPTIONS SIGNAL A BROADER PROBLEM

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There has yet to be a systematic analysis of antimalarial prescription errors in the United States. Utilizing the U.S. Military Health System's (MHS) outpatient medical records, all chloroquine (CQ) prescriptions from military facilities to the pediatric patients during the period 2006 to 2010 were reviewed. Adherence to destination specific prescribing guidelines from the CDC and MHS was assessed. Records from 547 encounters were reviewed, yielding 440 with destination information. Results were stratified to compare travel medicine clinics vs. primary care clinics. Travel medicine clinics in the MHS are operated by pediatric/adult infectious disease, preventive medicine and allergy & immunology specialties, all others were considered non-travel medicine clinics. Errors were categorized as either contraindicated (CI) for areas with CQ resistance or not indicated (NI) for areas without a recommendation for chemoprophylaxis. Travel Clinics provided 42% (n=185) of patient care. No CI errors were made at travel clinics. Non-travel clinics made CI errors in 6.3% (n=16) (p=0.001) of prescriptions. The overall rate of CI errors was 3.6%. Overall error rates (CI+ NI) for travel clinics was 6.5% (n=12) and non-travel clinics was 11.4% (n=29) (p=0.08) for a weighted average of 9.3% based on proportions of total chloroquine prescriptions. Travel Medicine specialty clinics have significantly lower rates of prescribing errors that place patients at risk for malaria. CQ-NI risks are common across clinic types and offer little to no benefit to the patient and exposes them to increased risk for adverse drug effects. These results suggest other aspects of care may also show disparities in quality of care. Strategies to improve travel medicine care, particularly among non-specialists are needed.

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HIGH PREVALENCE OF HYPOGLYCEMIA AMONG PEDIATRIC PATIENTS ADMITTED TO A RURAL MOZAMBICAN HOSPITAL AND RISK FACTORS FOR DEATH

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Hypoglycaemia is a common and life-threatening problem in African children, and is associated with a wide variety of diseases. We describe the prevalence and incidence of hypoglycaemia among admitted African children, establishing risk factors for a poor outcome. We retrospectively reviewed 13 years of systematic clinical data collected through a morbidity surveillance system ongoing in a rural Mozambican hospital. Data from all paediatric admissions were analysed, and independent risk factors assessed for 1) Hypoglycaemia and 2) death in children with

hypoglycaemia. Additionally, we used a glucose sensor to continuously monitor interstitial glucose levels (every 5 minutes) during the first 72 hours of admission for a subset of malaria patients. From January 2001 to December 2013, 45,593 children <15 years had a glycaemia determination performed on admission. 1477 children (3.2%) presented hypoglycaemia (glycaemia<3mmol/L), of which about 2/3 (971) with levels < 2.5mmol/L. Independent risk factors for hypoglycaemia on admission included being male (OR 1.17; p=0.033); having respiratory distress (OR 1.30; p=0.001), anorexia (OR 2.60; p<0.001), a positive blood culture (OR 1.69; p<0.001), malaria (OR 1.41; p<0.001), being a newborn (OR 2.50; p<0.001) or being severely malnourished (WAZ<3SD; OR 1.28; p=0.006). Hypoglycaemic children were significantly more likely to die than normoglycaemic ones (OR 6.8; p<0.001), with an associated CFR of 19.4% (245/1266). Independent risk factors for death among hypoglycaemic children included having respiratory distress (OR 2.75; p=0.031), anorexia (OR 3.55; p=0.015) and being severely malnourished (OR 5.07; p=0.002). We also aim to present data on continuous monitoring of hypoglycaemia (first 72 hours) for 80 malaria patients, which suggests that up to a third of admitted children have at least one hypoglycaemia episode detected through continuous monitoring. Hypoglycaemia remains a frequent and hazardous condition for African children, not only on admission but also throughout hospitalization, with many episodes remaining undetected. Symptoms, signs or conditions found to be associated with an increased risk of hypoglycaemia should trigger the verification of glycaemia, and the implementation of life-saving corrective measures.

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TRAVEL RELATED VACCINATION COVERAGE RATES IN A COHORT OF DEPARTMENT OF DEFENSE BENEFICIARIES SEEKING PRE-TRAVEL MEDICAL CONSULTATION

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Vaccine-preventable diseases are significant threats to international travelers. We evaluated the rates of travel-related vaccinations in Department of Defense beneficiaries seeking care at military travel clinics who enrolled in the TravMil cohort. 1323 patients were enrolled at 3 military travel clinics; 321 (24%) were active duty personnel, and 1002 (76%) were retirees or dependents. The most common destinations for travel were South/Central America and the Caribbean (31%) followed by Southeast/North Asia (28%) and Africa (28%). The median time between pre-travel consultation and departure was 26 days. Active duty personnel had higher immunization rates prior to travel when compared to retired military and dependents (hepatitis A: 97% vs 66%; hepatitis B: 80% vs 45%; Td/Tdap: 97% vs 74%; influenza: 90% vs 67% (p<0.05 for all)). The typhoid vaccine was the most common vaccine prescribed; 462 of 478 travelers to high-risk regions in Asia (97%) had current vaccines or were newly vaccinated. 26% (89/343) of at-risk travelers to destinations with endemic Japanese encephalitis (JE) did not receive vaccination, most often due to insufficient time to complete the series in 53% (47/89) of cases. Yellow fever vaccination was not prescribed in 14% (68/482) of patients traveling to endemic areas either due to low risk of exposure or presence of relative contraindications. 97% (156/161) of travelers to meningococcus-endemic regions of Africa received appropriate meningococcal vaccinations. The rabies vaccine was prescribed for 72 (5%) participants, 20 of whom were traveling for > 28 days. No vaccine-preventable diseases were identified in participants during post-travel follow-up visits. Travelers should be encouraged to schedule pre-travel visits at least 6 weeks prior to departure to allow for sufficient time to complete vaccination series such as JE or rabies. The pre-travel visit is a good opportunity for providing routine vaccines to retirees and dependents.

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POTENCY OF POLYVALENT ANTI-SNAKE VENOM SERUM AGAINST VIPERS AND ELAPIDS VENOMS IN GHANA

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Parenteral preparations "Inoserp® Pan-Africa" (from Veteria Labs, Mexico for Inosan Biopharma, Spain) for activity against venoms of some snakes common in Ghana was evaluated for safety, potency and sterility. *In vitro* demonstration of antibody/toxin reactivity using immunological methods and determination of effective neutralization doses, as well as, *in vivo* demonstration of biological activity by neutralization of snake venom toxins in laboratory mice (ICR strain) were carried out. Results of the experiments revealed that samples of the product submitted for testing are sterile and contains no contaminating microbes. The product contains antibodies that are capable of binding toxins in the venom of the representative Elapidae and Viperidae snake species tested namely *Naja nigricollis* (Spitting cobra), *Bitis arietans* (Puff adder) and *Echis ocellatus* (Saw-scaled viper). The product was thus found to contain antibodies that could neutralize both Elapidae and Viperidae venoms *in vivo* and thus may therefore be used clinically as post-exposure prophylaxis in snake bite victims.

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COST EFFECTIVENESS OF SURVEILLANCE FOR BLOODSTREAM INFECTIONS FOR SEPSIS MANAGEMENT IN LOW RESOURCE SETTINGS

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Bacterial sepsis is a leading cause of mortality among febrile patients in low- and middle-income countries, but blood culture services are not widely available. Consequently, empiric antimicrobial management of suspected bloodstream infection is based on generic guidelines that are rarely informed by local data on etiology and patterns of antimicrobial resistance. To evaluate the cost-effectiveness of surveillance for bloodstream infections to inform empiric management of suspected sepsis in low-resource areas, we compared costs and outcomes of generic antimicrobial management with management informed by local data on etiology and patterns of antimicrobial resistance. We applied a decision tree model to a hypothetical population of febrile patients presenting at the district hospital level in Africa. Focusing on patient survival as the outcome of interest, we used a probability of death of 20.0% for appropriately treated sepsis and 50.0% for inappropriately treated sepsis. Laboratory and treatment cost data were obtained from Tanzania and costs of antimicrobials were derived from World Health Organization data. Costs were inflated to 2011 U.S dollars. Based on the literature, a 13.4% prevalence of sepsis among febrile patients was assumed. Using susceptibility patterns from bloodstream infection studies in Africa obtained by systematic review, we estimated that 44.0% of organisms were covered by a regimen tailored to local etiology and patterns of antimicrobial resistance of bloodstream infections whereas 12.0% of organisms were covered by a generic regimen. We found that the evidence-based regimen saved an additional 1,067 lives per 100,000 persons with fever at a cost of US$43.14 per life saved. Based on World Health Organization recommendations that interventions below a threshold of US$5,705 per Disability-Adjusted Life Year avoided should be pursued, our findings indicate that routine surveillance for bloodstream infections is likely a cost-effective service in the African context.

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EVALUATION OF CHILDREN ADMITTED IN KOROGWE DISTRICT HOSPITAL, TANZANIA FROM 2005 TO 2012 WITH INTENTION TO TREAT FOR MALARIA

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Malaria has been documented over years to be the leading cause of hospital admissions in Tanzania. It has recently been reported that malaria is declining but fever episodes are still the common feature of admission. The study aimed at monitoring children admitted in Korogwe District Hospital (KDH) with intention to treat for malaria. Children below five years admitted with a primary diagnosis of malaria from outpatient department from January 2005 to December 2012 were enrolled into this study after obtaining informed consent and collecting clinical features and performing laboratory investigations which included blood smear for malaria parasites, levels of haemoglobin, lactate and glucose. Treatment was based on the initial assessment at the admission desk and changed accordingly after obtaining laboratory results. A total of 3,260 children were admitted into KDH with an intention to treat for malaria between 2005 and 2012. Out of these, 1,243 (38%) had a confirmed diagnosis of malaria; and 395 (12.12%) had severe malaria. There was a trend of declining malaria admission across years from 2005 to 2012. Febrile episodes remained high across years providing evidence that there are multiple causes of febrile episodes that are non-malaria. A total of 2623 (80.46%) admitted children had fever and 189 (5.8%) had respiratory distress syndrome, 79 (2.5%) were hypoglycaemic and 524 (45.9%) had high lactic acidosis. Moderate and severe anaemia were 42% (95% CI: 40.9% - 44.4%) and 13.9% (95% CI: 12.7% - 15.2%), respectively. The risk of death was associated with day of admission, odds ratio on day of admission being 275 (9% CI: 601 - 1243, p <0.001), glycaemic levels, 18 (95% CI: 6 - 54, p <0.001) for hypoglycaemia, 9 (95% CI: 2 - 31, p<0.001) for hyperglycaemia, 2.4 (95% CI: 1.2-5, p=0.014) for lactic acidosis and respiratory distress, 4.7 (95% CI: 1.8 - 12.2, p=0.001). However, anaemia and malaria parasitaemia were not associated with the risk of death, odds ratio being 2.4 (95% CI: 0.28 - 19.66, p = 0.43) for anaemia and 3.2 (95% CI: 0.5 - 20.0, p = 0.213), respectively. In conclusion, this study has provided evidence that there has been a progressive decline in malaria morbidity for children who are admitted in the paediatric ward. The management of malaria and severe anaemia in this study conformed to the highest standard of care that can be found in a District Hospital in a resource constrained society.

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EFFECTS OF DOXYCYCLINE ADJUVANT THERAPY ON PRIMARY KNEE OSTEOARTHRITIC PATIENTS IN IRAQ EVALUATED USING THE WOMAC INDEX

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Osteoarthritis (OA) is a common chronic joint disease that involves degeneration of articular cartilage and its risk factors are many, including gender, obesity, low vitamin D and increased age. There are limited options to treat or prevent OA in Iraqi subjects, especially those in lower socioeconomic categories. Pre-clinical data suggested that doxycycline might act as disease-modifying agent for the treatment of osteoarthritis with the potential of slowing cartilage degeneration. To examine the short-term effects of doxycycline on knee OA we designed a double blind, placebo controlled study to determine the effects of daily doxycycline 100 mg BID on OA. One hundred forty patients were enrolled and equal numbers were randomly assigned to either treatment or placebo groups. Seventy patients received an oral doxycycline capsule 100 mg twice daily and controls received a starch containing capsule twice daily. The efficacy outcome measured was the change in the WOMAC (Western Ontario and McMaster Universities) index of knee OA over the course of three monthly visits. Our results demonstrated that there were no significant differences between the drug and placebo groups in pain score at the baseline visit (p=0.63) or at the first visit one month later (p=0.29). However, at the second visit, there was a significant reduction in pain score in the drug group compared to placebo group (p=0.028). At three months, the difference between treatment and placebo groups became more significant (p=0.013). Joint stiffness and physical function scores measured at each visit showed no significant differences between study groups. Mean WOMAC scores were reduced at each visit compared to baseline values in doxycycline group compared to the placebo group, and the reduction in mean WOMAC score by the third monthly visit was significantly much greater in the treatment group (8.37±2.1) than in controls (3.83±3.1, p<0.001). In conclusion, oral twice-daily dosage of 100 mg doxycycline demonstrated significant symptomatic benefit in patients with primary knee OA in terms of pain reduction and total WOMAC score.

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RESULTS OF BASELINE OPHTHALMOLOGICAL EXAMINATIONS CONDUCTED IN *ONCHOCERCA VOLVULUS* INFECTED PARTICIPANTS FROM ONCHOCERCIASIS MESO- AND HYPERENDEMIC AREAS IN NORTH-EAST DRC AND GHANA IN A STUDY OF THE EFFECTS OF A SINGLE DOSE OF 8 MG MOXIDECTIN VS. IVERMECTIN

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A study comparing the safety and efficacy of a single dose of 8 mg moxidectin vs. a single standard dose of ivermectin (150 µg/kg) treated 762 males and 411 females, including 79 children of 12-17 years, with ≥10 microfilaria/mg skin in Nord-Ituri (DRC, N=460), Nord-Kivu (DRC, N=472) and Nkwanta North district (Ghana, N=241). Ophthalmological pre-treatment examinations included: detailed history, visual acuity, anterior segment examination with up to x25 slit lamp, dilated fundus examination by direct and indirect ophthalmoscopy, color vision, intraocular pressure, visual field with FDT perimetry, ocular mobility, pupillary reflex, external ocular structures, and number of dead and life microfilaria in the anterior chamber and the cornea. Medical histories were to be reported into the data base if, in the investigator's judgement, they were relevant for assessing post-treatment effects. They were coded according to the Medical Dictionary for Regulatory Activities. All ocular examination results were entered into the data base and their severity graded according to the Onchocerciasis Chemotherapy Research Center Common Toxicity Criteria [grade 0 (normal) - grade 4] or for events not included in these criteria, by method specific grading systems, NCI CTC version 2 or a generic grading system. Substantial variations between the three sites were observed in the percentages of participants with grade 3 or 4 abnormalities in visual acuity and visual field (range 6-16% and 21-40%, respectively). Different types of abnormalities of the anterior and posterior segments were diagnosed in 0-11% and 0-15% of participants,

respectively. Ocular symptoms were reported for 37-75% of participants. The most frequent symptoms (beyond those related to vision itself) reported by the participants or detected at the baseline examination were eye pruritus (6.6-40.2%), blurred vision (6.1-30.3%), corneal disorders (0-8.0%), eye pain (2.5-6.6%), conjunctivitis (0-1.9%).

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DELAY IN HANSEN'S DISEASE DIAGNOSIS IN THE UNITED STATES

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The burden of Hansen's disease (HD) (leprosy) has declined worldwide but it remains endemic in many less-developed countries. In developed countries, HD is rare, mostly associated with immigrants from endemic areas. In the United States, 72% of 213 HD cases reported in 2009 were foreign born. Untreated HD can progress to a severely debilitating disease, involving functional loss of extremities and disability. Risk of impairment increases with longer delay between onset of symptoms and disease diagnosis. We identified risk factors associated with longer interval between HD symptom onset and diagnosis in the United States. HD cases registered with the National Hansen's Disease Program and diagnosed during 2000-2010 (n=1,241) were included in the analysis. Cox proportional hazards models were used to assess median times between symptom onset and diagnosis. Foreign-born persons whose symptoms began before their arrival in the United States had a longer delay in diagnosis than U.S-born and foreign-born cases with onset after arrival (3.3 years vs. 1 year, $p<0.05$). U.S-born patients residing in states with an HD specialized ambulatory care clinic experienced a shorter delay to diagnosis, compared with those with no clinic nearby (0.9 vs. 2.3 years) ($p<0.05$). Proximity to a HD clinic did not affect delay to diagnosis among the foreign born. Among the U.S-born delay in diagnosis was significantly ($p<0.05$) longer for those whose symptoms began when they were 30-44 years old (2.3 years) compared to other age groups. For the foreign-born the longest delay (3 years) was for those with onset with onset at ≤15 years old (3 years). Better understanding of the reasons for delayed diagnosis is needed, particularly for younger foreign-born patients and for those with symptom onset before U.S arrival. The role of enhanced HD screening and education during immigration-related medical examination needs to be assessed. For the U.S-born, improved access to HD clinics should be explored. Increased HD awareness and outreach to physicians are also needed, especially in states without HD clinics and in areas with large foreign-born populations.

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PHYSIOLOGICAL ROLE AND CHEMOTHERAPEUTIC POTENTIAL OF THE ECDYSONE RECEPTOR HOMOLOGUE OF THE HUMAN PARASITE *BRUGIA MALAYI*

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A homologue of the ecdysone receptor (Ecr), a master regulator of development in insects has previously been identified and shown to be responsive to 20-OH ecdysone in transfected *Brugia malayi*. As the EcR is not found in vertebrate animals, it and the regulatory pathways it controls represent an attractive potential chemotherapeutic target. To further delineate the physiological role that the EcR plays in filarial parasites, adult female parasites were treated with 20-OH ecdysone in culture and microfilarial output and embryograms monitored in treated and control parasites. Females treated with 20-OH ecdysone produced significantly more microfilaria that control worms, implicating the EcR in regulation of microfilarial development. RNAseq of the transcripts of adult females treated with 20- OH ecdysone was also conducted to observe changes in gene expression. RNAseq identified 67 genes whose expression was genes significantly up-regulated in the treated parasites compared to untreated controls. A mammalian two hybrid system was used develop a high throughput assay to identify agonists and antagonists of the filarial EcR. This assay was based upon a mammalian two hybrid system involving BmEcR Gal4 and RXR VP16 fusion constructs and a GAL4 Gaussia luciferase reporter. The fusion constructs heterodimerize and when bound to the cognate ligand, activates transcription of the luciferase reporter. On induction with 20-OH ecdysone, transactivation of the luciferase gene was seen in triply transfected mammalian cells, with a signal to noise ratio of roughly 6 and Z' of the assay is 0.7. This assay will be employed to screen natural product libraries and a collection of ecdysone analogs in the near future.

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INVESTIGATING *ONCHOCERCA VOLVULUS - WOLBACHIA* BACTERIAL ENDOSYMBIONTS COPY NUMBER VARIATIONS AFTER IVERMECTIN TREATMENT

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Onchocerca volvulus - causative agent of onchocerciasis - harbour *Wolbachia* bacterial endosymbionts (wOv), whose activities are vital for worm survival. For instance, doxycycline treatment caused parasite mortality due to wOv death. In addition, co-evolution wOv and worm genomes, and lateral gene transfers have been reported. Despite this survival benefit, "un-interrupted" worm autophagy mechanisms regulate wOv copy numbers to achieve a homeostatic balance. Here, we investigate whether interruptions in worm biology, such as ivermectin (IVM) treatment, can lead to changes in wOv copy number. We used a relative real time quantitative PCR assay to measure wOv copy number variations (CNV) between IVM treated and naïve worms. The *Wolbachia* surface protein gene (wsp) for wOv and the glutathione reductase gene (gr) for worm are single copy, therefore gene copy numbers were equivalent to genome copies. In all, 143 adult parasites (34 naïve and 109 treated), sampled from Ghana, were assayed. Treated parasites had significantly reduced variance of wsp/gr ratio compared to naïve ones ($P = 0.001$). Also, treated parasites had a lower median wsp/gr ratio (4.25, S.E. = 2.31) than naïve (10.36, S.E = 10.11), though difference not statistically significant ($P = 0.531$). These results may be indicative of IVM treatment influencing the worm wOv copy numbers.

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RAPID, SENSITIVE DETECTION OF FILARIAL DNA WITH MINIMAL EQUIPMENT

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Currently, molecular xenomonitoring efforts for lymphatic filariasis (LF) rely on the PCR or RT-PCR-based detection of *Brugia malayi, Brugia timori* and *Wuchereria bancrofti.* Typically, extraction of DNA from infected mosquitoes is performed using the column-based DNeasy Blood and Tissue Kit from Qiagen. However, this extraction is both time consuming and expensive, and the diagnostic testing which follows requires expensive thermal cyclers or Real-Time PCR instruments. Such expenses make these molecular tests impractical for laboratories within many endemic areas. Accordingly, in such locations, there exists a substantial need for an inexpensive, equipment-minimizing diagnostic option. In this work, we evaluate a crude, NaOH extraction method for the isolation of template DNA that minimizes the presence of inhibitors, in conjunction with an isothermal DNA amplification method that requires minimal laboratory equipment. This simple extraction reduces the total cost of the DNA isolation by 50%. We show that for pools of up to 25 mosquitoes, spiked with one infected *B. malayi* or *W. bancrofti* L3 worm, detection

by real-time PCR, and tHDA isothermal amplification are equally sensitive using both NaOH extraction, and Qiagen-based extraction methods. Furthermore, as a portable and simple diagnostic assay, tHDA isothermal amplification can be coupled with HybriDdetect® test strips, enabling detection of amplified *B. malayi* DNA with equal sensitivity to gel electrophoresis product visualization. By eliminating the need for expensive equipment without compromising sensitivity, this assay and product detection combination provides a diagnostic alternative for endemic locations lacking the means to perform the molecular assays currently employed for xenomonitoring purposes.

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APPLICATION OF RECOMBINANT HUMAN ANTIBODY FOR VALIDATION AND QUALITY CONTROL OF SEROLOGICAL ASSAYS IN SUPPORT OF ONCHOCERCIASIS ELIMINATION PROGRAMS

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Monitoring progress toward onchocerciasis or river blindness elimination has largely relied on the detection of microfilariae in skin snips of infected patients, a technique that presents challenges: acceptability, timing with mass drug administration (MDA) interventions, and sensitivity when the disease burden is low. The detection of antibodies to *Onchocerca volvulus* (Ov)-specific antigens provides an alternative and proven approach that requires only capillary blood from a finger stick and where the sensitivity of antibody detection is not dependent on timing with recent MDA. One challenge with serological assays is the availability of a reliable and consistent source of positive control. To address this issue, a recombinant, monoclonal human IgG4 antibody was developed to the antigen Ov16. With anti-Ov16 IgG4 ELISA-based serological assays already widely used to confirm elimination in the Americas and parts of Africa, the recombinant Ov16-specific human IgG4 antibody could be used for standard curves on ELISA-based assays and as a quality control reagent for an Ov16 rapid diagnostic test (RDT). The performance of two ELISA tests for anti-Ov16 human IgG4 was assessed when using the recombinant IgG4 antibody, plasma samples, and dried blood spots. The data demonstrates that use of a horseradish peroxidase-based (HRP) ELISA results in a lower limit of detection than the currently used alkaline-phosphatase-based ELISA format. A dried-down formulation of the recombinant antibody was developed and used under field conditions without refrigeration as a quality control for Ov16 RDTs. Stability was demonstrated for over 15 weeks, unrefrigerated. The recombinant human anti-Ov16 IgG4 antibody will be useful for inter-laboratory validation of ELISA assays and as a quality control reagent for RDTs at different points of the supply chain from manufacturer to field use.

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EXPRESSION PATTERNS OF NICOTINIC ACETYLCHOLINE RECEPTORS (NACHRS) IN *BRUGIA MALAYI* FILARIAL WORMS

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Nematode nicotinic acetylcholine receptors (nAChRs) are the targets for a number of anthelmintics including levamisole, pyrantel, morantel and oxantel. The levamisole AChR type is composed of five subunits, Cel-unc-29, Cel-unc-38, Cel-unc-63, Cel-lev-1 and Cel-lev-8 in C. elegans. Nine nAChRs are present in the *Brugia malayi* genome including orthologues of Cel-unc-29, Cel-unc-38, and Cel-unc-63. We studied expression these genes by qRT-PCR in *B. malayi* adult female, male, and microfilariae. Six of eight nAChRs genes studied were differentially expressed. Four were more highly expressed in males, 1 in females, and 1 in microfilariae. We performed *in situ* hybridization with cRNA probes to localize expression of five *B. malayi* nAChRs genes in adult worms. Most of them had similar expression patterns with signals in developing embryos, spermatogonia, the uterine wall, vas deferens, and lateral chords. For example, Bm1_35890, an orthologue of cel-unc-29 encoding the subunit of levamisole-sensitive receptors that had equal expression in male and female worms by qRT-PCR, was strongly expressed in both male and female worms. In females, strong expression signals were detected in the ovary, developing embryos and lateral hypodermal chords, with moderate expression in the uterus wall adjacent to stretched microfilariae. Expression signals in males were strong in spermatogonia and in the wall of vas deferens. Expression patterns for the novel gene Bm1_48815 (with no orthologue in other nematodes and equal expression in males and females) were similar to those of Bm1_35890. Anti-peptide antibody to Bm1_48815 bound to the same tissues that were labeled by *in situ* hybridization, but the antibody also bound stronngly to body muscle in both male and female worms. Increasing evidence suggests nicotinic receptors regulate developmental events in the nervous system and neuronal AChRs may play important developmental roles as the receptors are expressed early during embryogenesis in vertebrates. The expression patterns of these genes suggest that they are involved in reproduction, and this may explain the effect of drugs that target nAChR on reproduction in filarial worms.

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HIGH CONTENT IMAGING: MORE THAN A PRETTY PICTURE

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The A·WOL consortium aims to find a novel macrofilaricidal drug to treat the debilitating diseases lymphatic filariasis and onchocerciasis, through targeting the *Wolbachia* bacteria that reside within these parasitic nematodes. Typically, screening chemical libraries directly against parasitic nematodes is cumbersome, low throughput and relies on animal reservoirs. By focusing on the endosymbiont, we have been able to utilise an insect cell-based screening approach that originally relied upon a quantitative polymerase chain reaction readout, but now employs a High Content Imaging readout running on the Perkin Elmer Operetta® platform. This assay uses texture analysis of cells stained with SYTO®11 (fluorescent DNA stain) as a direct measure of bacterial load and allows the consortium to screen up to 10x 384 well plates per day; a radical increase in throughput from the qPCR screen. Further to its use as a screening tool, the Operetta® is also being used for more fundamental biological investigations such as experiments surrounding the infection dynamics within host cells and, as part of a separate project aiming to create a novel cell-based screen for discovery of a macrofilaricide, the development of a filarial nematode cell line. The nature of the anti-*Wolbachia* screening approach will be presented in addition to preliminary data from other High Content Imaging investigations surrounding *Wolbachia,* host cells and cell line development.

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A•WOL MACROFILARICIDAL DRUG DISCOVERY AND DEVELOPMENT - OPTIMIZATION OF ANTI-*WOLBACHIA* EFFICACY

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There is an urgent need to develop a novel treatment for filariasis, and targeting *Wolbachia* provides safe macrofilaricidal activity with superior therapeutic outcomes compared to standard anti-filarial treatments. The Anti-*Wolbachia* (A·WOL) Consortium has developed both *in vitro* and *in vivo* assays, to screen chemical libraries for anti-*Wolbachia* activity. The outputs from the A·WOL program are now being pursued as part of A·WOL II Macrofilaricide Drug Discovery & Development programs. Screening of >10000 compounds from the BioFocus library and chemoinformatic analysis have generated six independent lead series chemotypes with the potential to enter a medicinal chemistry "hit-to-lead" and lead optimization program. A·WOL Drug Discovery is now progressing these lead series through a rigorous lead optimisation and candidate selection process, using iterative cycles of medicinal chemistry and biological testing in order to deliver at least one novel pre-clinical candidate and a chemically distinct back-up, aligned with our Target Product Profiles for an anti-*Wolbachia* macrofilaricide. In addition, ongoing screening of large diversity-based libraries (150-500k compounds) aims to provide additional, chemically diverse hits, with one-order improvement in absolute potency or significant shortening of treatment time, in order to expand the structural diversity of anti-*Wolbachia* chemotypes. A·WOL Drug Development is optimising regimens of anti-*Wolbachia* monotherapy and combination treatment of registered anti-*Wolbachia* and anti-filarial drugs *in vivo* using an adult *Brugia malayi* mouse model. This efficacy testing is driven by a rational PK/PD modelling approach which supports dosage regimens, in order to identify the best treatment regimens to test in field trials.

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THE DEPLETION OF *WOLBACHIA* FROM *BRUGIA MALAYI* MICROFILARIAE AND SUBSEQUENT EFFECT ON THE DEVELOPMENT OF INFECTIVE LARVAE IN *AEDES AEGYPTI*

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The filarial nematode *Brugia malayi* is a human pathogen that harbours the bacterial endosymbiont, *Wolbachia.* Tetracycline treatment causes the depletion of *Wolbachia* leading to infertility and early death in the adult worm. If tetracycline treatment is ongoing at the time of infection, development from the L3 stage to adult is inhibited. The depletion of *Wolbachia* in microfilariae (Mf) has been shown to affect development to L3 infective larvae in the intermediate host *Litomosoides sigmodontis.* To assess the effect of tetracycline treatment on *B. malayi* microfilariae and development to the L3 infective stage within the mosquito, *Aedes aegypti,* infected gerbils (*Meriones unguiculatus*) were treated for 2, 4 and 6 weeks. At each time-point, Mf were extracted and fed to mosquitoes and the subsequent development to L3 larvae was assessed versus controls. To further understand the relationship between *Wolbachia* and L3 development, live Mf were directly visualised within the mosquito midgut and also proteomic analysis was conducted of secretory/excretory products from treated and control Mf. The results show the significant effect of *Wolbachia* depletion on the ability of Mf to progress to L3 stage and treated Mf are unable to escape the mosquito midgut. The retardation of L3 development by tetracycline may have an important additional blocking effect on the transmission of filarial parasites and understanding the mechanism by which this occurs may offer further insight into the important role *Wolbachia* play in each of the life-cycle stages of *B. malayi*.

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MICRORNAS IN PARASITIC NEMATODES - DEFINING A FUNCTION

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We previously identified a large number of microRNAs in the filarial nematode *Brugia* species and in *Haemonchus contortus*, a sheep nematode related to human hookworms. Recent experiments have sought to define the function of two microRNAs, *Bpa-mir-5364* and *Hco-mir-5352*. The Brugia miRNA was selected for study on the basis of an intriguing expression pattern, being ~x12-fold increased between vector derived L3 and L3 recovered at 24 h post-infection. This miRNA is a novel member of the *let-7* family. Using target prediction programs combined with a comparative genomics approach we sought to identify predicted mRNA targets, and selected three of these for further analysis. We established a dual luciferase assay in transfected HEK cells to examine the relationship between *Bpa-mir-5364* and the 3'UTR of the predicted targets and used anti-sense oligonucleotides to attempt to inhibit *Bpa-mir-5364 in vitro*. For *H. contortus*, we focused on a single miRNA that is parasite-specific and only found in nematode parasites that live in the gastrointestinal tract (irrespective of nematode clade). *Hco-mir-5352* is one of a cluster of four microRNAs that is conserved in a number of gastrointestinal nematodes. We could detect *Hco-mir-5352* in adult worm excretory-secretory products and in tissues from infected sheep, indicating that it may be released by the parasite *in vivo* and thus could interact with a host mRNA. A predicted target of *Hco-mir-5352* is mammalian CD69 and we have used the dual luciferase assay in an attempt to confirm this relationship. An improved understanding of the function of parasite microRNAs casts novel light upon the intricacies of the host-parasite relationship.

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DIRECT VEGF-SPECIFIC ANTI-ANGIOGENIC ACTIVITIES OF THE ANTI-*WOLBACHIA* DRUGS, DOXYCYCLINE AND MINOCYCLINE, IN AN *IN VITRO* MICROVASCULAR BLOOD AND LYMPHATIC ENDOTHELIAL CELL CULTURE SYSTEM

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Lymphatic filarial infection (LF) is the cause of elephantiasis and hydrocoele lymphoedemas (LE). LE is initiated as a result of episodic inflammatory damage and remodelling of parasitized lymphatics. Heightened inflammatory responses in LE patients are associated with pro-angiogenic/lymphangiogenic molecules in circulation, such as vascular endothelial growth factors (VEGFs), which suggests inflammatory angiogenesis is relevant in the pathophysiology of LF. Current mass drug administration treatments for LF have mainly microfilaricidal activities and do not help alleviate the suffering of current LE patients. As such, there is an unmet need to identify new therapeutics to help reduce LF morbidity. Encouragingly, in two recent trials, 6-week doxycycline therapy was identified to improve LE grade after 12-24 months. The anti-morbidity mode of action (MoA) of doxycycline has not been defined. LE grade is improved both in doxycycline treated patients with and without active LF infection, suggesting a separate MoA to that of targeting the filarial inflammatory endosymbiont, *Wolbachia.* We developed an *in-vitro* blood and lymphatic endothelial cell culture system to assess the VEGF-specific anti-angiogenic activities of the anti-*Wolbachia* compounds, doxycycline and minocycline. Utilising a 96 well/200 µL based Operetta™ (Perkin Elmer) fluorescent bio-imaging system we have stimulated the proliferation of

human adult dermal microvascular blood endothelial cells (HMVECd) and equivalent lymphatic endothelial cells (HMVECdLy), using VEGFs targeting VEGF receptors 1-3. Titrations of both doxycycline and minocycline toward physiological plasma levels have demonstrated a dose-dependent reduction in both VEGF-specific HMVECd and HMVECdLy proliferation. This data supports a direct anti-angiogenic MoA of second-generation tetracyclines in use for the treatment of LF, which may target pathological inflammatory VEGF processes. The bio-imaging platform has been validated for onward screening to identify new candidate compounds with VEGF-specific anti-angiogenic activities.

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ONCHOCERCIASIS: TRANSCRIPTOMIC AND PROTEOMIC APPROACHES FOR IDENTIFYING BIOMARKERS ASSOCIATED WITH THE PRESENCE OF VIABLE ADULT FEMALE PARASITES FOR POST-CONTROL SURVEILLANCE

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Control of onchocerciasis or 'river blindness' has had considerable success in Africa. As efforts shift from control to disease elimination better tools are needed to identify viable adult females (OvAF) for the "end game" needs. The goal of this project is to identify host- and/or OvAF-specific biomarkers for use in rapid field-friendly tests during post-control surveillance programs. By taking advantage of the recently sequenced genome and assembly of Onchocerca volvulus (~97.4Mb) organized by optical mapping into chromosomes, we have overlaid RNAseq analyses of the important mammalian stages of O. volvulus (Ov) L3, adult males, adult females and microfilariae. Of the predicted 12994 genes, a total of 12416 transcripts were identified from all stages, with over 9600 adult female transcripts. Analysis of transcripts across all the stages resulted in the identification of 75 select gene products that were abundant in OvAF and absent or minimally expressed in other stages. In parallel, shotgun proteomics of the somatic extracts from each of the Ov mammalian stages (including excretory-secretory proteins of OvAF) are in progress using UPLC MS/MS (Thermo Easy nLC 1000 UPLC coupled to Thermo Q-exactive quadrupole-Orbitrap mass spectrometer). Of even more interest is the proteomic analysis of O. volvulus infected human sera in which hundreds of Ov-specific proteins have been identified, of which four are among the female-specific proteins found during the transcriptomic analysis. Recombinant antigens and monospecific polyclonal antibodies are being made to test the validity of these targets as biomarkers for use in post-control surveillance assay systems.

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THE MUTUALISTIC SYMBIOSIS OF *WOLBACHIA* AND THE FILARIAL NEMATODE *BRUGIA MALAYI* - UNRAVELLING THE PROTEOME AND TRANSCRIPTOME

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The parasitic nematode *Brugia malayi* is a causative agent of lymphatic filariasis, a disfiguring disease affecting over 120 million people worldwide. *B. malayi* exists in a mutualistic symbiotic relationship with the α-proteobacterium *Wolbachia*. We have applied a global proteomic profiling approach to investigate the molecular basis of this symbiosis. Adult female *B. malayi* in the mammalian host *Meriones unguiculatus* were sampled at multiple time-points post-antibiotic depletion. Deep proteome mining combined with high-resolution mass spectrometry was used for comprehensive proteome profiling of *Wolbachia*/worm at these selected time-points. *Wolbachia*/worm ratios were also monitored by qPCR. Using a combination of extensive peptide pre-fractionation and established proteomic workflows we observed improved proteome coverage by an increase in peptide/protein identification. Such proteomic approaches coupled with reversed phase liquid chromatography and analysis by high-resolution mass spectrometry provides a powerful tool for global proteome profiling. Proteins of interest from this initial global proteomic 'screen' have been further investigated using a targeted proteomics approach. Following basic analysis through established bioinformatics pipelines, transcriptomic, proteomic, and published datasets will be integrated in a systems biology approach with the objective of understanding the molecular basis of the mutualistic *Wolbachia*/*B. malayi* symbiosis.

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IDENTIFICATION OF CHEMOSENSORY RESPONSES IN THE FILARIAL WORM, *BRUGIA MALAYI*

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Lymphatic filariasis (LF) is a disease caused by mosquito-borne filarial nematodes including *Wuchereria bancrofti* and *Brugia malayi*. Although over 120 million people suffer from this disfiguring disease, chemotherapeutic options for LF are limited to three drugs: diethylcarbamazine citrate, albendazole and ivermectin. The threat of drug resistance combined with the inefficacy of these drugs against adult parasites highlights the need for new anthelmintic drugs. Chemosensation is an essential behavior used by multi-cellular organisms to interact with the environment. In the free-living nematode, *Caenorhabditis elegans*, chemosensation plays a crucial role in development, avoiding noxious conditions and finding food and mates. In parasitic nematodes, chemosensation is thought to play a critical role in host-seeking and host-invasion behaviors making genes involved in this system attractive targets for drug or vaccine development. However, little is known about the chemosensory system in filarial parasites of animals. In this study, we sought to elucidate chemosensory-induced behavior in *B. malayi* during the infectious L3 stage of the parasite. Scanning electron microscopy revealed that amphids, the major chemosensory organs of nematodes, are present in both juvenile and adult stages of *B. malayi*. In addition, orthologues of several genes known to be involved in chemosensory behavior in *C. elegans* were identified in *B. malayi* based on sequence homology. Finally, we identified over 10 chemical compounds that were either attractive or repellent to *B. malayi* L3 stage parasites using chemotaxis assays. This research is the first study to demonstrate that *B. malayi* has a responsive chemosensory pathway. In addition, the results obtained indicate that the chemosensory response in *B. malayi* plays an important role in host-seeking and host-invasion behavior, and therefore is a prime candidate for chemotherapeutic intervention.

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GENOMICS OF *WUCHERERIA BANCROFTI* FROM FOUR ENDEMIC REGIONS OF INFECTIVITY: HAITI, KENYA, MALI AND PAPUA NEW GUINEA

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Lymphatic filariasis (LF) is a major threat to human health in the tropics, leading to the disfiguring and debilitating conditions hydroceles and

elephantiasis. There are approximately 1.2 billion people living in LF endemic countries where 120 million people currently suffer from the disease. The parasites that cause LF are separated into two genera, *Brugia* and *Wuchereria*, with *W. bancrofti* (Wb) responsible for ~90% of LF cases. Until recently, Wb has been neglected in genomic studies due to complications in sample collection and preparation, such as lack of adult stage samples. Our previous work has shown it is possible to concentrate Wb microfilaria from an infected blood sample resulting in high quality genomic sequence. Here we expand our previous studies in Papua New Guinea to three more endemic areas of Wb infectivity: Mali, Kenya, and Haiti. We report on thousands of unique single nucleotide polymorphisms (SNPs) and identify genes that are highly variable among localities. We utilize discovered SNPs to *i)* construct an assay to identify the geographic source of new Wb outbreaks, *ii)* explore the historical context of admixture between endemic areas, retracing the possible routes of Wb migration, and *iii)* root the gene trees of Wb to identify the species origin. Our results provide a new context for studying Wb infection by identifying endemic areas of high genetic diversity that may hinder elimination.

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CHARACTERIZATION OF PUTATIVE *BRUGIA MALAYI* ACETYLCHOLINE-GATED CHLORIDE CHANNELS

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The neglected tropical disease, lymphatic filariasis (LF), affects over a million people and an even greater number of individuals are at risk for this disease in endemic tropical regions. The filarial nematodes, *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori*, can cause severe lymphedema leading to gross disfigurement in the infected human host. Mass Drug Administration (MDA) programs deliver chemotherapeutics diethylcarbamazine, ivermectin, and albendazole, annually to endemic areas to manage and treat this disease. Despite success with MDA, growing concerns of resistance combined with the inefficacy of these drugs against the adult stages of the parasites emphasizes the need for novel and broadly effective antifilarial chemotherapeutics. Cholinergic neuro transmission is a proven source of effective anti-nematodal drug targets. Although much is known about Na+ conducting nicotinic acetylcholine receptors, the family of acetylcholine-gated chloride channels (ACC) is poorly understood. We used reverse genetics to investigate *Brugia malayi* acetylcholine chloride channels (BACCs), which are a novel family of ACCs. Transcriptomics, homology based searches, and 5' and 3' RACE-PCR identified 8 putative BACCs. qRT-PCR results revealed that three BACCs, BACC-1, BLGC-46, and BLGC-47, are expressed in both juvenile and adult stages. Results from *in situ* hybridization demonstrate that BACCs are distinctly localized. We are further probing the potential function of BACC-1, BLGC-46, and BLGC-47 by using RNA interference and microfluidics assays. Our results show that BACCs have similar characteristics to other ACCs thus these genes are prime candidates for further investigation as novel chemotherapeutic targets.

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CASE REPORT: FOUR-YEAR-OLD FEMALE FROM INDIA WITH NIGHTTIME COUGH, PULMONARY INFILTRATES AND AN ABSOLUTE EOSINOPHIL COUNT OF 53,000

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A four-year-old female with a prior history of failure-to-thrive, who recently emigrated from a lymphatic filariasis endemic area in Northern India, was admitted to the pediatric floor of Mount Sinai Medical Center with low-grade fevers and night-time cough for over four weeks, diffuse bilateral centrilobular ground glass opacities on chest CT and an absolute eosinophil count in excess of 53,000. Blood smear examination was negative for parasites. Filariasis Bm14 ELISA (IgG4) performed at the Centers for Disease Control was positive at 100 units per microliter (upper limit of normal 12.4 units per microliter). Clinical presentation was most consistent with Tropical Pulmonary Eosinophilia secondary to *Wuchereria bancrofti*. Serology was also strongly positive for *Strongyloides* and *Toxocara,* and was weakly positive for *Trichinella*. She was treated with two days of oral ivermectin 200 micrograms/kilogram per day for the *Strongyloides*, then 21 days of oral diethylcarbamazine 6 milligrams/kilogram divided three times a day for the suspected *W. bancrofti* and finally 14 days of albendazole 15 milligrams/kilogram per day for the *Toxocara*. Seven weeks after completing therapy, the patient clinically improved and her absolute eosinophil count was reduced to 3300. Tropical Pulmonary Eosinophilia is a rare diagnosis in children in the United States. Blood smears for *W. bancrofti* are negative in the setting of Tropical Pulmonary Eosinophilia, so history, identification of epidemiologic risk, and antibody testing are the keys to appropriate diagnosis. The case also demonstrates the importance of checking for other parasites in patients from an area co-endemic for multiple parasites who have been identified to already have a particular parasitic disease, as polyparasitism is not uncommon.

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A CASE REPORT: SUBCONJUNCTIVA EYE WORM

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We report a case of ocular *Loa loa* (10/9/2013) in a 46 year old Cameroonian Male who reported a history of a motile foreign body sensation in right eye for five years. Examination of patient's right eye showed live Loa Loa worm in subconjunctiva space. The worm was extracted by making a small incision in the conjunctiva with a scalpel and removing the worm with small forcep. Patient was treated with Albendazole after the worm removal.

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HIGH THROUGHPUT SCREENING FOR ANTI-*WOLBACHIA* DRUGS TO TREAT ONCHOCERCIASIS AND LYMPHATIC FILARIASIS

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The Anti-*Wolbachia* consortium (A•WOL) at Liverpool School of Tropical Medicine (LSTM) has partnered with the Global High Throughput Screening (HTS) Centre at AstraZeneca in the first open access HTS project for the World Intellectual Property Organization's (WIPO) Re:Search program against Neglected Tropical Diseases. The A•WOL consortium aims to identify novel macrofilaricidal drugs targeting the essential bacterial symbiont (*Wolbachia*) of the filarial nematodes causing onchocerciasis and lymphatic filariasis. The project aimed to scale-up the throughput of the A•WOL cell-based screening assay using AstraZeneca's leading automation, screening technologies and expertise. The A•WOL screen at LSTM uses a DNA fluorescent (SYTO®11 stained), *Wolbachia* infected C6/36 *Aedes* albopictus cell line imaging assay (running on the Perkin Elmer Operetta® platform) to screen up to 10x 384 well plates per day. The development of a higher throughput screen at AstraZeneca has dramatically evolved the assay protocol, through the optimisation of the cell culture, assay analysis, washing methodology and plate reading technologies. The process is now fully automated from cell and compound addition through to data analysis. The validation of this assay will allow screening of AstraZeneca's chemical library of compounds in

a single screening activity, dramatically increasing our throughput. Hits identified will then be progressed through the A•WOL drug discovery and development programme for new macrofilaricides.

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ECONOMIC COSTS AND BENEFITS OF MORBIDITY MANAGEMENT AND DISABILITY PREVENTION FOR LYMPHATIC FILARIASIS

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Lymphatic filariasis (LF) is endemic in 73 countries, with 120 million people infected, of whom 40 million suffer serious disability, including lymphedema of the legs (15 million), arms, and breasts; hydrocele in 25 million men; and acute attacks of fever and disabling pain that last several days, termed adenolymphangitis (ADL), which contribute to worsening lymphedema and substantial productivity loss. The Global Programme to Eliminate Lymphatic Filariasis (GPELF) has two goals: interrupting LF transmission by 2020 and caring for people already infected through morbidity management and disability prevention (MMDP). While 53 countries have ongoing mass drug administration, only 27 had begun MMDP by 2013, in part, due to the perceived high cost and low return on investment for MMDP. To address this concern, we estimate costs and benefits of MMDP for the global population affected and at risk over the lifetime for affected cohorts. MMDP changes the distribution of disability and productivity over time. Actual program costs are based on 3 delivery modes: community-based care (CBC), referral to primary care clinics, and specialty clinics. We calculate societal costs of untreated morbidity due to acute attacks, chronic lymphedema and hydrocele, consequent lost wages, and other costs. In each country with cost comparisons, research to date suggests societal cost of untreated LF far exceeds cost of MMDP. In India, patients enrolled in CBC morbidity management averaged 29 fewer lost work days per year. In Haiti, ADL was reduced substantially after simple training in self-care. A program in Togo, after 3 years of MMDP, reported stabilization of lymphedema stage and slight decrease in ADL. In Ghana, patients reported substantial improvement in work capacity after hydrocele surgery. We will combine results from these and other countries and adjust costs and benefits to each context, to estimate savings in direct costs, productivity loss, and marginalization that provide economic rationale in addition to the ethical mandate for MMDP, the second pillar of GPELF.

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LOA LOA IN A CONGOLESE REFUGEE WOMAN IN MINNESOTA

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An estimated 13 million people are living with the neglected tropical disease Loiasis. The *Chrysops* deerfly vector can be found in rainforest canopies of Central and West Africa. Loa loa microfilaria migrate into subcutaneous tissue, maturing into adult worms, mating, and producing more microfilaria for more than a decade in a human host. This could cause a refugee to experience intermittent symptoms for many years after resettlement to the US. Calabar nodules (pruritic, non-tender skin swellings) and white worms moving across the visual field should lead to the search for *Loa loa*. A 49 year old Congolese refugee presented with 2 days of a "needly" sensation in her right eye after 6 days of left eye pain with periorbital swelling. She had temple pain and intense pruritis in her palms and soles. She reported numerous episodes of migratory pruritic swellings on her left forearm and foot and a sensation of threads moving across her vision in the previous 2 years. While living near Kinshasa 9 years earlier, she was treated with an herbal poultice that allowed tiny white worms to be removed from her eyes. Her husband has been treated for onchocerciasis in the past. An NIH onchocerciasis antibody test was negative, and a slit-lamp exam found no evidence of coinfection. Her filarial serologies were consistent with a past or acute filarial infection. She was treated with 3 weeks of DEC, causing only mild nausea. Her eosinophilia decreased from an AEC of 800 to 200 after treatment. There is a 50% chance that this course will have treated her filarial infection definitively. This woman suffered from Loiasis, an illness that can plague patients with intermittent symptoms for over a decade. Although short-term travelers are rarely infected, the current influx of Congolese refugees to the US heightens the importance of Loiasis awareness. As onchocerciasis can be endemic in the same regions, coinfection must be considered when selecting treatment for Loiasis to avoid the severe Mazzotti inflammatory reaction that occurs when given DEC while infected with onchocerciasis.

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ORAL FLUBENDAZOLE: A POTENTIALLY USEFUL MACROFILARICIDE

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The search for a macrofilaricide that can enhance the goal of elimination of filarial infections, and the diseases they cause, is a current and relevant goal. The most attractive approach to macrofilaricide discovery is to re-purpose agents already in use in humans. High amongst these potential candidates is flubendazole, a drug that was shown in a screening survey in the early 1980s to be effective against *Onchocerca volvulus* in humans but was difficult to administer due to the properties of the formulation used. The need to reformulate this agent to overcome the issue of insolubility and make it suitable for use as an oral administration was a goal regarded as important for field program use of this anhelminthic in filariasis. We will discuss here results obtained with a new formulation of flubendazole that achieves, after a single oral doses of 2-6 mg/kg, a plasma concentration of between 1-3µg/ml with a peak plasma level at 3-5 hr which decreases to control levels by 12-20hr in rodents. Using the *Litomosoides sigmodontis* model in gerbils, we have achieved >90% killing of adult worms when the animals were assessed 9 weeks after treatment with 6 mg/kg of the oral formulation of flubendazole given daily for 5 days. *In vitro* incubation of *Brugia sp.* with pharmacologically-relevant concentrations of flubendazole caused alterations in the internal organs of the adult worms. Histological observations in both filarial species exposed to flubendazole *in vitro* and *in vivo* showed that several structures in the worms were affected by the drug; the early developing forms in the uterus were the most affected component, and hypodermal cells were also significantly damaged when worms were incubated at concentrations of flubendazole equivalent to the plasma levels obtained after oral dosing. The beneficial effect of treatment with oral flubendazole on host tissue pathology induced by the presence of the parasite was marked when comparing untreated animals to flubendazole-treated animals 9 weeks after dosing. The degree of tissue and organ changes were scored subjectively, and the histopathology occurring examined and scored histologically. The very significant reduction in pathology in animals treated with 6 mg/kg flubendazole suggests that the death of adult worms induced by this drug is not likely to invoke severe adverse events in the host. Our study shows that oral flubendazole can safely kill the majority of adult filariae in this experimental model.

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ONCHOCERCA LUPI: AN EMERGING ZOONOSIS IN NORTHERN EUROPE AND THE UNITED STATES?

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The geographical distribution of cases caused by *Onchocerca lupi,* a common parasitic worm in wolves, has widened during the last decade. Moving from Southern European countries such as Greece and Turkey, an increasing number of cases have begun to emerge in Northern Europe and the Americas. Common arthropod vectors for *O. lupi* include blackflies (e.g., *Simulium yahense* and midges (e.g., *Culicoides* spp.). *O. lupi* commonly causes ocular problems such as conjunctivitis, photophobia, and excessive lacrimation. In recent years, however, clinicians have identified cases that have involved extra-ocular sites such as the spinal canal. As of 2014, more than sixty cases of *O. lupi*, ocular and otherwise, have been identified throughout Europe and the United States. This presentation will examine not only the global epidemiology of *Onchocerca lupi* but potential surveillance measures for this emerging zoonosis.

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OPTIMIZATION OF THE *ACANTHACHEILONEMA VITEAE* LIFE CYCLE FOR INTENSIVE PRODUCTION

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The filarial nematode *Acanthacheilonema viteae* is of great interest to the filariasis community because it lacks the endosymbiotic *Wolbachia* bacterium found in several filarial parasites of humans. The NIH/NIAID Filariasis Research Reagent Resource Center (FR3) began distributing A. viteae to its users in 2011. The FR3 strain of A. viteae and of its tick vector *Ornithodoros tartakowskyi* were originally acquired from TRS Laboratories and the life cycle is currently being optimized for intensive production to meet increasing demand. Variables targeted in this optimization include 1) the microfilaremia of gerbils used for infecting ticks, 2) the life stage and sex of ticks used for infections, 3) the number of subcutaneous injections used to infect hamsters with third-stage infective larvae (L3), and 4) the medium used for subcutaneous infection of gerbils with L3. By feeding only adult stage ticks on gerbils selected for high microfilaremias we increased our yields from 13 L3/tick (n=497) to 126 L3/tick (n=107 ticks). Similarly, the average recovery of adult A. viteae from hamsters has risen from 51 ± 45 (n=13) to 90 ± 48 (n=22), partially due to the finding that worms isolated in Hanks' Balanced Salt Solution (HBSS) produce patent infections whereas those isolated in RPMI 1640 do not. Methods to infect ticks with *A. viteae* via artificial membrane feeding and inoculation are currently being developed, and a recent trial resulted in recovery of 208 L3s/tick inoculated by enema with microfilaremic gerbil blood (n=4 ticks). The increased recovery of *A. viteae* from host animals has allowed the FR3 to meet higher demand from the filariasis research community for all life stages of the worm.

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ACTIVATING AUTOPHAGY AS A NOVEL ANTI-*WOLBACHIA* MODE OF ACTION FOR MACROFILARICIDAL DRUG DISCOVERY

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River blindness (*Onchocerca volvulus*) and elephantiasis (*Wuchereria bancrofti* and *Brugia malayi*) affect over 150 million people in more than 80 countries, with a further 1 billion at risk of infection. Each of these nematode parasites has evolved a mutualistic association with the bacterial endosymbiont *Wolbachia*. Depletion of *Wolbachia* with the antibiotic doxycycline arrests development, fertility and viability and delivers potent macrofilaricidal efficacy in clinical trials. In order to identify alternative anti-*Wolbachia* drugs with a more rapid activity we have exploited the host nematodes immune regulation of *Wolbachia* populations through autophagy to discover drugs with a wolbachiacidal mode of action. We have screened libraries of 100 autophagy inducing drugs and compounds against *B. malayi*. Selected 'hits' are then screened against transgenic *C. elegans* and human embryonic kidney (HEK) cells expressing a fluorescent autophagy marker, ATG8 to identify drugs, which are selectively more potent against nematode versus human autophagy activation. Hits ranked by relative nematode potency are progressed through the A·WOL drug discovery and development screening pipeline to identify pre-clinical lead candidates and optimized combinations of anti-*Wolbachia* drugs to reduce treatment timeframes.

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PHARMACOKINETIC/PHARMACODYNAMIC MODELLING OF ANTI- *WOLBACHIA* CHEMOTHERAPEUTIC AGENTS IN A LYMPHATIC FILARIASIS MURINE INFECTION MODEL

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An estimated 120 million people are infected by lymphatic filariasis throughout the tropics leading to a profound public health and socio-economic burden in severely affected communities. *Wolbachia* is an essential endosymbiont of the filarial nematodes *Wuchereria bancrofti, Brugia malayi* the causative agents of lymphatic filariasis. Doxycycline is currently the gold standard for the targeting of *Wolbachia* in lymphatic filariasis chemotherapy. However, the current drug regimen is a 100-200 mg/day doxycycline dose given for 4 to 6 weeks to patients. The A·WOL consortium plan to reduce the current treatment time to 7 days or less to improve drug regimen adherence and to reduce drug resistance and costs of treatment. To achieve a rapid 7-day or less kill rate of *Wolbachia*, a number of drug combinations will be employed. These include different tetracyclines (Doxycycline and minocycline) rifamycins (Rifampicin or Rifapentine), Moxifloxacin as well as anti-helminthic drugs. The complexity of multiple drug combinations necessitates a rational approach in the identification and choice of the best treatments in *in-vivo* models and translating the animal treatments in the lab into clinical trials on the field. We have done series of PK-PD models and simulations using parameter and non-parameteric population PK-PD modelling software programs to further dissect and quantify the dynamics of anti-bacterial activity of these drugs in the treatment of Lymphatic Filariasis and Onchocerciasis. As an example here, we identified the PK parameters of doxycycline, minocycline and rifampicin in *in-vivo* PK studies in the SCID *Brugia malayi* model and used the PK data along with pharmacodynamic output to interpret the PK-PD relationships in light of the effect of each drug upon *Wolbachia* viability in parasites. The data display the power of PK-PD modelling in quantifying the PK-PD relationships of different drugs whilst giving insight to predictions of drug dynamics and interaction with *Wolbachia* viability.