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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.

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DEVELOPMENT OF A SMALL ANIMAL MODEL OF ONCHOCERCIASIS FOR THE SCREENING OF MACROFILARICIDAL DRUGS

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Onchocerciasis affects >37 million people with approximately 800,000 suffering visual impairment (river blindness). Development of macrofilaricides for onchocerciasis is hampered by the lack of a facile animal model. Our laboratories and others have demonstrated protracted survival of non-murine filariae in immune-compromised mice. Therefore we tested whether cattle-sourced *Onchocerca* could persist in Severe-Combined Immuno Deficient (SCID) mice. *O. ochengi* nodules were harvested from the skins of infected cattle while infective L3 were produced from blackflies fed on cattle in Cameroon. Motile male *O. ochengi* were isolated following culture of disrupted onchocercomas, prior to surgical implantation into the peritoneal cavity of SCID mice. Viable macrofilariae were recovered in 100% of recipients assessed between 1-5 weeks post implant (mean survival=49.22% ±5.08, n=22). Therefore, the implant model was assessed for suitability to test for efficacy of direct anti-nematodicidal drugs using the standard macrofilaricide, flubendazole suspension (FBZ). FBZ or vehicle control (VC) was administered parenterally (10mg/kg sc, +5 days) into groups of SCID recipients (n=7-8) of 15 male *O. ochengi*. FBZ induced an almost total macrofilaricidal response assessed 4-5 weeks after implant (mean survival FBZ=1.67%±1.09 vs VC=43.81% ±11.44, p=0.0089). *O. ochengi* L3 larvae were also inoculated into SCID mice. However, only 1 viable larva could be recovered after 7-14 days following sc or ip injection of between 40-100 L3 (n=26). In conclusion, we have developed a small animal model of onchocerciasis using adult *O. ochengi* parasites that is sufficiently robust to commence screening of macrofilaricidal candidates. We are currently validating this model for additional screening of anti-*Wolbachia* candidates. Further, we have commenced development of a murine onchocercoma xenograft model to evaluate whether male and female macrofilariae and released microfilariae can survive in SCID recipients for periods necessary to further validate efficacy of macrofilaricidal drugs.

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AN INTERACTIVE WEB BASED COMPUTER SIMULATION FOR THE PREDICTION OF MASS DRUG ADMINISTRATION OUTCOMES IN ONCHOCERCIASIS CONTROL AND TREATMENT

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Here we show for the first time a user friendly, interactive, internet based computer simulation that will help in the prediction of mass drug administration outcomes in onchocerciasis treatment and control. The model is being developed within the A·WOL consortium, to predict the impact of anti- *Wolbachia* drugs currently being advanced. The simulation program focuses on how different treatments, such as microfilaricidal, macrofilaricidal or combinations, would reflect upon the overall outcome of treatment regimes in a specific endemic area. We utilise dynamic models using to form an interactive platform where the user can experiment with different scenarios with complete control over parameters such as degree of endemicity, drug modes of action, drug pharmacokinetic profiles, vector biting rates and the application of vector control and drug resistance. The model also puts into consideration population variations in response to the drug which aids in predicting worst case scenarios and developing alternative plans. It can also be used to simulate the impact of applying combination therapies, drugs with different pharmacokinetics parameters and different potencies and modes of action. As an example, we show a case study where the model predicts the treatment outcomes in endemic areas when using ivermectin alone or a combination of ivermectin and doxycycline. The modelling shows that the use of a combination of ivermectin and doxycycline treatment vastly increases the chances of elimination success. The use of a macrofilaricidal drug such as doxycycline shows a much higher potential in achieving overall elimination than microfilaricidal drugs (such as ivermectin) alone which agrees with previous computer models

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LASER DIFFRACTION ASSAY FOR LOW RESOURCE QUANTIFICATION OF MICROFILARIAL BURDEN

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Efforts to eliminate lymphatic filariasis and to control onchocerciasis by mass drug administration (MDA) in West and Central Africa have been hampered by the risk of severe adverse reactions among persons harboring high-level loiasis infections (>8,000 microfilariae per mL of blood). A test-and-NOT-treat strategy has been proposed for excluding persons with high-level loiasis from MDA. This will require low-resource, real-time, point-of-care diagnostics that can be used at the time of MDA to identify persons with high-level loiasis and exclude them from treatment. Using *Caenorhabditis elegans* L1 larvae as surrogates for microfilariae, we have developed a low-resource laser diffraction assay for microfilarial quantification. The prototype apparatus is composed of a low-power red laser (similar to a laser pointer) directed through a sample chamber (cuvette, flow cell, or capillary tube) towards a photodiode connected to a laptop-powered oscilloscope. Preliminary studies show the assay is capable of quantifying worm concentrations between 500 and 50,000 L1 larvae/mL. Assay sensitivity is dependent on depth of the sample chamber: a 1 cm chamber depth gives maximum sensitivity while a 0.05 cm depth gives lower sensitivity but greater precision. Cellular blood components interfere with the diffraction assay, and low-resource methods to separate larvae from blood components prior to quantification are being developed. These preliminary data suggest that a portable, battery-powered, low cost laser-diffraction device may be capable of rapid, point-of-care quantification of microfilarial burden in the setting of MDA.

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FIELD EVALUATION OF STANDARD DIAGNOSTICS' ONCHOCERCIASIS IGG4 RAPID DIAGNOSTIC TEST PROTOTYPES

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The progress that has been made toward the elimination of onchocerciasis, or river blindness, in the Americas and Africa has been the culmination of decades of commendable dedication to controlling this disease. Over the past few years, the use of annual or semi-annual community-based ivermectin distribution through mass drug administration (MDA) programs has moved the goal from control to elimination. As elimination goals are being reached, appropriate diagnostic tools are needed to identify exposure to Onchocerca volvulus (Ov) for purposes of identifying possible recrudescence. A rapid diagnostic test (RDT) that detects IgG4 antibodies

specific to the antigen Ov16 has been developed and will be manufactured by Standard Diagnostics, Inc. Prior to commercial availability, the test has been evaluated in the PATH laboratory and in rural settings in Togo with 1,500 participants during routine Ov epidemiological surveillance activities. In the laboratory setting, our data indicate that the RDT has a sensitivity of 89% and a specificity of 99%, when compared to an Ov16-specific ELISA. The read window, an important indicator of test result stability, is highly consistent over a 24-hour time period. We will also present the performance of the RDT relative to the Ov16 ELISA and sensitivity as compared to skin-snip derived microfilaria status when tested in real-world settings in Togo that include factors such as exposure to high temperature and humidity. Finally, we describe possible facilitators and barriers for integration of the test into routine surveillance practices. Our data verify the performance of the test in field settings prior to a commercial launch of the Ov16-based RDT and informs how it should be used in current onchocerciasis control and elimination efforts.

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SURVIVOR COPYCAT BEHAVIORS AND AUTOCHTHONOUS HELMINTHIASES IN THE U.S.

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Popular *Survivor* TV programs glamorize rugged outdoorsmen surviving wilderness adventures by seeking natural sustenance. To identify risk factors for autochthonous helminthiases resulting from *Survivor* copycat behaviors, Internet searches identified 54 cases of helminthiases transmitted by the raw consumption of animals recommended as safe, natural foods by *Survivor* series programming during the period, 1984-2013. Cases were defined by positive microscopic, serologic, or molecular diagnostics. Continuous variables were analyzed for significant differences by unpaired t-tests; categorical variables were analyzed by chi squares. Statistical significance was indicated by p-values ≤0.05. 38 autochthonous cases of neuroangiostrongyloidiasis (NAS) with eosinophilic meningoencephalitis and 16 cases of autochthonous paragonimiasis (PG) with hemorrhagic pneumonitis were reported following consumption of raw intermediate hosts infected by causative parasites, the rat lungworm (*Angiostrongylus cantonensis*) and the American lung fluke (*Paragonimus kellicotti*) respectively. The mean age of NAS cases was 21.5 years; most cases were in males (P = 0.039) from Hawaii (P = 0.039), 37% of whom reported consuming raw snails or frogs or eating unwashed greens harboring infective larval snails (P = 0.003). The mean age of the PG patients was 27.3 years; most cases (93%) were reported in males (P < 0.0001) from Missouri, most of whom (67%) had consumed raw crayfish (P < 0.0001), often while intoxicated (47%) on camping, paddling, or floating trips within the Mississippi River Drainage Basin (73%, P = 0.028). There was one death in a 71-year-old male with PG. The most significant risk factors for autochthonous helminthiases from *Survivor* copycat behaviors included male gender; and consumption of raw, wild animals in parasite-endemic regions, especially while intoxicated outdoors. Recommended preventive-behavior interventions included proper food preparation of self-harvested wild animals, wilderness survival training, and alcohol avoidance during outdoor recreation.

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EPIDEMIOLOGY OF SOIL TRANSMITTED HELMINTHS AND *PLASMODIUM FALCIPARUM* AMONG SCHOOL CHILDREN IN BUMULA DISTRICT, WESTERN KENYA

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Soil-transmitted helminthiasis and malaria continue to be important health problems among Kenyan school children, many of whom are at risk of coinfection. It has been suggested that the immune response evoked by helminth infections may modify immune responses to plasmodia species and consequently alter risk of infection; however previous studies have been inconclusive. As part of baseline activities of a current trial investigating deworming impact on risk of clinical malaria among school children in Bumula District in western Kenya (ClinicalTrials.gov: NCT01658774), a cross-sectional survey was conducted to investigate the prevalence, intensity and geographic distribution of soil-transmitted helminth (STH) and *Plasmodium falciparum* infections among 4,842 school aged children in purposively selected 22 primary schools. Single stool samples were collected and examined in duplicate for STH (hookworm, *Ascaris lumbridoides* and *Trichuris trichiura*) using the Kato Katz method. A finger-prick blood sample was taken and examined for *P. falciparum* by expert microscopy. Overall, 23.4% of the children were infected with *P. falciparum* and 26.5% with one or more STH species, with hookworm (16.4%) and *A. lumbricoides* (14.3%). The prevalence of STH and *P. falciparum* infections varied significantly by age group and sex and by geographical location. After adjusting for age, sex and clustering within schools, the risk of *P. falciparum* infection was higher among children with any STH infection (odds ratios= 1.2, 95% confidence intervals [CI] (1-1.4), p=0.03), although there was no significant association between *P. falciparum* and individual STH species. Interestingly however, *P. falciparum* infection was positively associated with medium to high intensity *A. lumbricoides* infections (odds ratios= 1.5, 95% CI (1.1-2.1), p=0.006). Coinfection with *P. falciparum* and STH is common among school children in western Kenya, supporting the need for integrated helminth and malaria control. There is also a need to investigate the consequences of deworming on the risk of malaria.

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PHYTOCHEMICAL, EFFICACY AND SAFETY PROFILES OF *EMBELIA SCHIMPERI* VATKE

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Embelia schimperi fruits are among the most common traditionally used agents to teat parasitic / helminthes infections in Ethiopia. Despite the plants widespread use, scientific evidence on constituents responsible for the biological activity, efficacy and safety is not well documented. A 96 well micro titer pale assay technique was used to evaluate the *in vitro* anthelmintic properties of crude hydro alcoholic extract and solvent fractions of *E. schimperi* fruits against hookworm and strongyloides spp. The *in vivo* anthelmintic activity of the extract was determined in albino mice experimentally infested with *Hymenolopis nana* worms. The percent killing activity of the crude extract was from 92-100% when tested at a concentration of range of 50-400mg/ml against hook worm and *Strongylodes* spp. The percent motility inhibition against hookworm larvae was from 66.6-86.6% when fractions were tested at a concentration of

50mg/ml. 100% adult parasite clearance was observed from the mice intestine when the crude extract was administered at a single oral dose of 1kg/kg. Phytochemical screening indicated the presence of steroids, polyphenols, quinone and alkaloids. Preliminary sub chronic toxicity study was also conducted to make gross observation on measurements of change in body weight, hematological, biochemical parameters and histopathology of kidney and liver of albino mice. The toxicity study indicated that the crude extract of *E. schimperi* fruit has wide safety margin in all tested parameters and the plant may be relatively safe as an oral anthelmintic medication if further investigations is carried out. This study also indicates the presence of active principles in *E. schimperi* fruits with anthelmintic activity that may support the usage of preparations from the plant by local people to treat parasite helminthes infection. In this study average worm expulsion tine and possible mechanism of action of the extract is also suggested. However, further research is required to study long term toxicity, effect on beneficial micro biota, mechanism of action, identification of marker compounds and effectiveness of the plat *in vivo* / human model/ and clinical trails before it can be recommended for human use.

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THE EFFICACY OF ALBENDAZOLE AND LEVAMISOLE DRUG COMBINATION IN INDIVIDUALS WITH REDUCED EFFICACY FOR SINGLE-DOSE ALBENDAZOLE TREATMENT AGAINST HOOKWORM INFECTIONS

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The Soil-Transmitted Helminth (STH) infections control programme has been on-going in Ghana since 2000 with mass drug administration (MDA) of albendazole (ALB) to school children. However, our research revealed, 100% cure rate with albendazole-levamisole (ALB-LEV) combination in hookworm population responding sub-optimally to ALB treatment in four endemic communities in the Northern region of Ghana. This was an eighteen months longitudinal study. A total of 421 school children between the ages of 2-17 years were randomly selected from four endemic communities in Kpandae district of the Northern region of Ghana. One hundred and three (103) patients positive for hookworm were categorised into four groups: Control, ALB only, LEV only and LEV - ALB drug combinations with 26 patients in each group. Coprological assessment for parasites was based on the Kato-Katz technique. Parasites recorded were hookworm (*Ancylostoma doudenale* and *Necator americanus*), *Trichuris trichiura, Hymenolepis nana*, and *Taenia* sp. in the communities selected. Overall, the highest cure rate after 21 days of treatment was recorded for LEV-ALB treatment (100%) followed by LEV alone (92.31%) and ALB alone (88.46) whiles the Control was zero. Also, faecal egg count reduction rate was 100% (LEV-ALB), 96.15% (LEV alone), 91.84 (ALB alone) and 9% for Control.Cumulative re-infection rates were 12, 32 and 48% and 23, 50 and 69% respectively for LEV-ALB, LEV alone and ALB alone at 6 months and one year after treatments. Hookworm still remains a significant public health burden in Africa. The result shows a significant efficacy for LEV - ALB combination in hookworm population with reduced ALB treatment (100% FEC) after 21days treatment and therefore can be used to augment ALB for clearing possible resistant parasites of hookworm infections. All parasitic samples are currently being analysed to identify polymorphism related to benzimidazole resistance in hookworms.

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MOLECULAR DIAGNOSIS OF SOIL-TRANSMITTED HELMINTH INFECTIONS USING A NOVEL ISOTHERMAL AMPLIFICATION METHOD

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Ascariasis, Trichuriasis, and Hookworms are the three main soil-transmitted helminth infections, causing human morbidity in tropical areas of the world. Diagnosis of human soil-transmitted helminths (STHs) relies on microscopy to identify eggs in the feces. The precise detection of STHs by microscopy requires training and is labor intensive and can lead to false negative results. As a result of the limitations of microscopic methods, it was of interest to develop new methods to identify different STH species. Accurate diagnostic is not only essential for epidemiological studies but is vital for monitoring the possible emergence of drug resistance and for the development of new anthelmintics since the efficacy of different drugs shows variation among the species. For a variety of infections, studies have shown that molecular diagnostic tools can be more reliable and sensitive. Therefore, development of a simple, rapid and sensitive molecular tool for STH species diagnosis is desirable. We developed novel diagnostic assays based on the Smart Amplification method (SmartAmp) to detect STH infections under isothermal condition. This isothermal method uses asymmetric primer design to prevent non specific amplification. Control plasmids were employed to develop and optimize the assays. The assays were applied to analyze fecal samples using species-specific primer sets. Real-time PCR monitoring of the amplification was achieved within 20-40 min with complete suppression of the background amplification. SMartAmp assays were developed for diagnosis of STH infections and the reliability of the method was validated using the conventional PCR method.

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IDENTIFICATION AND CHARACTERIZATION OF THE IMMUNOMODULATORY MOLECULE(S) IN EXCRETORY-SECRETORY (ES) PRODUCTS DERIVED FROM THE GASTROINTESTINAL NEMATODE *HELIGMOSOMOIDES POLYGYRUS*

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Heligmosomoides polygyrus (Hp) is a murine gastrointestinal (GI) nematode used as a model of helminth parasites that infect humans and livestock. We previously showed that Hp-derived excretory-secretory products (HES) potently suppress effector $CD4^+$ Th cell responses to an unrelated antigen by modulating the antigen presenting function of dendritic cells (DC) to induce an anti-inflammatory network involving regulatory T cells. Interrogation of mass spectrometry (MS) data from 1D-SDS PAGE and LC-MS/MS analysis of HES with transcriptomic information indicated that HES contains over 200 proteins, including several with known immunomodulatory effects. To better define the immunomodulatory molecules(s) in HES, we used biochemical approaches and assessed the suppressive activity of the separated fractions by measuring IL-12p70 secretion by bone marrow-derived DC (BMDC) pre-treated with HES fractions prior to stimulation with the TLR9 ligand CpG-ODN. First, we performed size exclusion chromatography to separate HES proteins based on their molecular weight and identified 12 fractions that suppressed DC secretion of IL-12p70 in response to CpG-ODN. The suppressive fractions were pooled and subjected to further separation by anion-exchange chromatography followed by testing on CpG-ODN-stimulated BMDC. We identified 3 fractions that suppressed IL-12p70 secretion. Analysis of these fractions by LC-MS/MS identified 21 candidate proteins. To further narrow down the immunomodulatory candidate(s), HES was resolved by cation-exchange chromatography. Only one fraction

was identified to have suppressive activity in the BMDC assay; LC-MS/MS analysis is underway to identify the proteins in this fraction. Our goal is to clone and purify the candidate immunosuppressive protein(s) and to test the ability of recombinant protein to modulate DC polarization of effector $CD4^+$ T cell responses *in vitro* and *in vivo*. Together, our findings provide novel information on biochemical and immunomodulatory aspects of GI nematode-derived ES products.

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DEXAMETHASONE DOWNREGULATED THE EXPRESSION OF MATRIX METALLOPROTEINASE 2, 9 AND TH2 CYTOKINES IN MICE WITH EOSINOPHILIC MENINGITIS CAUSED BY *ANGIOSTRONGYLUS CANTONENSIS* INFECTION

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Angiostrongylus cantonensis, also known as the rat lungworm, is the major cause of eosinophilic meningitis in the Pacific Islands and Southeast Asia. Rats serve as the definitive host of the nematode. Humans are infected incidentally and lead to eosinophilic meningitis. Previous BALB/c animal study had demonstrated increased matrix metalloproteinase 9 (MMP-9) expressions and blood brain barrier damage in mice infected with *A. cantonensis*. Steroids have been shown to be one of the effective treatment options for eosinophilic meningitis caused by *A. cantonensis* infection. However, the mechanism of how steroids can influence on eosinophilic meningitis are still unclear. We hypothesize that the beneficial effect of steroid on eosinophilic meningitis are mediated by decrease MMP and cytokines expressions. In a BALB/c mice model, mice were orally infected with 40 *A. cantonensis* L3 via an orogastric tube and were sacrificed every week for 3 consecutive weeks after infection until the end of the study. Dexamethasone was injected via the intra-peritoneal routine from 7th days of infection until the end of the study. Evans blue method was used to measure the blood brain barrier changes and the serum/CSF and brain homogenates expression of MMP-2, 9 and cytokines were analyzed by gelatin zymography, western blot, ELISA and reverse transcriptase polymerase chain reaction (RT-PCR) respectively. There were an increased MMP-2 and MMP-9 expressions in CSF and brain homogenates by western blot and gelatin zymography following 2-3 weeks of infection. Dexamethasone administration could down-regulate the expression of MMPs. These changes were parallel to the blood brain barrier disruption as evidence by the Evans blue extravasation following infections. Furthermore, the brain homogenates cytokines , such as IL-5, IL-10 and INF-γwere also elevated following 2-3 weeks of infection. Dexamethasone could decrease the expression of IL-5, IL-6, IL-10 and TNF-αby using ELISA. The brain homogenates mRNA expressions of IL-5, IL-6, INF-γ and TNF-α were decreased gradually by RT-PCR. All of these findings suggested that the Th2 cytokines play an important role in mice with eosinophilic meningitis caused by *A. cantonensis* infection and provided the evidences supporting steroid effects on *A. cantonensis* infection by inhibiting MMP-2, 9 and Th2 inflammatory cytokines expressions.

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THE IMPACT OF COMMUNITY DEWORMING AGAINST SOIL TRANSMITTED HELMINTHIASES ON PREVALENCE, WORM BURDEN AND MORBIDITY IN A HIGH PREVALENCE AREA OF ARGENTINEAN GRAN CHACO

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Soil-transmitted helminthiases (STH) are the most prevalent infections of human-kind, relevant to public health due its morbidity and role in poverty perpetuation. Tartagal, Gran-Chaco, Argentina is a high prevalence area where no preventive anthelminthic chemotherapy was recently applied. This study, conducted in Tartagal since March 2012, investigates the results of community-based mass drug administration (MDA) and lasting until 2015. Population: ~3600 individuals. Surveillance of a statistically representative random group, with fresh stool sample analysis, complete blood count and detection of IgG against S.stercoralis with NIE-ELISA. Treatment intervention: single dose albendazole + ivermectin. Survey: n=157 stool; n=149 blood for baseline and n=103 stool; n=152 blood 6 months later. Baseline prevalences for any STH at baseline and follow-up were 55 and 16% respectively; and for hookworm (HKW) 50 and 13% respectively ($p<0.05$). HKWs main species was A.duodenale (85%). Baseline S.stercoralis prevalence was 13% and at follow-up 8% ($p=0.22$). T.trichiura and A.lumbricoides were rarely found. Worm burden revealed 3 heavy HKW infections at baseline and none at follow up. At baseline 56% of the individuals were anemic, mean Hgb value 11 gr/dL (SD=1.6; minimum 4.9) which improved to 10% anemic (all young women), mean Hgb value 13 gr/dL (SD=1.5; minimum 9) at follow up ($p<0.05$). HKW infection was significantly associated with anemia ($p=0.001$), risk ratio (RR)=1.54 (CI 95% 1.03-2.30) at baseline, however at follow up it was no longer significant ($p=0.58$). 75% had eosinophilia at baseline and 43% after MDA ($p<0.05$). No significant difference in S.stercoralis seroprevalence was found between baseline (51%) and follow up (44%) ($p=0.25$). MDA coverage: 80%. One round of community-based MDA was effective in reducing the prevalences of STH infection, anemia and eosinophilia and in decreasing anemia severity and HKW infection burden. Individuals who remained anemic after MDA were women of reproductive age, with other probable causes of anemia.

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PLANT NATURAL PRODUCTS SHOW *EX VIVO* ACTIVITY AGAINST ADULT HOOKWORM

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Hookworms, blood feeding intestinal parasites, remain a major health burden with approximately a billion people infected in tropical and subtropical regions of the world. In these geographical areas, they are a major contributor to iron-deficiency anemia, weight loss, stunted growth and malnutrition. Control strategies have relied on mass treatment with benzimidazole drugs. However, recent reports have described the resistance of hookworms and other soil-transmitted nematodes to these drugs. Considering that in several endemic areas, local populations use plant products to treat several ailments including parasitic diseases, we

tested compounds from plants for their anthelminthic activity against the adult stage of the hookworm, *Ancylostoma ceylanicum*. The present study screened extracts from five plant species and chromatographically-enriched fractions of the most active one. These plants were collected from the western United States. Extracts from two of the plants namely *Dalea ornata* and *Oemlaria cerasiformis* showed anthelminthic activity (mortality and/or reduced motility) of their crude extracts and enriched fractions against *A. ceylanicum*. Associated worm mortality rates ranged from 25% at 24 hours to 100% at 120 hours, after incubating worms with the test compounds. Three concentrations of the compounds were tested (100, 50, 10 mg/mL). Our study revealed a dose-dependent activity where the lowest concentration (10 mg/mL) achieved 100% mortality 120 hours post exposure while the same activity level was obtained at 48 hours with 100 mg/mL. Our data show that plants represent a relatively untapped source of potentially effective anthelminthic molecules. Studies are underway with the aim of purifying and testing active components of the extracts *ex vivo* and *in vivo*. The anthelminthic activity of these compounds in the animal model of the disease will be evaluated using clinical, parasitological and immunological parameters such as weight gain, anemia, egg output, worm burden, immune cell proliferation indices, and immune cell population types and sizes by flow cytometry.

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CYTOKINE EXPRESSION PROFILES AND IMMUNITY TO MEASLES VACCINATION IN KENYAN CHILDREN WITH HELMINTH INFECTION

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Helminths are among the most prevalent infections in resource-limited countries, with an estimated one billion school-age children worldwide living in areas at high risk for helminth transmission. Children are particularly susceptible to high-burden infections, which can result in derangements in host cytokine expression. These derangements, in turn, lead to both immunosuppression and pathologic inflammation in the host. The disruption of cytokine signaling by helminths has been proposed to impair the ability of children to mount protective immune responses to vaccination. We are currently enrolling children in a cross-sectional study to test the hypothesis that helminth infection is associated with a modified Th2 cytokine response, which is permissive for chronic helminth infection and reflected by an elevated IgG4/IgE ratio. Furthermore, we will explicitly test the prediction that helminth infection is associated with impaired vaccine-induced immune responses, specifically for measles vaccination. This study is nested within a large pediatric surveillance cohort at three clinical sites in Western Kenya. Plasma and stool samples are collected at the time of enrollment from eligible children aged 1 through 5 years with documented measles vaccination. Shortly after collection, stool samples are tested by Kato-Katz and formol-ether concentration methods to diagnose helminth infection and quantify parasite burden. Plasma from children with and without helminth infection will be analyzed by ELISA to compare serum levels of Th1 and Th2 cytokines, as well as titers of IgE, IgG4, and anti-measles antibodies. Preliminary enrollment demonstrates a helminth infection prevalence of approximately 25% in our Western Kenya cohort. Improving our understanding of helminth-mediated immune dysfunction will facilitate the rational design of both anti-helminth vaccines and novel adjuvants to enhance vaccine efficacy in helminth-endemic areas.

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TOXOCARIASIS IN NORTH AMERICA: A SYSTEMATIC REVIEW

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Toxocariasis is an important neglected tropical disease that can manifest as visceral or ocular larva migrans, or covert toxocariasis. All three forms pose a significant public health problem in areas of high prevalence. To determine the burden of toxocariasis in North America we conducted a systematic review of the literature following PRISMA guidelines. We found 18 articles with original prevalence, incidence, or case data for toxocariasis. Of these articles, 5 reported data for Canada, 8 reported data for the United States, and 5 reported data for Mexico. One article reported only prevalence data, no articles reported incidence data, 5 articles reported only affected cases, and 12 articles reported both prevalence data and number of affected cases. 63% of articles with seroprevalence estimates for the United States tested blood samples collected during the 1988-1994 National Health and Nutrition Examination Survey. The most commonly cited risk factors for toxocariasis included male sex, pet ownership, particularly if the animals are allowed to live outdoors and eat other animals or otherwise consume unconventional pet food, African American race, age less than 18, low level of education, poverty, foreign birth, living in the southern United States, and playing at parks or in sandboxes where dogs and cats have defecated. Of the studies that reported prevalence data by sex 73% found a statistically significant greater prevalence in males compared to females, while 27% of studies found no significant differences between genders. No studies found females to have significantly higher prevalence of Toxocara infection than males. Further research is needed to determine the true current burden of toxocariasis in North America, however the prevalence estimates gathered in this review suggest that the burden of disease is significant.

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THE ROLE OF SOIL-TRANSMITTED HELMINTH INTENSITY AND CO-INFECTION IN THE SPATIOTEMPORAL VARIATION OF CHILDHOOD ANEMIA: RESULTS OF A 5-YEAR MDA PROGRAM IN BURUNDI

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We investigated the role of *Ascaris lumbricoides, Trichuris trichiura* and hookworm infection and coinfections in the anaemia burden in school age children as a result of a 5-year mass drug administration (MDA) program in Burundi. The aim of the study was to investigate for the first time the impact of the MDA program on the spatiotemporal variation in anaemia burden in the country. Longitudinal parasitological data was collected from 40,553 children from 2007 to 2011 in 31 schools in Burundi. These data included faecal egg counts for *A. lumbricoides, T. trichiura* and hookworm, coinfections with these parasites and blood iron levels, age, sex, weight and height. Locational data included the GPS coordinates of the schools, annual mean land surface temperature, NDVI, precipitation and the distance to perennial water bodies. Spatiotemporal Bayesian geostatistical models of anaemia were built adjusting for age, sex, helminth intensity and co-infections, malnutrition and physical environment factors associated with the geographical location of the school. Our results indicate that the geographical distribution of the prevalence of anaemia did not change significantly during the 5-years. Our results indicate an association between the spatiotemporal variation in anaemia burden in children in Burundi and STH intensity and co-infections and environmental

conditions. Our study demonstrates the important role of malnutrition in anaemia burden over the five year period. The findings of this study suggest that MDA has played an important role in the control of anaemia in school age-children in Burundi. The results also suggest the need to integrate nutritional interventions to the current MDA programme to achieve the desired level of anaemia reduction in Burundi. Our maps can be useful to guide future MDA campaigns to achieve this reduction.

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DOMESTIC ANIMALS MECHANICALLY TRANSMIT *NECATOR AMERICANUS* HOOKWORM TO HUMANS

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Necator americanus hookworm infections cause serious impairment in millions of children across the tropics. While humans are the only definitive host of *Necator americanus*, epidemiological evidence from South America, Africa, and Asia suggest that risk for hookworm infection is increased by contact with dogs and pigs. Building on experiments showing that *N. americanus* eggs can survive passage in the pig digestive tract, we demonstrate that pigs and dogs in twelve rural communities in central Ghana harbor and excrete viable human hookworm eggs in their stool. Using logistic and ordinal regressions, we show that pig ownership and the proportion of households owning dogs in one's village significantly predicted hookworm prevalence and infection intensity. In these communities, 67% of dog feces and 55% of pig feces contain hookworm eggs. Using PCR, we confirmed a majority of collected animal stools contained *N. americanus*. Further, infective third stage hookworm larvae developed from all of the samples (n=41), demonstrating egg viability after natural passage through an animal's digestive tract. The presence of high rates of antibodies to *Toxocara canis* in their serum suggests humans in these communities are frequently exposed to helminth parasites through contact with dogs. We introduce a new hookworm transmission model that indicates that animal-mediated transmission of hookworm eggs may play an important role in human disease. Hookworm control strategies embracing the 'One Health' concept by interrupting hookworm transmission from animals should be developed.

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A PARADIGM FOR STUDYING ANTHELMINTIC COMBINATIONS *IN VIVO*

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Soil-transmitted helminth (STH) nematode parasites (hookworms, *Ascaris* and *Trichuris*) are key contributors to morbidity and poverty worldwide. Few anthelmintics are available for treatment, and only anthelmintic, albendazole, is commonly used in mass drug administrations, even though there are more than 1 billion people are infected. New anthelmintics and treatment strategies are greatly needed, in particular as albendazole resistance is inevitable given its current method of usage and as commonly occurs with veterinary use of this drug. Our group has identified Cry5B made by *Bacillus thuringiensis* as a promising new anthelmintic for treating STH parasites. Apart from developing Cry5B as a much needed new class of anthelmintic, we are also interested in preserving the potency of Cry5B and other anthelmintics as much as possible—i.e., preventing resistance. To achieve this aim, combination therapies with anthelmintics is an excellent approach. The challenge with anthelmintic combinations is defining a good combination and at what ratio drugs can be productively combined. Although we have published combination studies using the nematode *Caenorhabditis elegans*, it was not clear how these translate *in vivo* in infected mammals. Surprisingly, little work has been done to define the characteristics of anthelmintic combinations *in vivo*. By using the hookworm (*Ancylostoma ceylanicum*) infections in hamster, we establish a new and powerful *in vivo* paradigm for studying anthelmintics combinations—defining not only how well two drugs combine but also providing some optimization of the ratio for combinations. Here, we will update you our latest results of the anthelmintic combinations with Cry5B and other anthelmintics, e.g., tribendimidine. We show that, like in C. elegans, these two drugs can be combined to give powerful effects *in vivo*. Work on other *in vivo* combinations is also proceeding. Our paradigm here provides a powerful means on how to examine and demonstrate the anthelmintic combination therapies.

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THE ROLE OF SOIL-TRANSMITTED HELMINTH INFECTIONS IN THE GEOGRAPHIC RISK OF FUNCTIONAL ILLITERACY OF SCHOOL-AGED CHILDREN IN THE PHILIPPINES: SPATIAL VARIATION AND NUMBERS AT RISK

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We aimed to understand the contribution that soil-transmitted helminth (STH) infections make to functional literacy status of school-aged children in the Philippines and investigated, for the first time, the geographical distribution of prevalence of functional literacy indicators adjusting for STH infections. STH infection data were collected from 29,919 individuals during the most recent National Schistosomiasis Survey conducted in 2005 to 2007 in 177 locations throughout the Philippines. Data were also provided by the National Statistics Office on three functional literacy indicators (i.e. ability to compute, read and comprehend) of 19,673 school-aged children in the Philippines. Our study was conducted in two phases: first, Bayesian geostatistical models were developed to predict the complete geographical distribution of *Ascaris lumbricoides*, *Trichuris trichiura* and hookworm co-infection and intensity of infection across the Filipino population. Second, Bayesian geostatistical models were built for each of the functional literacy indicators, adjusting for the location and the predicted co-infection and intensity of *A. lumbricoides*, *T. trichiura* and *hookworm* generated during the first phase. Our results indicate that *A. lumbricoides* and *T .trichiura* co-infection is an important contributor to the spatial variation of functional illiteracy of school-aged children. We identified significant spatial heterogeneity in functional literacy indicators between regions of the Philippines and remarkable spatial variation in functional literacy indicators within different regions of the Philippines accounted by STH co-infection and intensity of infection. Our results demonstrate the important role of STH co-infection and intensity of infection in the geographical variation of prevalence of functional illiteracy in the Philippines. The findings of this study can be useful to guide the Integrated Helminth Control Program to improve the health and functional literacy in the Philippines by reducing STH infection levels in school-aged children.

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SCHISTOSOMIASIS AND SOIL TRANSMITTED HELMINTHS IN SCHOOL-AGED CHILDREN: EPIDEMIOLOGICAL PROFILE IN KINSHASA AND BAS-CONGO PROVINCES OF THE DEMOCRATIC REPUBLIC OF CONGO

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The lack of epidemiological data on schistosomiasis (SCH) and soil-transmitted helminthes (STH) in the Democratic Republic of Congo (DRC) hampers effective disease control, although these diseases have a significant impact on population health. In 2009-2010 we conducted a random survey in school-aged children (3rd grade) in 11 health areas of the provinces Kinshasa and Bas-Congo. We collected socio-demographic data and examined stool and urine samples of each child. A total of 2399 children (1559 children from Kinshasa and 840 from Bas-Congo) were included. The overall prevalence of SCH was 13.5 %; CI95%: 12.1-14.8. The highest prevalence of SCH was found in Bas-Congo province (32.1; CI95%: 29-35.3). A total of 61.3 % (CI95%: 59.4-63.3) school-aged children were infected STH with a predominance of A. lumbricoïdes. This prevalence was higher in Kinshasa (64%; CI95%: 61.6-66.4) compared to Bas-Congo province (56.3%; CI95%: 53.4-60). The data generated in this study provide baseline data for the formulation of control strategies on SCH and STH infections in Kinshasa and Bas-Congo. More work is needed in other provinces.

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DETERMINANTS OF INFANT-FEEDING CHOICES OF HIV-POSITIVE WOMEN ATTENDING PREVENTION OF MOTHER-TO-CHILD TRANSMISSION CLINICS IN OYO STATE, SOUTHWESTERN NIGERIA, 2013

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The Nigerian National HIV Guidelines recommends avoidance of all breastfeeding when replacement feeding is acceptable, feasible, affordable, sustainable and safe.This study determined factors influencing the infant-feeding choices of HIV-positive women enrolled in Prevention of Mother-to-Child Transmission (PMTCT) of HIV clinics in Oyo State, Nigeria. A cross-sectional survey of 450 HIV-positive women who had received infant-feeding counselling prior to delivery using a two-stage sampling technique. A semi-structured questionnaire was administered to obtain data on socio-demographics, infant feeding choice and factors influencing these choices. We defined mixed feeding as addition of other food or water to breast milk in the first six months of life. Four Focus Group Discussions (FGD) were conducted. Data was analyzed using Epi-info software version7.Detailed content analyses of the FGDs were done. The mean age of the mothers is 31 ± 3.5years. Exclusive breastfeeding (EBF), exclusive replacement feeding (ERF) and mixed feeding (MF) were 62.0%, 25.0%, and 13.0% respectively. Determinants of ERF were mode of delivery (AOR 2.3, 95%CI 0.8-4.3) and the desire to reduce the risk of transmission (AOR 5.4, 95%CI 2.8-6.4). For EBF, household income (AOR 3.6, 95%CI 1.8-5.4) and health workers influence (AOR 2.5, 95%CI 1.2-3.8), while for MF, non-disclosure of HIV status to spouse (AOR= 4.3,95%CI 1.5-12.8), Neighbours' advice ((AOR 1.8,95%CI 1.5-4.7) and infant illnesses (AOR= 2.9, 95%CI2.3-7.8) were the predictors. FGDs revealed pressure from family members as the major determinant of mixed feeding practice. In conclusion, pressure from family/neighbours to practice mixed feeding should be discouraged. Incorporation of family members into programs promoting safer infant feeding options in mothers living with HIV/AIDS and male involvement is imperative. Keywords: infant food, HIV, Women, Nigeria

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EVALUATION OF THE IMPACT OF HIV INFECTION ON THE DIGESTIVE FLORA OF CHILDREN

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The recurrent bacterial infections and affections observed in HIV positive infants could be explained by the imbalance in their digestive flora. The study aims at evaluating the impact of HIV infection on the digestive flora of infants aged 3-24 months, since there have been no studies addressing this issue in Cameroon. A cross-sectional and case-control study was carried out in two hospitals in Cameroon. Stool sample was collected from each of the proxy-consented HIV positive and HIV negative children. These stools were cultured using aerobic, strict anaerobic, 10% CO_2 and microaerophilic conditions. Identification of the bacteria species were done by biochemical characterization. Out of the 80 children enrolled for the study, 33 (41.25%) were HIV positive children and 47 (58.75%) were HIV negative children. 15 different types of bacteria species were isolated from HIV positive infants with high presence of *Lactobacillus* spp. (96.97%) *Streptococcus* spp. (84.85%) and *Bifidobacterium* spp. (81.81%). Opportunistic bacteria like *Shigella* spp. (24.24%), *Staphylococcus aureus* (15.15), *Klebsiella* spp. (12.12%), *Acinetobacter* spp. (3.03%), *Pseudomonas* spp. (3.03%) and *Proteus* spp. (3.03%) were also identified. Statistically, *Clostridium* spp. (p=0.009), *Shigella* spp. (p=0.002), *Enterococcus* spp. (p=0.000) *Staphylococcus aureus* (p=006) and *Streptococcus* spp. (0.015) were significantly more present in HIV positive infants than in HIV negative infants. Bacteria species like *Proteus* spp. *Pseudomonas* spp. *Acinetobacter* spp. *Staphylococcus aureus* were isolated only in HIV positive infants and absent in HIV negative children giving a frequency rate of 24.2%. HIV positive infants at stage 3 and 4 harbored more opportunistic bacteria. We remarked the imbalance in bacteria flora of HIV infected infant's harbouring quantitatively more isolated bacteria than in HIV non-infected children. Systematic stool culture would significantly benefit for the follow-up of HIV infected children to reduce the risk of recurrent bacteria infection.

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A THEORETICAL PERSPECTIVE OF THE LIVED EXPERIENCES OF HIV INFECTED MOTHERS TOWARDS ADMINISTERING DAILY COTRIMOXAZOLE PROPHYLAXIS TO THEIR HIV EXPOSED BUT NOT INFECTED CHILDREN IN MALAWI

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Although new HIV infections among adults in sub Saharan Africa have declined by 34% since 2001, the epidemic continues to disproportionately affect this region bearing 70% of all new infections in 2013. However, the sustained scale up of interventions for prevention of mother to child transmission of HIV has led to fewer newly HIV infections in children. The World Health Organization recommends that all HIV exposed infants be started on cotrimoxazole prophylaxis (CPT) until breastfeeding is stopped and HIV infection is excluded to provide adequate prevention against early opportunistic infections. Adherence may be challenging when CPT is given to very small infants who need to take it for long periods of time. Previous studies have only focused on adherence to PMTCT services hence it is

important to build a grounded theory of the social and cultural influences of CPT adherence in this group. Three focus group discussions and seventeen In depth Interviews were conducted with HIV infected mothers administering daily CPT to their HIV exposed but uninfected infants in a semi urban district in Malawi. A thorough literature search supplemented the concepts that emerged from the data. Findings We propose a fusion of the ecological theory of perception and the Health Belief Model to explain adherence to CPT in this study. The women themselves, their families, the communities in which they live and the health care system relate and influence each other through social interaction which play a role in influencing actions either positively or negatively. Motivators such as a supportive family help someone to take a positive action and factors such as shortage of drugs at the health facility act as deterrents. Interestingly, the data in this study demonstrates that despite negative influences that might arise at any level, the determination of the individual to take a health related action and the realisation that the recommended action will prevent any negative outcomes motivated the mothers to believe that they could successfully do something to prevent any negative health outcomes on their children. In conclusion, these findings could be used to design individualised interventions on HIV disease counselling as well as importance of treatment adherence in infants. The most currently used theory of Behavioural Learning lacks this capacity as it ignores effects such as past behaviour, habits and lack of acceptance of one's diagnosis.

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KNOWLEDGE, ATTITUDE AND PRACTICES OF SEXUALLY TRANSMITTED INFECTIONS AMONG WOMEN OF REPRODUCTIVE AGE LIVING IN KATANGA SLUM KAMPALA, UGANDA

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Sexually transmitted infections (STIs) still stand as one of the commonest health problems affecting women of reproductive age especially in slums. These have further been idenified as cofactors of HIV transmission, multiple complications such as, abortion, infertility and ectopic pregnancy among others.The burden of STIs continues to remain high in Uganda at 69%. In order to design appropriate preventive measures ,there is need to establish the profile of knowledge, attitudes and practices of sexually transmitted infections among susceptible populations such as women of reproducitive age living in slums like Katanga in Kampala Uganda.This was a descriptive crosssectional study with 339 participants.A consecutive sampling method was done. Using a standardized questionnaire, woman in Katanga slum who met the eligibility criteria were interviewed and data collected from them. Data was coded, cleaned, transcribed and double entered using EPI-data 3.1 and analyzed using SPSS 17.0. Coded data was summarized using frequencies for categorical data and medians for continuous data.In this study76.7% knew what STIs are with 41.9% giving Syphilis, Candida, HIV and gonorrhoea as examples. Most of the participants (99.1%) were between the age of 25-49 years with the majority (31%) being between 25-29years. The commonest symptoms known to the participants were genital itching (59.2%) and genital rash (14.5%).Only 2.9% did not know about the predisposing factors for STIs, however most mentioned multiple patners (63.7%) and unprotected sex (50.7%).Only 51% could identify STIs by the signs and symptoms with 71.9% knowing that they are treatable and curable.40% of the participants lacked knowledge on the effects and complications of STIs on their health and body. Although knowledge on methods of prevention was high (92.3%), it was not followed by appropriate behavioural patterns since 18.8% were found positive for STIs using the symdromic approach and 82% mentioned having suffered from STIs in the past 06 months more than once.The level of knowledge about STIs and their prevention is not matched by sexual behavioural patterns. Many women in Katanga slum still dont practice the approppriate preventive measures for STIs . There is a need to lay strategy on how the preventive measures which are well known by this vulnerable population can actually be effectively adopted and practiced.

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CROSS SECTIONAL ASSESSMENT ON THE PREVALENCE OF HIV AMONG DOTS CLIENTS IN ADDIS ABABA ADMINISTRATIVE REGION 2011-2012

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TB and HIV are the major public health problems in Addis Ababa, considering this fact; Health institutional based cross-sectional study was undertaken in Addis Ababa administrative region from September 2011 to February 2012. The objective of the study was to determine HIV sero prevalence among registered tuberculosis patients in Addis Ababa public DOTs clinics. The HIV antibody was determined using a single ELSA technique. A total of 417 tuberculosis patients in 26 Dots (intensive care) clinics aged 16 years and above was enrolled in the study. The over all HIV sero prevalence rate among tuberculosis patients was 32.5%. The highest rate was observed in the age group 30 to 39 years. Almost equal proportions of male 49.3% were found to be HIV sero positive compared to the females 50.7%. Being unmarried was found to be associated with HIV positive test result ($p<0.005$). Those divorced and widow/widower patients had high proportion of HIV positive. Daily labourers and patients who are living alone were found to be significantly infected with HIV ($p<0,001$). The HIV positive rate was higher among pulmonary tuberculosis patients compared to extra-pulmonary tuberculosis cases. Smear positive pulmonary patients 25% were found to be significantly associated with HIV sero positive test result compared with smear negative pulmonary tuberculosis cases15.6%. It was concluded that HIV infection is highly prevalent among smear positive TB, higher schooling, daily labourers, young age group, unmarried TB- clients, people who had history of STD, history of multiple sexual partners. Finally, additional qualitative supportive study on KAP on TB patients towards HIV/AIDS is recommended.

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MYCOBACTERIUM TUBERCULOSIS-SPECIFIC CD8+T CELL RECALL IN CONVALESCING TB SUBJECTS WITH HIV CO-INFECTION

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Memory T cell populations recover following phase I chemotherapy for tuberculosis (TB) and augment the effectiveness of antibiotics during the continuation phase of treatment. For those with human immunodeficiency virus (HIV), the CD8+T cells may have an especially important role in host defense to Mycobacterium tuberculosis (M.tb) as CD4+T cell function and/or numbers decline. Here we performed a preliminary study to investigate the impact of HIV infection status on CD8+T cell effector function during the convalescent TB period. Peripheral blood samples from convalescent HIV+ and HIV- TB subjects were used to determine CD4+T cell count and monitor antigen-specific CD8+ T cell activation of effector function (lymphoproliferation, IFN-γ;, granulysin) in response to M.tb antigen. Our preliminary results suggest that HIV co-infection is associated with moderate suppression of the M.tb-specific memory CD8+T cell compartment in many subjects convalescent for TB. Interestingly, highly activated CD8+T cells were observed in recall experiments using peripheral blood from several HIV+ subjects that had low (<200 cells/mm3) CD4+T cell counts. Further investigation may provide important information for development of novel approaches to target M.tb-specific CD8+T cell memory to protect against TB in HIV-endemic regions.

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PREVALENCE AND RISK FACTORS OF PULMONARY TUBERCULOSIS AMONG HIV/AIDS PATIENTS IN IHIALA, ANAMBRA STATE, NIGERIA

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The prevalence and risk factors of pulmonary tuberculosis among HIV/AIDS patients were determined in this study. A cross-sectional study design was adopted and hospital records from 2007 to 2011 of 375 HIV/AIDS patients attending Highly Active Antiretroviral Therapy (HAART) Centre of Our Lady of Lourdes Hospital Ihiala, Anambra, Nigeria, were reviewed. A standard proforma was used to generate data on other risk factors and diagnostic records. The prevalence rate of PTB among HIV/AIDS patients was 6.1%. The mean age, BMI and CD4 levels were 35.53 years (SD 9.37), 22.22Kg/m^2 (SD 2.23) and 429.85cells/mm^3 (SD 268.41) respectively. Pulmonary TB prevalence was highest among HIV/AIDS patients aged 30-39 years (60.9%), married patients (60.9%), patients with normal BMI (100%) and patients with CD4 levels <200cells/mm^3 (60.9%). Lower CD4 levels <200cells/mm^3 (P<0.0001), age 30-40 years (P <0.019) and marital status (being married; P<0.005), respectively, were significantly associated with the occurrence of PTB among HIV/AIDS patients in this study. Co-treatment of TB/HIV/AIDS (DOTS-HAART), lifestyle modification and education to minimize exposures to risk factors should be scaled up and encouraged.

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THE IMPACT OF NUTRITIONAL STATUS ON FIRST LINE HAART FAILURE IN HIV-INFECTED CHILDREN IN CAMBODIA

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While the impact of antiretroviral treatment on growth in children is well established, the influence of prior nutritional status on the response to treatment is not well known. The objective of this study was to assess impact of nutritional status on treatment failure in HIV infected children receiving first line highly active antiretroviral therapy (1st line HAART). Nutritional status was assessed by height-for-age, weight-for-age and weight-for-height Z scores using the 2006 World Health Organization growth reference. CD4 cells counts and viral load (VL) were measured every 6 months or when CD4 drop-down has been observed, consequently minimally twice per years. Laboratory parameters and genotypic resistance tests were performed in case of clinical or immunological failure by National Paediatric Hospital laboratory affiliated to Institute Pasteur Cambodia, Phnom Penh. First line therapy was consisted of Zidovudine + Lamivudine + Nevirapine or Efavirenz in those with concomitant tuberculosis infection. In our study group of HIV infected children treated with HARRT (n=98) the median Z score for HAZ, WAZ, BAZ at baseline of HAART was -3,3 (IQR = -4,4/-2), -3,4 (IQR=-4,1/-1,4), -1,4 (IQR=-2,3/-0,4) and decreased during study period -2 (IQR=-2,7/-1,5), -2,1 (IQR=-2,4/-1,5), -0,9 (IQR=-1,8/0,2) respectively. Concerning risk factors for treatment failure, we compared 77 (79%) children on 1st line HAART with 21 (21%) children with treatment failure (treated with second line regiments). Low CD4 cell percentage (<5%) and wasting (WAZ > - 3) at the baseline were not significantly associated with treatment failure. In our study, baseline poor nutritional status was not associated with failure of 1st line HAART among HIV-infected Cambodian children. All of our children were placed in 2 orphanages with good nutrition (five times a day) on inpatient basis, which may contribute to low failure rate. We advise the development of further studies to assess the nutritional status of children with HIV/AIDS using anthropometric measurements.

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DECREASING OCCURRENCE OF BACTERIAL SEXUALLY TRANSMITTED DISEASES AFTER INTRODUCTION OF VOLUNTARY COUNSELING AND TESTING HIV-PROGRAM IN ELDORET, KENYA IN 2009-2013

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Incidence of bacterial sexually transmitted diseases (STD) correlates with HIV as well with Hepatitis B and C and introduction within integrated program usually has impact on multiple diseases. The aim of this research is to assess the impact of community based integrated health program focused on HIV/AIDS and tuberculosis (TB) on the occurrence of bacterial STD in rural community of about 50 000 inhabitants in north Eldoret, Kenya, in area of HIV prevalence of 8-14 %. Among all outpatient department (OPD) visits during last 4 years (2009-2013) in Bl. Ladislaus Batthyany-Strattmann Clinic serving for about 50 000 people, incidence of STD and HIV in infections cases was assessed and correlated. HIV VCT program has been established in 2008 as VCT center next to the clinic with one VCT counselor and 1 trained nurse. All VCT/HIV program and all OPD visits for STD were recorded monthly. Syphilis and gonorrhea were evaluated as STD. Among 42711 OPD visits in last 4 years, STD was diagnosed in 1446 patients (3.39 %) and HIV in 462 patients (1.1 %). However, 10 years ago, when the clinic started its work, HIV prevalence in males was 8.6 % and in females 11.9 %. Dramatic decrease of HIV was correlated with sustained decrease of bacterial STD´s (syphilis, gonorrhea). While in 2009, 505 cases of bacterial STD and 110 new cases of HIV were detected, 421 STD´s were diagnosed in 2010, 176 in 2011 and 201 in 2012 and 148 in 2013 were recorded followed decrease of HIV from 110 in 2009 to 53 in 2013. Unfortunately, proportion of adults with HIV and STD was decreasing in 2009-2013, more then 3-fold, while pediatric SDT in children <5 years increased from 0 % to 1 % in 2009 , 3 % and 6% in 2010 and in 2013, respectively. Integrated HIV/STD community program led to more 3.3 - fold decrease of STD ad 2.1-fold decrease of AIDS in rural community of Eldoret after 5 years of the introduction of VCT. Moreover, increasing prevalence STD in children <5 years of age is of great concern.

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BACTERIAL INFECTION AND MALARIA IN HIV POSITIVE CHILDREN ADMITTED TO HOSPITAL IN NORTHEAST TANZANIA; RESULTS OF A ONE-YEAR OBSERVATIONAL STUDY

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Over three million children under 15 are now infected with HIV in sub-Saharan Africa, with profound health consequences. Both HIV infection, HIV treatment and malaria infection affect the incidence of invasive bacterial disease in children and the range of causative organisms, yet few studies have provided these data systematically. We present data from an observational study of paediatric admissions between June 2006 and July 2007 to a district hospital serving a rural population in northeast Tanzania. Children aged 2 months to 13 years admitted to the hospital during study hours with fever, or history of fever, were considered eligible.

Enrolled children underwent a standard clinical examination and panel of diagnostic blood tests including a research blood slide and rapid-test for malaria, blood culture and HIV serology. Children aged less than 18 months with positive serology were tested for HIV-1 RNA. CD4 T-cell counts were performed by flow cytometry on-site. Results from this study have been published, but data pertaining to the range infections in HIV positive patients are presented her for the first time. 3,704/4,334 (85.5%) of those children admitted during study hours were eligible, consented and enrolled. The data on 3,639/3704 (98.2%) were complete. HIV infection was diagnosed in 142/3,639 (3.9%). The most common clinical diagnosis in children admitted with HIV was very severe pneumonia (33/142 (23.2%)). In HIV positive children the prevalence of *Plasmodium falciparum* by blood slide was significantly lower (48/142, 33.8%) than in HIV negative (2,147/3,497, 61.4%, p<0.001), though the median parasite density in HIV positive (22,731 parasites/µl) and HIV negative children (36,758 parasites/µl) were similar (p=0.128, Wilcoxon rank-sum). HIV positive children had a higher prevalence of invasive bacterial disease (27/142, 19.0%) than HIV negative children (314/3,497, 9.0%, p<0.001). In children negative for HIV the most common bacterial isolate was non-typhi *Salmonella*, in HIV positive children *Streptococcus pneumoniae* was the most common isolate. HIV was implicated in over 1 in 8 inpatient deaths. We were unable to show such an association between HIV infection and severe malaria and malarial death in this population.

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PUBLIC-PRIVATE DIAGNOSTICS AND REFERRAL SERVICES FOR HIV/TB CO-INFECTED PATIENTS: A SYSTEMATIC REVIEW

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In 2012, there were an estimated 8.6 million new infections and 1.3 million deaths due to tuberculosis (1). Tuberculosis is the second leading cause of death due to infectious diseases worldwide (2, 3), and is the leading cause of death among people living with HIV (3). The successful integration of tuberculosis and HIV services in both the public and private sector is essential to the fight against the dual burden of TB and HIV. The WHO Stop TB department has called for a collaboration of public and private healthcare providers (often referred to as public private mix [PPM]) to maximize TB/HIV integrative services while minimizing costs. Many challenges remain for engaging all care providers into integrated HIV/TB services, particularly in regards to co-current diagnostics and referrals. HIV patients must be screened for tuberculosis, and tuberculosis patients must be screened for HIV. An efficacious public private partnership has the potential to integrate tuberculosis and HIV diagnostics and referral services to improve health outcomes and reduce patient costs. This study was undertaken to determine the frequency of public-private diagnostic and referral services for HIV/TB co-infected patients in high burden countries? Using several electronic databases and WHO indictors, we will conduct a systematic review to assess the degree of integration of various public-private partnership models for co-current HIV/TB diagnostics and referral services. We would like to evaluate the efficacy of various PPP models using published indicators of successful integration of services, and discuss the subsequent policy implications of our findings.

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PREVALENCE OF HIV/AIDS AND TOXOPLASMOSIS IN NIGERIA

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Human Immunodeficiency Virus (HIV) has been infecting people all over the world at an alarming rate and giving rise to full blown AIDS (Acquired Immune Deficiency Syndrome). Its prevalence in Nigeria is a concern. It is at present believed to infect 3.1% of a population of 150 million. Associated with HIV/AIDSs are opportunistic infections. Some of these are cryptosporidiosis, *Pneumocystis carinii* infection and Toxoplasmosis. Toxoplasmosis is one of the most serious opportunistic infections in HIV/AIDS patients that lead them to their early demise. For HIV/ AIDS patients, cerebral toxoplasmosis causes encephalitis which worsens the patient's condition. Human beings get infected by eating raw or undercooked meats containing *Toxoplasma gondii* cysts. Such meats come mainly from pigs, sheep and goats, especially the last two which Nigerians eat a lot and most commonly during festivities like Christmas celebration, birthday celebration, Muslim festivals, etc and are sold commercially. There is scarcity of information about co-infection of HIV/AIDS and Toxoplasmosis in Nigeria. A review of available literature on the prevalence of toxoplasmosis in humans in Nigeria shows that there are about 17 reports between 1960 and 2010. Of these only three show co-infection of HIV/AIDS and toxoplasmosis. Even so, the infection rate is fairly high (38.8%/ 219 and 43.4%/60 and a positive case report). There is a need in Nigeria to step-up activities involving the screening of HIV/AIDS patients for toxoplasmosis and treating those found infected as there is treatment for toxoplasmosis. This will prolong the lives of patients.

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POVERTY, FOOD INSECURITY AND LOW BODY MASS INDEX, ARE ASSOCIATED WITH POOR QUALITY OF LIFE FOR PEOPLE LIVING WITH HIV IN RURAL HAITI

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Poverty, food insecurity, and HIV/AIDS often co-occur and have a synergistic negative effect on health. However, the impact of these factors on quality of life (QOL) has only recently begun to be explored for people living with HIV (PLWHIV) in resource-poor settings. This study investigates the relationships between poverty, food insecurity, body mass index (BMI), and QOL in a cohort of 524 HIV-positive patients in rural Haiti. Multivariate logistic regression models were fitted to identify predictors of high QOL based on a survey containing a validated poverty score, the Household Food Insecurity Access Scale, and three domains of the AIDS Clinical Trials Group Health-Related Quality of Life Scale: general health, physical functioning, and role functioning. Of 489 respondents, 246 (50%) reported severe food insecurity and 428 (88%) reported some food insecurity. Mean QOL scores were 45 (Health), 62 (Physical), and 65 (Role), out of 100. Not being severely food insecure (OR=0.453, p=0.035; OR=0.387, p=0.008), having less poverty, (OR=0.957, p<0.001; OR=0.969, p=0.005), and having a higher BMI (OR=1.1, p=0.002; OR=1.08, p=0.007) were each independently associated with higher health and physical QOL. Having access to food from a garden (OR=1.79, p=0.05; OR=2.01, p=0.016) and younger age (OR=0.978, p=0.039; OR=0.97, p=0.005) were associated with higher physical and role functioning QOL. Food insecurity is highly prevalent at 88% in this population of PLWHIV in rural Haiti. This population's mean QOL scores were also substantially lower than scores reported from other cohorts of PLWHIV in resource-poor settings. Food insecurity, low BMI, and poverty are independently associated with poor QOL in PLWHIV and must be addressed as an integral component of comprehensive HIV programs in this setting.

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EVIDENCE OF A DISTINCT PROFILE OF METALLOPROTEINASES 2 AND 9 AND THEIR INHIBITORS IN CARDIAC REMODELING OF CHAGAS DISEASE

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Dilated chronic cardiomyopathy (DCC) in Chagas disease is associated with myocardial remodeling and interstitial fibrosis, resulting in significant extracellular matrix (ECM) modifications. ECM remodeling is regulated by proteolytic enzymes such as matrix metalloproteinases (MMPs). The main objective of this study was to evaluate the involvement of MMPs 2 and 9 and their inhibitors (TIMPs) in indeterminate (IND) and cardiac (CARD) clinical forms of Chagas disease. We evaluated, for the first time, the serum levels of MMPs 2 and 9 and TIMPs 1 and 2, as well as the main cell sources of MMPs 2 and 9 in the peripheral blood from patients presenting IND or CARD clinical forms of Chagas disease. Our results showed that MMP-9 serum levels are associated with the severity of Chagas disease. The serum levels of TIMP-1 were not different between the studied groups; however the serum levels of TIMP-2 were higher in CARD group. The correlation analysis showed a possible specificity of TIMP-1 for MMP-9. The analysis of MMPs production by T lymphocytes showed that CD8+ T cells are the main source of both MMP-2 and MMP-9 molecules. Using a new 3-dimensional model of fibrosis we also observed that serum from patients with Chagas disease induced an increase in the extracellular matrix components in cardiac spheroids obtained from mice cardiomyocytes. Furthermore, MMP-2 and MMP-9 have showed different profile of correlation with matrix proteins (laminin and fibronectin) and inflammatory cytokines (TNF-alfa and IL-1beta) in patients with Chagas disease. Our results suggest that MMP-2 and MMP-9 show distinct activities in Chagas disease pathogenesis. While MMP-9 seems to be involved with the inflammation and cardiac remodeling of Chagas disease, MMP-2 does not show any correlation with inflammatory molecules. In conclusion, our data stress the involvement of MMP-9, and not of MMP-2, in heart disease and, for the first time, its participation in chagasic cardiomyopathy. These data are innovative and represent an advance in the knowledge of the mechanisms involved in the establishment/maintenance of the Chagas heart disease pathology.

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EFFECTS OF INFECTION AND *TRYPANOSOMA CRUZI* SOLUBLE ANTIGEN EXPOSURE ON A HUMAN ASTROCYTE CELL LINE

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Trypanosoma cruzi can compromise the human central nervous system (CNS), especially during the acute phase of infection or in immune-suppressed hosts. Astrocytes could be essential cells in the pathogenesis of *T. cruzi*'s CNS infection. It is known that protozoans such as Toxoplasma gondii and *Leishmania* spp. secrete proteins that facilitate invasion and intracellular survival. This work evaluated the changes induced in a human astrocyte line when: a) infected with *T. cruzi* trypomastigotes, b) exposed to the live parasite separate by membrane filter, or c) exposed to a soluble antigen from trypomastigotes. The number of cells and morphology were evaluated by light microscopy on Giemsa-stained cells; HLA molecule expression and cell cycle were assessed by flow cytometry. An increase in the number of cells was observed, proportional to the amount of soluble antigen used in culture from 3.95x105 cells/ml in control cells to 7.0x105 cells/ml with 10 µg/ml, and 9.9x105 cells/ml with 100 µg/ml (p=0.0174). The percentage of cells in G2/M phase of the cell cycle was higher in cultures exposed to soluble antigen (8.43% and 9.36% at 10µg/ml and 100 µg/ml, respectively) than control cultures (6.06%, p=0.018). *T. cruzi* infection at day four post-infection increased the intensity, but not the percentage of HLA class I expression (mean fluorescence intensity 9,634 ±2,260 for infected cultures vs. 2,986 ±1,877 for control cells, p=0.0000126); and raised the percentage of HLA class II molecules in infected cultures (11.71% ± 5.63%) compared to control cultures (1.4%± 0.17% p=1x10-8). Exposure to the soluble antigen increased expression of HLA class II (7.71% ±4.04 with 10 µg/ml, 11.36% ±8.36 100 µg/ml) in comparison to cells without antigen (1.93% ±0.9, p=0.0168), and no changes in HLA class I were detected. Infection by *T. cruzi* and exposure to a parasitic soluble antigen induce cellular proliferation as well as changes in HLA molecule expression patterns of human astrocytes line, implicating astrocytes as participants in the local cellular response during CNS infection.

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EVIDENCE OF HOMOGENEOUS DISTRIBUTION OF *LEISHMANIA* AMASTIGOTES IN ULCERS OF CUTANEOUS LEISHMANIASIS

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Polymerase chain reaction (PCR) testing of skin lesion specimens currently provides the most sensitive method for diagnosis of cutaneous Leishmaniasis (CL), a disease highly endemic in Latin America. Previous studies that have compared the sensitivity of *Leishmania* detection either by microscopy or PCR using different sampling methods and sites on the lesion showed discrepant results. Sensitivity can be variable and dependent upon the infectious agent load and its dispersion in the lesion. We applied a quantitative real-time PCR assay targeting *Leishmania* (*Viannia*) minicircle kinetoplast DNA to quantify the parasite load in biopsy, scraping and cytology brush specimens obtained from the ulcer center, base and raised border. A total of 31 patients with parasitologically confirmed CL were enrolled: 29 males and 2 females, with median age of 34 years and median disease duration of 2 months. Parasite loads in skin samples varied between 1.1E+00 and 5.7E+06 parasites per µg of tissue DNA. Median parasite loads did not differ significantly among the three sites of the ulcer in all sampling methods, but there was a trend towards fewer parasites in the ulcer border where the diagnostic specimen is usually obtained. Compared to biopsies, a greater amount of parasites could be quantified from dermal scrapings of the border (p=0.007) and from cytology brushes of the ulcer base (p=0.02) and center (p=0.01). Parasite load measurements on biopsies versus scrapings or cytology brushes were highly correlated [Spearman's rho range 0.75-0.95, p<0.0001]. There was no significant difference in parasite load according to the infecting species (p=0.39), with L. braziliensis and L. peruviana the predominating species. Our results suggest that in recent onset CL, *Leishmania* amastigotes distribute homogenously within the ulcer; thus, samples can be easily and safely obtained from ulcer centers and bases preserving diagnostic performance. The use of scrapings and cytology brushes outperforms invasive biopsy. Further studies with larger samples and with longer disease duration are needed.

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3D MODELS OF SAND FLY SALIVARY PROTEINS

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Sand flies (Diptera: Phlebotominae) are vectors of *Leishmania* (Trypanosomatidae), the causative agents of cutaneous and visceral Leishmaniasis. During the blood feeding, sand fly females inject saliva into the host skin to overcome host haemostatic mechanism. Many proteins with distinct functions were found in cDNA library of sand fly salivary glands. 3D models of salivary proteins would allow us to better understand the protein function in antihaemostatic and immune responses. Our study was focused on yellow-related proteins, the high affinity binders of the host prohemostatic and proinflammatory biogenic amines such as a vasoconstrictor serotonin. In *Lutzomyia longipalpis*, it has been shown that salivary yellow-related proteins bind serotonin into their binding pocket located in the central part of the molecule, thereby hamper its physiological function and allow blood feeding of sand fly females. We took advantage of the known 3D structure of *L. longipalpis* yellow-related protein. The predicted 3D structures showed interspecies variability in the amino acid residues inside the binding pocket. For example, the bond of serotonin is probably stronger in P. orientalis yellow-related protein PorMsp24 than in 3Q6K of *L. longipalpis* due to the substitution of phenylalanine for glutamine and the new possible hydrogen bond between serotonin and glutamine. Such substitutions may affect the binding ability of yellow-related proteins. In *L. longipalpis*, the binding ability differs also between the yellow-related protein variants within the species. Since there are several yellow-related proteins in each sand fly species, we constructed 3D models to predict also the interspecies variability in serotonin binding ability. Yellow-related proteins are also highly antigenic for hosts and are considered as candidates for transmission blocking vaccine against Leishmaniasis, which means the need to know their exact function and structure. 3D models of sand fly salivary yellow-related proteins were constructed in MODELLER and visualised in The PyMOL Molecular Graphics System.

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SPONTANEOUS HEART DISEASE IN THE ADULT CHIMPANZEE: THE ROLE OF *TRYPANOSOMA CRUZI*

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Heart disease is the leading cause of death in the United States, and kills over 650,000 people annually. A high incidence of heart disease, especially idiopathic cardiomyopathy, is seen in chimpanzees and is the leading cause of death in chimpanzees at the Southwest National Primate Research Center (Seiler 2009). The high prevalence of heart disease-related mortality in captive colonies makes chimpanzees a valuable animal model for studying heart disease. The aim of this study was to retrospectively examine potential contributing factors and causes of heart disease in chimpanzees with emphasis on *Trypanosoma cruzi* (*T. cruzi*). We reviewed necropsy reports of adult chimpanzees that died with heart disease as a histopathological finding. We examined age, sex and cause of death. The overall prevalence of heart disease in chimpanzees was 67.81% (Seiler 2009). Of these, 30.12% have myocarditis and the remainder died of cardiomyopathy. We divided the animals into two groups based clinical findings and histopathological characteristics. Group1: myocarditis and Group2: cardiomyopathy. Myocarditis was indicated by lymphocytic infiltration of the myocardium. Cardiomyopathy was characterized by diffuse myocardial fibrosis with minimal inflammation. By simple PCR, we tested 78 heart tissue samples from chimpanzees in these groups for the presence of *T. cruzi* DNA. Of the chimpanzees that died of heart disease, only 19% tested positive for the presence of *T. cruzi*. Of all of the animals tested, the myocarditis group was 23% positive for *T. cruzi* while 18% of the cardiomyopathy group tested positive. Combined, the percentage that tested positive was 19% (15/78). In some tissues (2/35), *T. cruzi* microorganisms were observed. The cause of heart disease in these animals is still under investigation. Based on these results, *T. cruzi* is one of the causes of heart disease in the chimpanzees at SNPRC, however it is not the main cause.

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INFECTION BY *LEISHMANIA BRAZILIENSIS* IS ALTERED IN U937 DERIVED MACROPHAGES EXPRESSING EXOGENOUS GFP

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Gene knock-down mediated by RNA interference is increasingly used during the study of host-parasite interaction. Often exogenous gene expression such as GFP is used as a reporter during functional assays. The aim of this work was to evaluate exogenous gfp as a positive control in order to verify RNAi mediated gene silencing, in the context of U937 derived macrophages infected by *Leishmania* braziliensis. Parental cell line U937 was genetically modified via lentiviral transduction with a construct encoding for constitutive expression of GFP, generating the cell line U937-GFP. In turn this cell line was transduced with vectors encoding for a shRNA targeting GFP or a non-relevant shRNA as negative control, to generate the cell lines U937-GFP/shRNA-GFP and U937-GFP/shRNA-NR, respectively. The three U937 derived cell lines were characterized in terms of GFP expression by Western blot, as well as their ability to be infected by *Leishmania* braziliensis. One-way ANOVA and Sidak test were applied for statistical analysis using STATA 13 software. Results showed a reduction of 88.9% in GFP levels in the cell line U937-GFP/shRNA-GFP while there was no change in GFP expression in the negative control U937-GFP/shRNA-NR. In functional terms, the U937-GFP cell line showed reduced infection rate as compared with the parental cell line U937. Interestingly, this infection parameter was reconstituted to parental levels, in the U937-GFP/shRNA-GFP cell line, but remained low in the negative control U937-GFP/shRNA-NR still expressing GFP.This suggests that expression of GFP is responsible for the reduction in infection rate observed in the U937-GFP cell line. Caution should therefore be considered when using GFP exogenous expression in this cellular model of human macrophages.

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GENETIC DIVERSITY IN *LEISHMANIA DONOVANI* FROM SRI LANKA: USE OF MINICIRCLE DNA FOOTPRINT ASSAY

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Leishmaniasis comprises a variety of syndromes primarily due to at least 16 species and subspecies of *Leishmania.* In humans, the clinical spectrum ranges from asymptomatic infections to high mortality, with three distinct forms being classically described: visceral (VL), cutaneous (CL) and mucocutaneous (MCL). Leishmaniasis is a recently established disease in Sri Lanka with over 3000 cases of CL distributed island-wide with a few visceral and mucosal cases reported during the past decade. A genetically distinct variant of the usually visceralizing *L. donovani* is the causative agent and has been studied here using mitochondrial minicircle footprint assay to further understand its genetic status. Extracted parasite DNA from skin lesions of 34 CL patients and bone marrow aspirates of 4 VL patients were subjected to kDNA minicircle PCR followed by its comparative sequence analysis through dendrogram using 6 reference *Leishmania* species as previously reported. Sri Lankan isolates from skin lesions made a separate cluster within other known *L.donovani*. There

were 4 distinct subclusters seen within the Sri Lankan group with isolates that demonstrated poor response to standard drug (intra-lesional sodium stibogluconate) forming a separate subcluster. No specific clustering of clinical isolates based on their geographical origins across Sri Lanka was apparent in the dendrogram. Distinct genetic mutations with specific functional characteristics are likely to induce drug resistance. However, correlations between *L.donovani* minicircle based strain specific sequence heterogeneity and distinct clinical characteristics needs further investigation. Table of Contents

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APPLICATION OF MOLECULAR TECHNIQUE FOR THE DETECTION OF VISCERAL LEISHMANIASIS IN BLOOD DONORS IN ENDEMIC AREA FOR LEISHMANIASIS IN FORTALEZA-CEARÁ, BRAZIL

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Leishmaniasis is caused by protozoa of the genus *Leishmania* and is endemic in 98 countries, affecting the poorest mainly in developing countries. In 1991 the World Health Organization (WHO) raised the issue that there is the possibility of transmission of *Leishmania* by blood donors. The conditions of storage of blood products allow the survival and maintenance of infectivity of *Leishmania* in blood banks and to date, serologic screening is not done in blood donors in Brazil. In areas of transmission, asymptomatic carriers represent a large contingent, it is important to evaluate the possibility of blood products are a potential risk for transmission of the parasite. In this study, buffy coat of blood donors of the Center of Hematology Ceará (Hemoce), were analyzed by conventional PCR to look for circulating *Leishmania* and PCR positive sample were randomized to perform sequencing for confirmation of the parasite. From May to November 2011, 351 samples of buffy coat of blood donors were analyzed. All of them had a negative serology for Chagas Disease, Syphilis, HIV I and II, HTLV I and II, Hepatitis B and C. For the conventional PCR reaction, specific primers (150 and 152) for the genus *Leishmania* were used, described by Oliveira, 2005, referring to a sequence of the minicircle fragments kDNA with 110pb. The presence and integrity of the human DNA was verified by amplifying the β-globin gene, generating a fragment of 252 bp described by Pizzuto, 2001. DNA of *Leishmania* was detected in peripheral blood from 15/351 (4.3%) donors. The sequencing of 6/15 (40%) of the positive PCR confirmed that the isolated gene is belonging to the genus *Leishmania*. The results indicate the presence of *Leishmania* in blood donors and this implies in potential risk of transmission by blood transfusion depending on the parasite load. The role of asymptomatic donors infected with *Leishmania* in the chain of transmission is still not well established, so the possibility of transmission of the parasite by asymptomatic carriers must be better evaluated.

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MOLECULAR CHARACTERIZATION AND DRUG SUSCEPTIBILITY OF *TRYPANOSOMA CRUZI* ISOLATES DERIVED FROM INFECTED HUMANS OF ARGENTINA

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The genotype of *Trypanosoma cruzi* was related to the transmission cycle and geographic region. Herein, we studied the phenotype and susceptibility to trypanocidal agents of 5 *T. cruzi* populations recently isolated from 3 chronically infected adults (AR-SE23C, BOL-FC10A and AR-FC553) and 2 children with congenital infection (AR-FC202113 and AR-FC195205) living in Argentina. Molecular analysis showed that all isolates belonged to DTU V. For drug sensibility studies, epimastigotes were cultured in LIT media (106/mL) and treated with 0.38-150 μM of Benznidazol (BZ), Nifurtimox (NX), Pentamindine isethionate (PENT) and dihidroartemisinin (DH) during 72 h; SylvioX10/4 clone was used as reference strain. AR-SE23C, AR-FC553 and AR-FC202113 isolates were as susceptible to BZ (IC50= 3.35±0.5; 7.66±0.0 and 2.25±1.0 μM respectively) and NX (IC50=5.06 ±0.2; 5.54±0.1 and 1.21±0.2 μM, respectively) as Sylvio-X10/4 (IC50= 4.22±0.4 and 5.29±1.3 μM for BZ and NX, respectively). In contrast, significantly higher dose of BZ (73.08±4.3 and 40.62±3.1 μM) was required to reach IC50 for BOL-FC10A and AR-FC195205 isolates, respectively, and of NX (14.11±2.3 μM) for AR-FC195205 isolate. AR-SE23C, AR-FC553, AR-FC202113 and AR-FC195205 were similarly susceptible to PENT, with ID50 ranging from 2.68±2.1 to 4.09 ± 0.1 μM; instead, 10.27±1.7 μM of this compound were necessary to reach IC50 for BOL-FC10A. All isolates were similarly resistant to DHIC50 as compared to BZ, NX y PENT. Preliminary studies on C3H/He mice infected with AR-SE23C trypomastigotes and treated with 100mg/kg/day BZ and NX during 30 days showed that conventional treatment is effective to diminish mortality rate and parasitaemia levels. Nevertheless, histopatological analysis revealed that neither drug was effective to clear tissue parasites and ameliorate the inflammatory process generated in the acute phase. In conclusion, we here confirm the predominant circulation of *T. cruzi* DTU V in Argentina, which includes subpopulations with a wide range of susceptibility to trypanocidal agents *in vitro*. MINCYT/FONCyT-PICT 2010-2148

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UTILITY OF DIFFERENT CLINICAL SAMPLES OF *LEISHMANIA (VIANNIA) BRAZILIENSIS* LESIONS TO CONDUCT MICROSATELLITE ANALYSIS

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Leishmania (Viannia) braziliensis (LVB) is the main cause of cutaneous and mucocutaneous Leishmaniasis in the Americas. Microsatellites are DNA sequences repeated consecutively and present in abundance in non-coding regions of the eukaryotic genome. The variation in the number of repeats creates different alleles, allowing estimation of the genetic diversity of populations and gene flow. DNA from in vitro culture has been commonly used in Leishmania microsatellite analyses because of the quality of DNA and ease of standardization. However, parasite culture is difficult to obtain in field conditions. Other clinical samples are easier to collect, transport and maintain, but their use in population diversity studies has not been evaluated. Therefore, we assessed the viability and repeatability of microsatellite analyses based on different clinical samples from the same patients. We studied 22 cutaneous Leishmaniasis cases by LVB confirmed by nested real time polymerase chain reaction (PCR) and tested 53 clinical samples including cultures (22), biopsies (13), filter paper imprints (9) and scrapings using lancets (9). Samples were collected in Lima and six other cities of Peru during one year. Genomic DNA was extracted using the Qiagen DNeasy Blood and Tissue kit. Nine previously described microsatellite markers were tested by PCR and fragments amplified were analyzed to identify alleles using capillary electrophoresis in a 3130xl Genetic Analyzer (AB). We tested if all clinical samples amplified the nine microsatellite markers equally frequently and if PCR products from biopsies, lancets and filter papers showed the same allele peaks (± 2 base pairs) as PCR products from cultures. On average, cultures amplified significantly more markers than the other samples (8.4 versus 5.6 to 6.3) but all clinical samples showed the same alleles peaks as cultures for the nine markers in >90% of the cases. Seven markers had the same alleles in >95% of the cases. Culture strains of LVB amplify more microsatellite

markers compared to other clinical samples, but the alleles identified are similar across all type of samples. Considering that cultures are difficult to obtain in field conditions, the use of other clinical samples may allow a better characterization of the population structure and genetic diversity in this important parasite.

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UNDERSTANDING THE MOLECULAR EPIDEMIOLOGY OF *TRYPANOSOMA CRUZI* I IN NORTH AMERICA: NEW ORLEANS FIRST APPROACH

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The most current nomenclature of *Trypanosoma cruzi* recognizes six discrete typing units (DTUs), TcI-VI and a seventh one named Tcbat. TcI is highly heterogeneous and can be divided into 5 distinct subgroups TcIa-TcIe, based on differential amplification of the miniexon intergenic region. Though *T. cruzi* is endemic to Latin America, it has a marked presence in the United States. Despite the presence of an adequate vector for *T. cruzi* in Triatoma sanguisuga and one of the United States' few areas with an autochthonous case of human Chagas disease, there exist few studies exploring the genetic variability of Southeastern Louisiana's *T. cruzi* parasite populations particularly with respect to the TcI haplotypes. In the presented study, 60 rodent hosts and 12 T. sanguisuga vectors were captured from the site of Louisiana's local human *T. cruzi* infection. DNA extractions were prepared from rodent tissues and from cultures established from vector feces. *T. cruzi* prevalence was determined by diagnostic PCR, subsequent PCR genotyping methods allowed for detection of specific TcI sub-genotypes. Amplification of *T. cruzi* satellite DNA revealed 76.6% of infection among sampled rodents. Twenty two *T. cruzi*-positive rodents could be genotyped by differential amplification of the mini-exon and 19 (86.4%) were found to be TcI. Eight samples of feces from vector (66.6%) were trypanosomatid- positive by direct microscopic observation. Six strains were successfully isolated and genotyped as TcI. All TcI samples were further typed as haplotype TcIa. The findings presented here corroborate existing literature on North American *T. cruzi* genetic distribution as well as the current proposed geographical distribution of the TcI haplotypes. Further studies are required to fully assess the broader applicability of this study; however, it provides an epidemiological snap-shot of the sampled region, enhancing our current understanding of regional *T. cruzi* phylogeography.

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GENETIC EXCHANGE IN *LEISHMANIA TROPICA*

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Leishmaniasis is a parasitic disease caused by protozoan intracellular parasites of the genus *Leishmania*. *Leishmania* species can show different clinical presentations, ranging from non-lethal cutaneous and mucocutaneous Leishmaniasis, which typically leave disfiguring scars, to visceral leishmaniasis, which can be lethal if left untreated. While traditionally thought to propagate asexually, hybridization has been unequivocally demonstrated to occur in the sand fly stages for L. major, and evidence has also been found more recently in L. donovani. L. tropica is an emerging Old World species that is responsible for severe cutaneous disease throughout its range, from India to Northern Africa. From a sample set of 36 isolates, 25 nuclear markers and 3 kinetoplastid DNA markers were amplified and sequenced for multi-locus genotype analysis (MLSA). The preliminary sequence data shows extensive heterozygosity, indicative of potential ongoing hybridization occurring in this species. From this set, 4 strains have been selected for introduction of three different drug resistance markers. Selection of double-drug resistant hybrids was performed by culturing midgut homogenates of co-infected sand flies in the presence of both drugs. Here we present genetic evidence illustrating patterns of inheritance and gene exchange in this Old World species.

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DIVERSITY OF *TRYPANOSOMA CRUZI* INFECTION IN PATIENTS CO-INFECTED WITH HIV AND CHAGAS DISEASE

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Chagas disease, caused by the protozoan *Trypanosoma cruzi*, causes an unusual and severe neurological syndrome in immunocompromised patients such as those infected with HIV. We characterized the genetic diversity of *T. cruzi* infection in several HIV-infected Bolivian patients with high-level parasitemia. Samples were derived from HIV-positive patients recruited into an epidemiological study of HIV/Chagas disease coinfection in Santa Cruz, Bolivia, and DNA was extracted from whole blood or guanidine-preserved samples using QiaCube extraction robot. *T. cruzi* infection was confirmed with RT-PCR. We used nested PCR to amplify a 327-base pair fragment of the polymorphic TcSC5D gene, which has previously been used for strain typing, and amplicon deep sequenced the region using an Ion Torrent PGM. We determined multiplicity of infection and genotyped the strains of *T. cruzi* using deep sequencing and conventional restriction fragment length polymorphism (RFLP) methods. Sequences were clustered to predict genotypes using a heuristic clustering algorithm. Within-host and within-population diversity indices were calculated using EstimateS. We have shown that deep sequencing of *T. cruzi* from clinical samples is possible. Furthermore, we have documented polyclonal infections in HIV-coinfected patients, which may have implications for the pathogenesis of the disease in this population. Further studies will examine parasite diversity in a larger sample size and compare *T. cruzi* strains found in blood and cerebrospinal fluid.

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ARTINM ADMINISTRATION DURING ACUTE EXPERIMENTAL INFECTION WITH *TRYPANOSOMA CRUZI* PROMOTES PROTECTION AGAINST THE PARASITE

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Chagas disease is caused by infection with *Trypanosoma cruzi*, and the profile of the host immune response is essential for the infection control. ArtinM, from *Artocarpus heterophyllus*, a lectin that binds to the manotriose, modulates immunity toward Th1 axis and confers resistance to intracellular pathogens. Then, we analyzed the ArtinM immunomodulatory property during acute experimental infection with *T. cruzi*: Male BALB/c mice (10 mice/group) were used at 10 weeks-old, and the infection was realized with *T.cruzi* (3000 trypomastigotes; "Colombian

strain"). The ArtinM administration (0.5µg/100µL; i.p.) was performed before infection (-5, -4 and -3 days) and post–infection (5, 10 and 15 days), and as negative control was used the vehicle (saline), followed by groups: Saline control (SC), Saline infected (SI) and Lectin infected (LI). Parasitemia was determined at 7th, 14th and 21th days post-infection. The hemogram, reticulocyte count, cytokines measurement (plasma and heart), histological analysis of heart (inflammatory infiltrate and nests of amastigotes) were performed after 23 days of infection. Inflammatory infiltrate was measured in H&E stained heart sections, through the point's grid with predetermined area that express the area (%) of inflammatory infiltrate. This method was used to determinate the number of nests of *T.cruzi* by immunohistochemistry. IL group shows significant decrease in parasitemia at 14th and 21th days of infection. IS and IL showed increased reticulocytes, total leukocytes, neutrophils, lymphocytes and monocytes, and the hematimetrics parameters were decreased, ArtinM administration promotes a significant reduction in the inflammatory infiltrate (% of area) and nests of *T.cruzi* in the heart. Moreover, low levels of IL-12 p40, IFN-γ and TNF-α were found after ArtinM treatment, and IL-10 production was elevated, verified in plasma and heart. Thus, ArtinM administration demonstrates a protective effect against T.cruzi during acute experimental infection.

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INFLUENCE OF LOAD PARASITIC IN INFLAMMATORY RESPONSE AND DEVELOPMENT OF INTESTINAL INJURY IN MICE INFECTED WITH *TRYPANOSOMA CRUZI*

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The acute phase of Chagas' disease is reported to be multifactorial; among the forms of evolution of the most common disease have cardiac and digestive forms, this last in the esophagus and colon. Therefore, this study aims to evaluate variations of antigenic concentrations in the intestinal lesions in mice with acute *Trypanosoma cruzi* infection. We used 40 male mice C57BL/6WT divided into control group (A) and infected groups with $3x10^2$ (B), $3x10^3$ (C) and $3x10^4$ (D) forms trypomastigotes of strain "Y" of *T. cruzi*. From a total of eight groups, four were euthanized after 7 (S) days of infection, and the remaining groups after14 (F) days. The parasitemia was performed daily from day 3 after infection until the time of euthanasia. In the morphometric analysis, we verified the area myositis, ganglionitis and periganglionitis in relation the area of the muscle layer of the colon. Also analyzed the width and thickness of the muscular layer and of the colon mucosa. By immunohistochemistry, we detected the presence of *T. cruzi* nests. The quantification of the cytokines were performed by CBA. In results, there was an increase in parasitemia dependent on the concentration of inoculum between the different groups. Immunohistochemistry showed antigenic labeling in all animals of the group DF and in no animal of the BS group. The periganglionitis, ganglionitis and myositis were growing between the groups (significant differences), except to groups C and D, seven and fourteen days. The mucosal thickness showed no differences between groups. The width of the colon did not change between groups, except for DF, where there was enlargement. The thickness of the muscle layer is not changed in groups euthanized at 7 days, but after 14 days of infection in CF was hypertrophy and a reduction in DF. In the results of the cytokines, there was a noticeable change in the highest inoculum having an IL-2 after 7 and 14 days, and IL-6, IFN-g and TNF-a only 14 days, in which IL-10 did not differ significant between groups. In conclusion, the parasite load and the time of infection directly influence in the number of nests of *T.cruzi* present in the colon, but not interfere with the mucosal thickness. There is a hypertrophy of the muscle layer in CF and decreased thickness of muscle in DF at the expense of enlargement of the colon, with alterations significants in production of citokines proinflammatory.

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LABORATORY FINDINGS OF ACUTE INFECTION WITH *TRYPANOSOMA CRUZI* FOR DIFFERENT CONCENTRATIONS OF THE STRAIN "Y" IN THE INOCULUM

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Hematological and urinary parameters are often used for screening and confirmation of various diseases. In Chaga's diseases highlighted the clinical and laboratory findings are the result of a multifactorial infection with *Trypanosoma cruzi*. Moreover, recent studies demonstrate that variations of the antigenic concentrations may influence the course of disease, as well as the functional response of the infected organism. This way, this study aimed to report the laboratory findings in acute experimental T. cruzi infection with different inoculums through hematological and urinary parameters. Mice C57BL/6, males, aged 8 to 10 weeks, weighing 20 to 25g were infected with different parasite loads ($3x10^2$, $3x10^3$ and $3x10^4$) through strain "Y" of *T. cruzi*. The curve of parasitemia was performed until the 12th day after infection. Hematological parameters were evaluated using the complete blood count (automated method - ABX MICROS 60) on days 6, 9 and 12 after infection. The analysis of urinary parameters was performed through physicochemical analysis and urine sediment after 9 days of infection. The onset of parasitemia curve varied with the concentration of parasites in the inoculums on the 3rd, 4th and 5th days, for high, medium and low, respectively. The period of peak occurred for the high, medium and low inoculums on days 10, 9 and 8 respectively after infection. Any assessment of the blood count (red blood count, reticulocyte count, white cells and platelets) and urinary parameters (sediment), we observed changes in the high-inoculums when compared with the control ($p < 0.05$). However laboratory evaluation by hemogram and urine routine are able to detect modifications, dependent on the parasitic load.

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EVALUATION OF METABOLITES NITROGEN IN MICE CBL57BL/6 INFECTED WITH DIFFERENT INOCULA OF STRAIN "COLOMBIAN" OF *TRYPANOSOMA CRUZI*

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Chagas' disease is a neglected tropical disease caused by *Trypanosoma cruzi*. Through increased catabolism, is associated also with renal disorders. This study aimed to evaluate the nitrogenous wastes from C57BL/6 mice infected with different inocula of *T. cruzi*. 70 mice male C57BL/6 were used at 12 weeks of age, weighing between 20 and 25g. The *T. cruzi* infection was performed with an inoculum of $3x10^2$, $3x10^3$ and $3x10^4$ trypomastigotes of strain "Colombian". The animals were divided in groups: non-infected control (GC); Infected with 300 forms (GIA); Infected with 3,000 forms (GIB) and Infected with 30,000 forms (GIC), both for 22 and for 31 days. Held parasitemia every 3 days, the 24-hour urine collection in individual metabolic cages and blood collection. Urea levels were made by ultra-violet method and creatinine by colorimetric kinetic method, both in blood and in urine 24 hours a spectrophotometer. There was a decrease in urinary volume on the GIC after 22 days of infection and GIB, GIC 31 days after infection, beyond the weight decrease of the GIC group of animals on both days. For the BUN there was an increase to of levels GIC (22 days) and GIC and GIA (31 days). Levels of urinary urea reduced in the group GIB (22 days) and in GIC (31 days). The plasma creatinine showed a tendency to increase in all groups after 22 days of infection, whereas after 31 days there was a reduction in the levels for GIB and GIC. Decrease in urinary creatinine levels in groups GIB and GIC

(22 days) and normal values for all groups 31 days. The BUN / creatinine ratio was elevated in GIC (22 days) and GIA and GIB (31 days). The levels creatinine clearance had decreased for GIB and GIC (22 days) while the group at 31 days showed normal levels. Conclusion: We conclude that there are changes in nitrogenous wastes in C57BL/6 mice infected with *T. cruzi*, dependent on the concentration of the inoculum.

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POPULATION-BASED SURVEILLANCE STUDY OF INFLUENZA AND COMMUNITY-ACQUIRED PNEUMONIA MORBIDITY SYNERGISM IN UKRAINE

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Historically, research of infectious diseases has focused on infections with single pathogens. However, infections with pathogens often occur in the context of other pre-existing viral and bacterial infections or pathological conditions. Clinically, this is of particular relevance for co-infections with pathogen *Streptococcus pneumoniae* and influenza virus, which both are the important cause of global morbidity and mortality in the world. However, the analysis of incidence data, representing the possible synergy between community-acquired pneumonia (CAP), which possible causative agent is S. pneumoniae, influenza and other respiratory diseases (RD) (chronic bronchitis, asthma, etc.) has never been presented before in Ukraine. The aim of the research was the model-based study of the official incidence data to identify the possible relationship between CAP, RD and influenza morbidity rates during 2007-2011 epidemic seasons in Ukraine via mathematical modeling. The official incidence data, published annually by Influenza Control Center and FG Yanovsky National Institute of Phthisiology and Pulmonology, was analyzed. As a result it was proposed conceptual synergy model of CAP, influenza RD morbidity among the population of Ukraine. The model parameters were found by the program developed in Java and based on the quasi-gradient method. The highest CAP morbidity rate in Ukraine was in 2009-2010 yrs., exceeding corresponding value for 2008 by 24.2 % and for 2011 - by 5 %. The analysis of incidence data showed significantly higher morbidity rates than average in Vinnytsia, Ivano-Frankivsk and Kyiv regions. The mathematical model implied the existence of functional relationship between the incidence of influenza and RD in their influence on the occurrence of complications such as CAP. Results of modeling showed that probably about 20% of CAP among the population of Ukraine occur as a complication after influenza (individually or against the background of bronchitis, asthma and other pathological conditions related to RD). In conclusion, it was proposed the meaningful approach to modeling that takes into account the functional relationship between RD and influenza cases that led to CAP and show strong association between these diseases and necessity of intensive preventive strategies for its control.

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THE FIRST ANTIGENIC/ANTIVIRAL CHARACTERIZATION OF INFLUENZA VIRUS ISOLATES RECEIVED DURING AUGUST 2009-SEPTEMBER 2012 FROM INFLUENZA SENTINELS SURVEILLANCE (ISS) IN ETHIOPIA

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Influenza is a respiratory disease caused by RNA viruses that affects birds and mammals. The vaccinations offered every flu season target types A and B Influenza Viruses. Detection of Influenza virus using Real Time-PCR technique started in Ethiopian National Influenza Reference Laboratory during the A (H1N1) 2009 pandemic and since then laboratory sends influenza positive samples to WHO collaborating center for Influenza at CDC Atlanta, for further antigenic characterization and drug sensitivity tests. A total of 145 samples that tested positive for influenza during August 2009-September 2012 were sent to CDC Atlanta for further antigenic characterization and drug sensitivity tests. In the reference Laboratory, the Influenza positive Isolates were characterized antigenically using hemagglutination inhibition test with a panel of post ferret antisera, and also tested for functional neuraminidase inhibition assay to assess susceptibility of the viruses to the neuraminidase inhibitors oseltamavir and zanamivir drugs. The results of 136(93.8%) isolates were available till September, 2012. 48.5 %(66) of the isolates were A/CALIFORNIA/07/2009-LIKE (H1N1) viruses,12.5 %(17) of the isolates were B/BRISBANE/60/2008-LIKE viruses, 4.4 %(6) of the isolates were A/PERTH/16/2009-LIKE (H3N2) GP viruses, 4.4 %(6) of the isolates were B/WISCONSIN/01/2010-LIKE viruses, 1.5 %(2) of the isolates were B/FLORIDA/04/2006-LIKE viruses, 2.2 %(3) of the isolates were B/VICTORIA/02/87,2.2 %(3) of the isolates were B/YAMAGATA/16L88 LINEAGE BY PCR, which is similar to B/Wisconsin/01/2010 virus. 21.3 %(29) of the isolates were unable to grow on the cell culture and was difficult for the characterization. 2.9 %(4) of the isolates were totally negative for Influenza Virus both by real time -PCR and cell culture techniques. All Influenza Positive Isolates were sensitive to Oseltamivir and Zanamivir on functional neuraminidase inhibition assay to assess susceptibility of the Influenza viruses to the neuraminidase inhibitors oseltamavir and zanamivir. All antigenically characterized Influenza Isolates were compatible with Influenza vaccine recommended by WHO. It also showed that diverse types of Influenza strains are circulating in Ethiopia. The inability of some positive isolates to grow in cell culture may possibly relate to sample collection, storage and sample transportation issues and need evaluations and improvements.

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SIMULATION MODELLING OF SOCIAL PROTECTION INTERVENTIONS FOR TUBERCULOSIS (TB) AND CHRONIC AIRWAY DISEASES (CAD) IN MALAWI

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Catastrophic health care expenditure has emerged as one of the concerns among global health practitioners particularly in low income countries. To address this concern, social protection has been used as a framework to address healthcare related poverty and vulnerability in these countries. This has been followed by a growing number of governments in low income countries developing and adapting social protection strategies in their health plans. Similarly, there is also a rising interest in social protection among global health and health system researchers. Despite global interests and adoption of health care related social protection interventions in low income countries, the implementation, uptake, equity and effectiveness of current social protection interventions are limited by; inadequate prevailing national health budgets which are often donor dependent; policies prevalent within the wider health sector and failure of integration of social protection intervention by government agencies and the health sector in general. The research was aimed at finding ways of improving social protection interventions aimed at protecting the poor and vulnerable populations in Malawi, especially those affected by chronic airway diseases (CAD) and TB. The overall objective was to improve delivery of CAD and TB services, by successfully engaging policy makers, healthcare providers and various stakeholders, in the generation of new evidence about effective ways to strengthen the provision, uptake, equity and effectiveness of social protection mechanisms in TB and CAD treatment. Simulation modelling, econometric analysis and process evaluation studies were used to evaluate the process and impact of existing social protection intervention and generate new knowledge. Preliminary results indicate that, the involvement as partners of major stakeholders directly responsible for social protection policy and interventions will ensure policy relevance of this research and its continued impact beyond the life of the research project.

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PNEUMOCOCCAL SURFACE PROTEIN A (PSPA) BASED PNEUMONIA VACCINE SHOWING ENHANCED PROTECTIVE IMMUNITY WHEN CONJUGATED TO VI POLYSACCHARIDE FROM *SALMONELLA TYPHI*

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Phase I data from trials using protein based pneumococcal vaccine antigens show poor immune response in humans. To address this problem protein antigen need to be presented in a way to induce a high immune response and subsequent protection in humans. We have developed a conjugate vaccine in which PspA family 1 and family 2 proteins from *Streptococcus pneumonia* are conjugated to Vi capsular polysaccharide from *Salmonella typhi*. The conjugation technology strongly boosts protective immune responses against *Streptococcus pneumonia* and *Salmonella typhi*.MethodWe have optimized a scalable high yielding method for fermentation and purification of Vi polysaccharide and two protein antigens of PspA Family. We have developed a method for conjugation of PspA Family 1 and 2 proteins with Vi polysaccharide and tested conjugates by ELISA for their immune response in mice. We have done preliminary challenge studies of the vaccine with pathogenic strains of *Streptococcus pneumoniae* to check vaccine efficacy.Results A series of Vi-PspA conjugates are prepared and tested in mice. A poor anti-PspA response was obtained when un-conjugated PspA was used as antigen but when conjugated to Vi a substantial increase in the responses were obtained. Immunized mice with selected Vi-PspA1 conjugate show 70-80% protection in the preliminary challenge studies done with Streptococcus pneumoniae. Further challenge studies with a Vi-PspA1 and Vi-PspA2 combination vaccine will be performed to enhance the protective immunity against both pathogens.ConclusionThe Vi-PspA production and conjugation process is developed for the purpose of Scale up and making a cost effective vaccine targeting developing countries.

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ELABORATE HEALTH RECORDS SURVEILLANCE FOR TB CASE FINDING IN AN INFANT COHORT STUDY IN PREPARATION FOR PHASE THREE TB VACCINE TRIALS IN WESTERN KENYA

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Finding every case of Tuberculosis in infant vaccine trials is important for efficacy endpoints. Health record surveillance could be a key source of TB suspects and therefore, TB Cases. We therefore, sought to evaluate the yield of health records surveillance for TB case finding in an infant cohort in preparation for vaccine trials.The study was a prospective, observational, cohort study. After enrolment, TB cases listed in TB registers were searched and matched with Health and Demographic Surveillance System data base to confirm residency and compared with the study database, if there was a march,further confirmation was done. Potential TB suspects were also generated from: Inpatient Department, TB laboratory,Patient Support Centre and X ray records.TB cases were found through both the active and passive detection systems. Interestingly the active case finding through scheduled TB screening follow-up visits generated only 8 cases (14.0%), whereas various "passive" surveillance systems located within the health system itself identified 72% of all TB cases. 12% of TB cases were diagnosed post mortem via verbal autopsy. In contrast to other infant cohort studies, incentivized individual health seeking behavior of parents likely played a significant role in increasing the effectiveness of the passive system. Elaborate Health records surveillance for TB case finding is labor and resource intensive, however, is likely to be more useful than routine TB screening to capture each endpoint in vaccine efficacy trials. Improvements are needed to reduce the proportion of TB cases detected post mortem

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SPECIES AND SEROTYPE DIVERSITY OF HUMAN RHINOVIRUSES FROM PATIENTS PRESENTING WITH INFLUENZA-LIKE ILLNESS IN KENYA IN 2008

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Human Rhinoviruses (HRVs) are the most common causative agents of respiratory infections. They are highly diverse (>100 serotypes) and display a high degree of sequence variation among individual serotypes. This occurs as a result of frequent recombination events and point mutations.There is paucity of information about the genetic diversity of HRV strains circulating in Kenya. In this study we analyzed HRV strains identified in samples collected across the country in 2008 to provide an insight into their genetic characteristics. 517 randomly selected archived samples from the ongoing country-wide influenza surveillance protocol were used in this study. These were nasopharyngeal specimens collected from persons > 2 months, who attended outpatient clinics in the year 2008 in hospitals located in 8 different regions in Kenya presenting with influenza like illnesses. Real-time RT-PCR was used to identify HRV and VP4/VP2 genomic region amplified followed by sequencing. The resulting nucleotide sequences of Kenyan HRV viruses were compared to those of homotypic prototypes to determine serotypic identities. Screening by real time RT-PCR detected HRV in 131 (25%) of the samples. Of these 33 (25%) amplified successfully by conventional RT-PCR, of which upon nucleotide sequencing 17 (50%) yielded usable sequences. Phylogenetic analysis based on the VP4/VP2 genomic region of Kenyan HRV strains, relative to HRV prototypes retrieved from Genbank, revealed separation of the sequences into three main clusters corresponding to HRV A, B and C species. Majority of the Kenyan strains (n=10) belonged to HRVA species and were identified as HRV A29, A47, A1 (n=2), A56, A49, A30, A106, A20 and A45 serotypes. Two Kenyan strains belonged to HRV B species. One of these was identified as HRV B6 while the other was segregated from the other strains. Only one Kenyan strain belonged to HRV C species and was identified as HRV C2. This study demonstrates circulation of Human Rhinoviruses in Kenya. It also shows their species and serotype diversity.Findings from this study suggest that HRV A strains played a key role in human respiratory infections in Kenya. Knowledge about circulating HRV strains is important as it may help guide development of therapeutic strategies against infections caused by these viruses.

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NEURAMINIDASE INHIBITOR SUSCEPTIBILITY OF INFLUENZA A ISOLATES OBTAINED IN KENYA, 2008-2011

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Neuraminidase inhibitors mainly oseltamivir and zanamivir function both as prophylactic and treatment agents. Currently there exists no data on antiviral susceptibility profile of influenza A isolates circulating within the Eastern African region. Here we characterized the antiviral susceptibility of the 2008-2011 influenza A viruses circulating in Kenya. RNA was extracted from virus isolates followed by PCR amplification of NA gene segments. Nucleotide sequencing of the NA amplicons was carried out using the BigDye chemistry prior to analyses using a suite of bioinformatics tools. IC50 values were determined using curve fitting software, Grafit 7.0. Out of 836 influenza A viruses 108 isolates were analyzed for markers of resistance to NA inhibitors. 7 of the 11, 2008 seasonal influenza A/H1N1 isolates depicted oseltamivir resistant marker H275Y while all 33 influenza A/H3N2 isolates had H275 hence were sensitive to oseltamivir. Similarly, genetic analyses of the A (H1N1) pdm09 strains in 2009 and 2010 showed that all had H275 marker. All the 14 influenza A/H3N2

isolates of 2011 had H275 marker. A total of 28 isolates were analysed for phenotypic susceptibility assay. The mean zanamivir IC_{50}s were 1.75nM, 2.53nM and 1.84nM for the subtypes H1N1, pH1N1 and H3N2 respectively. Most of the 2008-2009 (8) sH1N1 analysed showed highly reduced sensitivity to oseltamivir. The IC50s in the fluorescent assay ranged from 73nM-984nM. Pandemic A/H1N1 strains obtained between 2009-2011 indicated oseltamivir IC50 ranges of 1.60nM-6.32nM categorised as normal sensitivity. All the 8, influenza A/H3N2 isolates obtained between 2008-2011 were sensitive to oseltamivir with the IC50s ranging between 0.16nM and 0.94nM. The 2011, WHO range and median IC50 values for oseltamivir carboxylate were 0.4nM-10nM and 0.5nM; 0.1nM-5nM and 0.2nM; 0.2nM-10nM and 0.6nM for wild types sH1N1, sH3N2 and pH1N1 respectively. The 2011, WHO range and median IC50 values for oseltamivir carboxylate were 257nM-3455nM and 458.2nM; 132nm-2179nM and 191.3nM for mutant types sH1N1 and pH1N1 respectively. The WHO IC_{50} values for zanamivir both for mutant and wildtype strains ranged between 0.2nM-3nM for all subtypes with no significant difference between the mutant and wildtype strains for each subtype. H275Y mutation increased the IC50 in the 2008-2009 sH1N1 isolates by 50-100 fold. Resistance to NAI was found to be both drug and virus subtype specific.

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CHALLENGES FACING TB CONTROL IN INDIA FOCUSING ON THE ROLE OF XPERT MTB/RIF IN THE PUBLIC AND PRIVATE SECTOR

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Globally, tuberculosis (TB) remains a major public health issue with an estimated 8.8 million new cases and 1.3 million deaths reported in 2012. In India, this health issue is compounded by the failure to diagnose cases in the national surveillance system and the high incidence of TB drug resistance. Of the estimated 3 million cases missed by national notification systems globally, 31% were in India. Drug resistance is of increasing concern with 3.6% of newly diagnosed TB cases and 20% of previously treated patients having multidrug-resistant TB (MDR-TB), defined as Mycobacterium tuberculosis resistant to isoniazid and rifampin, with or without resistance to other first-line drugs. There were an estimated 99 000 cases of MDR-TB in India in 2009, including those outside the Revised National TB Control Program (RNTCP). The Indian RNTCP introduced the Programmatic Management of Drug Resistant TB (PMDT) to address the needs of this patient population and is rapidly scaling up its services. A key issue in the management of MDR-TB is timely and accurate diagnosis (including drug susceptibility testing). There is interest in the promise of point-of-care (POC) diagnostics for diseases of global health importance but a need for better appreciation of the POC testing process moving beyond the technology alone. One area of progress is the global scale up of Xpert MTB/RIF (Cepheid Inc.), a molecular test for TB that also enables rapid detection of rifampin resistance. However, a greater understanding of role that the large, unregulated private health care sector plays in providing TB care is essential. The Initiative for Promoting Affordable Quality TB Tests (IPAQT) endorses the use of WHO approved tests such as Xpert MTB/RIF in private labs at affordable rates. Focus group interviews were conducted with health care providers from the public and private health care sectors, the latter at sites involved with the IPAQT initiative, to ascertain current practices and perceptions and attitudes to inclusion Xpert MTB/RIF in the TB diagnostic algorithm. Perceived barriers to implementation include delays in obtaining results and cost.

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TUBERCULOSIS AND WAR IN THE U.S. MILITARY

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Studies which have demonstrated increased morbidity and mortality from tuberculosis (TB) during war have largely focused on civilian populations. TB also has a long and well-established association with the military, but the association of increased TB risk during armed conflict is less certain. The purpose of this study was to examine the U.S. military experience in times of war and armed conflict to better understand its impact on the risk of TB. This historical study estimates the risk of TB infection, disease and mortality during these conflicts, comparing those serving overseas with civilian and military populations remaining in the U.S. TB rates in the U.S. Army declined dramatically over the period from 1885 to 2012, from a high of 1,168 per 100,000 in 1917 to a low of 0.4 per 100,000 in 2012. Army rates were generally considerably lower than civilian rates, due in large part to the "healthy soldier" effect. Other than World War I, armed conflict showed very little impact on declining TB trends. Some of the focal risk groups known to have higher rates of TB included U.S. and enemy prisoners of war (POWs), the foreign born, and others found to be infected at induction into the military. Although the risk of TB in the U.S. military largely reflects that of the underlying U.S. population, the military also has unique exposures to tuberculosis during times of armed conflict. In order to protect the health of these troops and conserve military fighting strength, these unique exposures require additional surveillance and control measures.

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FEASIBILITY ASSESSMENT FOR ESTABLISHING SURVEILLANCE FOR EMERGING RESPIRATORY VIRUSES IN NON-PUBLIC HOSPITALS IN KENYA, 2013

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Influenza A H7N9 and Middle East respiratory syndrome coronavirus (MERS-CoV) are emerging respiratory viral infections with pandemic potential which can result in high fatality. As of March 2014, 395 cases and 125 deaths of influenza A H7N9 and 206 cases and 86 deaths of MERS-CoV had been reported worldwide. Kenya's high volume of international travel poses a risk for introduction of these pathogens. Public hospitals in Kenya conduct severe acute respiratory illnesses (SARI) surveillance. Little is known about SARI surveillance in non-pubic hospitals where majority of foreigners and travelers are likely to seek care. We conducted an assessment to determine the capacity of non-public hospitals to conduct SARI surveillance and to identify hospitals for implementation of MERS-CoV and influenza A H7N9 surveillance. In November 2013, we interviewed managers from 44 non-public hospitals in Nairobi and Mombasa counties. Respiratory illness accounted for a range of 0-18 admissions per day. A surveillance focal person was present in 32 (73%) hospitals, of whom 10 (23%) had received training in the last 12 months. Although requested by the Ministry of Health, national reporting of SARI was done in 18 (41%) hospitals; 12 (67%) reported monthly and 6 (33%) weekly. Among 28 (64%) hospitals which recorded patients' nationalities, foreigners accounted for a median of 10% (range: 0.5%-80%) of patients. Nasopharyngeal (NP) and oropharyngeal (OP) swab specimens were collected for clinical diagnosis in 27 (61%) and 29 (66%) hospitals respectively. In the last 12 months, 10 (23%) hospitals had trained their staff on NP/OP specimen collection, packaging, storage and transport to reference laboratories, and 27 (63%) had training on use of personal protective equipment. Non-public hospitals are an

important source of medical care for foreigners and the basic capacity for surveillance exists. These hospitals will be sensitized on the importance of national reporting of priority conditions, and some will be targeted for implementation of MERS-CoV and influenza A H7N9 surveillance.

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THE ACCURACY OF NON SEVERE PNEUMONIA DIAGNOSIS IN TANZANIAN CHILDREN: THE VALIDITY OF THE RESPIRATORY RATE

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Integrated Management of Childhood Illness (IMCI) guidelines recommend antibiotic treatment to all children presenting with cough or difficult breathing and an increased respiratory rate for their age. However, many pneumonia diagnoses in resource poor settings are done on the bases of sub-optimal condition; the environment may not be conducive for counting respiratory rates. We conducted a study to determine if respiratory rate was likely to be transiently raised by a number of contextual factors in a busy clinic leading to overuse of antibiotics. Respiratory rates were recorded in children aged 2 - 59 months presenting with cough or difficulty breathing to one of the two busy outpatient clinics and then repeated at 10 minute intervals over 1 hour in a quiet setting. A total of 167 children were enrolled with a mean age of 7.1 (SD±2.9) months in infant and 27.6 (SD±2.8) months in the older age group. The mean respiratory rate declined from 37.5 breaths per minute (bpm) at clinic to 35.8 bpm initial reading at quiet room and 35.0 bpm final reading ($p<0.02$). This resulted in 11(85%) being mis-classified with non severe pneumonia in infants and 2(13%) in older children. In a logistic model age group (infant or older child) was the only risk factor associated with over-diagnosis of non severe pneumonia. Over-diagnosis of non severe pneumonia in a busy clinic is significant and it tends to vary with age. Changing the respiratory rates cut-offs to higher threshold reduced the proportion of non-severe pneumonia mis-classification in infants. These findings have public health impact in managing these children as antibiotic over-use accelerates high levels of resistance. More studies of the accuracy and utility of respiratory rate as an indication for antibiotics are needed, especially as vaccines against bacterial pneumonia are introduced to many resource poor countries.

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MICROSCOPIC OBSERVATION DRUG SUSCEPTIBILITY (MODS): A RAPID DIAGNOSIS OF PULMONARY TUBERCULOSIS IN HIV/AIDS PATIENTS IN RESOURCE-SCARCE BOLIVIA

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The need for fast, reliable diagnosis of *Mycobacterium tuberculosis* (Mtb) infection is particularly acute in patients with HIV. This study examined the rates of tuberculosis (TB) and multidrug-resistant tuberculosis (MDRTB) in HIV patients in Bolivia. It evaluated the Microscopic Observation Drug Susceptibility rapid liquid culture (MODS) versus traditional Ziehl-Neelsen staining (ZN) and Lowenstein Jensen culture (LJ), and assessed the value of the string test and induced sputum in sputum-scarce individuals. The presence of Mtb in sputum of 107 HIV-positive patients was evaluated by ZN, LJ, and MODS. Mtb detection by string test was evaluated by MODS in 92 of these. The TB-HIV co-infection rate of HIV patients with respiratory symptoms by sputum sample was 44.9% (48/107). The mortality of TB+ hospitalized patients was 51.4% (18/35) and of ambulatory patients was 30.7% (4/13). The rate of MDRTB was 9%. Of 48 sputum samples positive by any diagnostic method, 63% were positive by ZN, 79% by LJ, and 96% by MODS. Median time to positive culture was 10 days by MODS versus 34 days by LJ ($p<0.0001$). In pts not able to produce sputum without induction, the string test had a sensitivity of 82% compared to induced sputum. Of the ten patients unable to produce a sputum sample, four were TB-positive by the string test. MODS was faster and more sensitive for Mtb detection when compared to LJ, and these differences are more pronounced in smear-negative patients, who are at the greatest risk for missed diagnoses. The string test, in conjunction with MODS, is a valuable diagnostic technique for HIV positive sputum-scarce patients. Nine percent of our patients had MDRTB, which reinforces the need for rapid detection of antibiotic sensitivity testing in HIV patients in Bolivia.

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HEALTH RISK ASSESSMENT OF PESTICIDES AND POLYCHLORINATED BIPHENYLS CONTAMINATIONS IN DAIRY PRODUCTS FROM SELECTED FARMS IN GREATER ACCRA REGION, GHANA

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The residual concentrations of synthetic chemicals such as organochlorines pesticides (OCPs), pyrethroids and polychlorinated biphenyls (PCBs) in dairy products (milk, yoghurt, cheese) from selected farms in Ghana were analyzed using Gas Chromatography (GC). A total of 50 samples of dairy products (9 cheese, 25 cow milk and 16 yoghurt) were analyzed for OCPs, pyrethroids and PCBs. Of the numerous pesticides evaluated, detectable levels of OCPs (β-HCH, endrin, heptachlor, endosulfan, p'p-DDT and methoxychlor);Pyrethroids (permethrin, allethrin, cypermethrin and deltamethrin) and PCBs (18, 28, 52,101, 153, 138, and 180) were found in all the dairy product samples analysed. Milk samples were found to be the most contaminated with respect to the OCPs and the levels ranged between 0.0001µg/ml and 0.0407µg/ml. β-HCH was the highest OCP with concentration of 0.0407µg/ml while Cyfluthrin was the highest synthetic pyrethroids recorded in yoghurt sample(0.0318µg/ml).The highest PCB 18 (2,2,5-Trichlorobiphenyl) recorded (0.2668µg/ml) in yoghurt samples. Data obtained from the field regarding safe use of pesticides and symptoms among farmers was very high. The estimated dose for γ-chlordane(8.5x10-5µg/ml), endrin(0.0114 µg/ml)p'p'-DDT(8.5x10-5µg/ml),DDE(8.5x10-5µg/ml),heptachlor(2.8x105µg/ml),dieldrin(6.8x10-5µg/ml) do not pose a direct hazard to human health, although present in milk samples since the values were lower than toxic threshold as well as,reference,doses (γchlordane: 0.0005 µg/ml; endrin:0.20µg/ml; p'p'DDT:0.50 µg/ml; DDE:0.50µg/ml; Heptachlor: 0.0001 µg/ml; and Dieldrin:0.005 µg/ml) and may indicate minimum risk to human. However, β-HCH(0.0375 µg/ml), endosulfan(0.0142 µg/ml), methoxychlor(0.0746 µg/ml) and PCBs (0.0498 µg/ml) levels exceeded the reference doses of (β-HCH:0.003 µg/ml, endosulfan:0.006 µg/ml, methoxychlor:0.005 µg/ml and PCBs:0.002 µg/ml) in children between the ages of 0-1 year, 1-11 years and adults indicating a great potential for systemic toxicity in all age groups especially children who are considered to be the most vulnerable population

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DESCRIPTIVE CHARACTERIZATION OF CHOLERA OUTBREAK CAUSED BY BREAK DOWN OF PUBLIC PIPE BORNE WATER, OKE ALAAFIA COMMUNITY, OYO STATE SOUTHWESTERN NIGERIA SEPTEMBER 2013

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Cholera is an acute illness with profuse watery diarrhea caused by *Vibro cholerae* serotypes 01 or 0139. In Nigeria, frequent outbreaks do occur. Effective interventions to control these outbreaks require the identification of the source and risk factors for infection. In August 2013, an outbreak of cholera occurred in Egbeda LGA. We investigated the outbreak to determine its magnitude, source, possible risk factors and initiate control measure. We reviewed cholera case-based line lists from health facilities, hospital records and conducted active case search for cases. We defined a suspected case as any resident of Egbeda, two years or above, with acute watery diarrhoea with or without vomiting between 26th August and 10th September, 2013. We used structured questionnaire to collect data on demographic characteristics, clinical informations, and risk factors. Data were analyzed with Epi-info software and Microsoft excel. Environmental assessment of water sources, water sampling, latrine use and waste disposal methods. We collected and analyzed 5 stool samples as well as 5 well water samples There were a total of 28 cases and 7 deaths case fatality rate of 25%. Twenty seven (96.4%) of cases were from Oke-alaafia community. Median age of cases 10.5yrs (range 2-65yrs); five of the deaths occurred among children 0-5years;(71.4%). Seventeen of the cases were males (60.7%). Major sources of water were wells (38.5%), 61.4% of respondents had no toilet facilities hence indiscriminate defaecation was common. Open dumping was the commonest (80.8%) waste disposal method. Vibrio cholerae 01 was isolated in 3 (60%) of stool samples analyzed. The outbreak probably occurred as result of drinking water from contaminated sources of water such as wells following break down of public pipe borne water. Chlorination of wells was done and we conducted intensive health education of Community members on proper storage and household water treatment.

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BACTERIOLOGICAL QUALITY OF WELL WATER USED FOR DRINKING PURPOSES IN GAROUA, NORTH CAMEROON

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Groundwater serves as a major source of drinking water in North Cameroon. Water quality is an important determinant of human health, considering in particular waterborne diseases in local communities. This study aimed at assessing the bacteriological quality and potential sources of well water contamination in Garoua, a metropolis of North Cameroon. The water quality of 23 wells was assessed through commonly used microbiological tests. Also analysed were physicochemical parameters of the water. For each well, monthly sampling was performed during 10 months; physical characteristics of sampling sites were documented and potential sources of contamination were identified. Results showed that the abundance of heterotrophic aerobic and mesophilic bacteria (HAMB) and bacterial bio-indicators in well water in Garoua all exceeded the WHO's drinking water standards. Total coliforms were present in all well water samples at high concentrations ($5.0x10^2$ to $4.8x10^4$ CFU/100 ml of water). The water harbours relatively high concentrations of faecal coliforms ($1.2x10^2$ to $2.3x10^4$ CFU/100 ml of water). *Escherichia coli* and faecal streptococci concentrations showed high spatial and seasonal variations from one well to another. The physicochemical analysis showed that, in 52.17% of wells, water was acidic with various mineralization. The principal component analysis (PCA) pointed out that seasonality had less influence on the majority of measured water parameters (pH, electrical conductivity, total dissolved solids, and salinity) than the location of the well water point. Human wastes from the traditional latrines system extensively used in this area and animal manure might have contaminated the wells.

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WATER RESOURCES DEVELOPMENTS IN ETHIOPIA: POTENTIAL BENEFITS AND NEGATIVE IMPACTS ON THE ENVIRONMENT, VECTOR-BORNE DISEASES AND FOOD SECURITY

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To satisfy the growing demand for electricity, Ethiopia plans to increase its electricity production five-fold between 2010 and 2015, mainly through the construction of dams. A literature review shows that while dams can boost power and agricultural production, promote economic development and facilitate flood control, they can also lead to environmental, ecological and socioeconomic changes. Several case studies show that dams may alter the composition and density of vectors and intermediate host species, increase the incidence of malaria schistosomiasis and possibly lymphatic filariasis and lead to eutrophication of reservoirs, soil erosion and earthquakes. There is evidence that dams and commercial irrigation schemes can increase soil and water degradation, vulnerability to drought and food insecurity in riverine and lacustrine areas downstream of dams. It appears that dams in Ethiopia are also vulnerable to high soil erosion rates and earth quakes. Consequently, the current and proposed large-scale dam construction program in Ethiopia requires in-depth research to improve our understanding of the unintended negative effects of projects and to guide the location, design and implementation of appropriate preventive and remedial programs .

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EXPLORING ADAPTATION MEASURES FOR INFECTIOUS DISEASES IN MACHALA, ECUADOR

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Ecuador is facing new challenges related to climate change impacts in the human health sector, especially for emerging infectious diseases, such as dengue fever. Although the public health sector is investing substantially in measures of surveillance and mosquito control, the effects of climate variability and change are not considered in the current strategies for reducing dengue virus transmission. Effective planning for adaptation measures in the public health sector would require the following: a) a better understanding of climate and environmental interactions with the vector and disease transmission in which a monitoring and surveillance climate-disease program is fundamental b) an evaluation of the effectiveness of current policies and measures in reducing the vector disease including 'what if' analysis in projected scenarios, and finally c) building scientific capacity in research institutions and looking for a permanent dialog with stakeholders and decision makers to discuss the adaptation measures on the climate change impacts on infectious diseases. With this perspective, a consortium of national and international institutions and local public health organizations is developing an interdisciplinary research program on dengue transmission in the city of Machala, Ecuador. This 3-year study will develop predictive models by

linking climate factors with local neighborhood social and environmental conditions to predict when and where dengue outbreaks will occur in Machala, Ecuador. This study aims to link multi-scale climate phenomena to neighborhood-level dengue risk, fundamentally changing how we think about climate-disease dynamics. At the same time, an iterative process of monitoring and evaluation of adaptation measures linked to decision making aids and tools is proposed for developing climate change policy in the public health sector.

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OCCUPATION-RELATED SELF REPORTED HEALTH PROBLEMS AMONG SOLID WASTE HANDLERS IN A RAPIDLY URBANIZING COASTAL COMMUNITY IN GHANA

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This study applied a mixed method design to investigate exposure to waste, use of personal protective gear, and self-reported health problems among solid waste handlers in a semi-urban township of southern Ghana. A total of 280 waste handlers were studied representing all types of waste handling practices including sweepers, collectors, transporters and disposers of waste. Most waste handlers engaged in multiple of these activities (69.3%). The most commonly reported health problems were bodily pains (56.4%), headache (38.6%), fever (35.7%), feelings of physical discomfort (28.2%), and diarrhoea (11.4%). In-depth interviews with 22 waste handlers also highlighted eye problems, stomach pains and non-specific symptoms such as stress and tiredness as commonly experienced. There was a correlation between exposure of bare body parts of waste handlers and disease, with a higher likelihood to report fever for those using bare hands during waste handling [odds ratio (OR) = 1.89 (95% C.I 1.37 - 2.56), $p < 0.0001$] and diarrhoea [OR = 6.25 (95% C.I 4.17 - 10.00), $p < 0.0001$] compared with those who used protective gear. Interviews showed that waste handlers generally had basic knowledge about the disease preventive purpose of wearing personal protective measures. However, observations revealed that most waste handlers did not use such measures consistently mainly due to discomforts, impracticalities of wearing it in hot and humid conditions, and not being supplied with protective gear by employers or not being able to invest in it themselves. The study indicates that waste handlers experience a burden of disease which may be consequences of their occupation. Our findings stress that waste handlers in rapidly urbanizing areas need protection against occupational diseases through the wearing of affordable and suitable protective gear. Waste companies and government institutions employing a growing number of waste handlers should train waste handlers in the proper use of protective gear, educate on how to protect their health and to provide such protective gear.

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VENO-OCCLUSIVE LIVER DISEASE IN CHILDREN AND YOUNG ADULTS: AN EMERGING PROBLEM IN DEVELOPING COUNTRIES

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Veno-occlusive liver disease (VOLD) is increasingly reported in young people from developing countries, particularly in the Arabian peninsula and in countries from the former Soviet Union. Many conditions can be associated with this multifactorial disease: parasitic diseases like CE and schistosomiasis, as well as TB, abscesses, cysts or the presence of a membraneous web that obstructs the terminal portion of the inferior vena cava . Poverty, malnutrition, recurrent bacterial infections, and filariasis have been suggested to be predisposing factors for inferior vena cava occlusion in developing countries , whereas myeloproliferative neoplasms, abdominal cancer and oral contraceptives are associated with this syndrome in developed countries. However, environmental toxic causes are described in children and young adult living in rural areas: particularly, pyrrolizidine alkaloids derivatives can cause the disease by microscopically damaging the hepatic venular bed. We report a case of a 25 year old male immigrant from a rural area of Morocco admitted to our Hospital for ascites, mild cholestasis and abdominal pain in October 2013. All tests resulted negatives except quanti FERON gold test, so the patient was unsuccessfully treated for TB for 2 months. The patient clinical conditions and LFT slowly worsened and after 2 inconclusive hepatic biopsies , a third biopsy combined with ultrasound and CT scan findings allowed the diagnosis of VOLD. All commonest causes of VOLD were ruled out, therefore, a toxic cause due to pyrrolizidines alkaloid derivatives was considered. The patient is currently on the wait list for liver orthotopic transplantation due to end-stage liver failure. This finding warrants more attention to the toxic environmental agents in developing countries , as they need to be considered in the differential diagnosis of liver failure also in immigrants to Europe.

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SOCIAL AND ENVIRONMENTAL DETERMINANTS OF CHILDHOOD DIARRHEAL DISEASE IN MALAWI: A NATIONWIDE, HOUSEHOLD-LEVEL, GEOSPATIAL ANALYSIS

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Diarrhea continues to be a leading cause of death among children under-five in developing countries. Despite increased access to improved water and sanitation in some settings, many areas of sub-Saharan Africa and Asia have been left behind. The complex and multifactorial nature of associations with childhood diarrheal disease complicate understanding of the more critical determinants of risk. We analyzed data from the 2010 Malawi Demographic and Health Survey to identify risk factors for diarrhea among Malawian children. A hierarchical logistic regression model and a GIS-based raster analysis using inverse distance weighted interpolation were used to evaluate the independent effects of sanitation on childhood diarrheal prevalence, and to characterize the distribution of diarrheal disease and its determinants across Malawi. Approximately one-quarter of all households reported at least one child under-five having diarrhea in the two weeks preceding the survey. Households with an average child age between 6-12 months had six times the odds of childhood diarrhea when compared to households with an average child age less than 5 months. While 79% of households had access to an improved water source, only 12% of households were using an improved sanitation facility, with an additional 11% lacking access to any sanitation facility. Households with no sanitation facility had 45% greater odds of childhood diarrhea as compared to those with an improved and unshared sanitation facility. In multivariable analyses, the significant effect of sanitation on diarrhea prevalence remained after adjusting for child age and maternal education. This study calls renewed attention to the persistent, yet preventable, burden of diarrheal disease among children in Malawi and reveals important District- and sub-District-level variation in the distribution of determinants that could be used to inform the targeting of future interventions.

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PERVASIVE EXPOSURE TO FECAL CONTAMINATION IN LOW-INCOME NEIGHBORHOODS IN ACCRA, GHANA

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Globally, diarrhea contributes to about 800,000 fatalities in children under five each year, and is a primary cause of mortality in developing countries. Rapid urbanization in low-income countries has led to a growing sanitation crisis. A need exists for more effective WASH interventions in low-resource urban environments that can minimize the transmission of feces and reduce the rate of diarrheal illnesses. Effective interventions require evidence-based research that highlights risk behaviors and perceptions of fecal contamination risk in people's daily lives. This study examines the context of fecal contamination during daily activities among residents in low resource urban settings of Accra, Ghana. Qualitative data were collected through 16 focus group discussions to understand the daily behaviors that place people at risk of fecal contamination. Data were collected and analyzed using a grounded theory approach to develop a conceptual framework of the context of fecal contamination in low income neighborhoods of Accra. MaxQDA10 software was used for data analysis. Results show that latrine use is low in these neighborhoods leading to a range of alternative methods of fecal disposal that contribute to fecal contamination throughout neighborhoods. Feces were further spread through refuse dumping, poor refuse collection systems, recreational activities, and occupational tasks of residents. These pathways of fecal contamination underscore the pervasiveness of risk for fecal contamination throughout low-income urban neighborhoods, suggesting the need for multi-pronged interventions that target multiple pathways of feces transmission.

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NEUROPSYCHOLOGICAL PROFILE OF CHILDREN IN NATIVE COMMUNITIES EXPOSED TO MERCURY CONTAMINATION IN MADRE DE DIOS, PERU

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Recent studies show that an adequate neuropsychological profile is associated to adequate levels of learning. For this reason we are interested in knowing which cognitive profiles in pediatric populations of native communities exposed to mercury contamination in Madre de Dios, which vulnerable to suffer neurocognitive deficiencies because there are located in areas of difficult access to education and present deficiencies in their basic needs so we have as objective to determinate the Neuropsychological Profile of children in native communities exposed to mercury contamination in Madre de Dios, Peru Transversal study of a representative sample taken from the native communities Ese'eja (Palma Real, Sonene, Inferno) of Madre de Dios, 64 children were evaluated regardless of gender, ages from 3 to 7 years old. For this evaluation we used Neuropsychological Test Maturity, CUMANIN. Parametric Kormogorov-Smirnov test of a sample was applied in order to check for a normal distribution in the study variables. Frequencies established variables, measures of central tendency and dispersion in qualitative variables were used. The test of variance analysis of factor was applied to check for differences between the study groups. The children of Infierno native community were 23,8%(n=15), Palma Real 47,6%(n= 30) and Sonene 28,6% (n=18). The mean age was 5,11 +/- 1,38. The percentiles about different parameters of Neuropsychological Profile of children were inferior of the p50 in Psychomotor 69.50% (n<P50=44), Language Articulatory 66.7%(n<P50=42), Expressive Language 71.40% (n<P50=45), Comprehensive Language 98.4% (n<P50=62), Space structure 69.80% (n<P50=44), Visopercepcion 69.80% (n<P50=44), Iconic Memory 34.9% (n<P50=22), Rhythm 87.3% (n<P50=55), Attention 90.50% (n<P50=57), Verbal Development 88.90% (n<P50=56), Nonverbal Development 77.8% (n<P50=49), Global Development 85.70% (n=<P50=54).The analysis of variance of a factor for Global Neuropsychological Development Profile respect to the three communities was 0.211 for the Verbal Development was 0.229 and Nonverbal Development was 0.248. In the present study we found a large number of children with less than p50 during different parameters of Neuropsychological Profile in other hand there was no difference between groups regarding their Neuropsychological Profile (Verbal and Non-Verbal Development)

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ASSESSMENT OF THE QUALITATIVE IMMUNE RESPONSE INDUCED BY THE CYD TETRAVALENT DENGUE VACCINE IN HUMAN VOLUNTEERS

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Clinical efficacy observed against dengue virus (DENV) serotypes 1, 3 and 4, but not DENV2 in the Phase 2b trial of the CYD tetravalent dengue vaccine candidate (CYD23; Clinicaltrials.gov NCT00842530), contrasted with the similar levels of antibody neutralization levels observed for all 4 serotypes in a Vero cell based $PRNT_{50}$ assay. To further investigate this finding, we assessed the quality of the vaccine-induced antibodies. Sera from a completed clinical trial (NCT 01134263) in volunteers who were flavivirus-seronegative at baseline, were depleted using virus-coated beads, and neutralization was assessed before and after depletion in a flow cytometry-based assay using U937-DC SIGN+ cells. These showed that in this naïve population CYD-TDV vaccination elicited mostly homotypic anti-DENV4 responses, while anti-DENV1, DENV2 and DENV3 responses included a significant heterotypic component. Antibody epitopes were then mapped in a few samples using recombinant DENVs displaying serotype-specific, E protein domain I/II hinge epitopes. Homotypic anti-DENV4 responses were seen to be directed against the DI/II hinge, while anti-DENV3 was not. This serotype-specific quaternary epitope was present and recognized by human monoclonal antibodies (mAbs) on the corresponding CYD-1, 2 and 3 serotypes, while cross-reactive anti-rE DI-II or DIII mAbs recognized all 4 CYD serotypes. Potentially enhancing anti-prM responses were not dominant in vaccinees' sera in Western Blots, and it was also observed from a FcγR+ CV1 cell-based assay (presented by Byers and others at this conference) that vaccine-induced anti-DENV2 responses were not more enhancing than against the other serotypes. In conclusion, our results suggest that despite the presence of key epitopes on the vaccine viruses, qualitative differences exist between vaccine-induced responses against serotype 4 compared to the other 3 serotypes, although it will be necessary to confirm the results obtained here in a larger number of sera. These results demonstrate nevertheless the interest of qualitatively assessing dengue vaccine immunogenicity and how it relates to protection. Studies using post-vaccination sera from clinical trials in endemic areas are ongoing, and results are expected in the coming 2 months

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INVESTIGATIONS OF THE OBSERVED EFFICACY OF THE CYD TETRAVALENT DENGUE VACCINE IN THE PHASE 2B TRIAL IN RATCHABURI, THAILAND

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The first efficacy trial (CYD23; Clinicaltrials.gov NCT00842530) of the CYD vaccine showed clinical protection against dengue virus (DENV) serotypes 1, 3, and 4, but not DENV2, while similar antibody neutralization levels (Vero cell based PRNT50 assay) were observed for all four serotypes. Post study investigations of this result included a broad array of analytical and experimental methods in four areas: host, virus, vaccine vector, and novel immunological assays. Antigenic diversity between parental vaccine virus and wild-type CYD23 isolates does not impact neutralization (PRNT50) using serum from either CYD23 vaccinees or placebo recipients, and an assessment of viremia differences between serotypes in CYD23 clinical cases was inconclusive. Pre-existing immunity impacts vaccine immunogenicity and exploratory analysis using logistic regression, suggests a relationship between probability of disease and PRNT titers. Using CV1 cells transfected with FcγRIIa, preliminary studies with post-vaccination sera from baseline-naïve subjects show no evidence of differential neutralization. Serotype-specific antibody depletion studies, and studies using recombinant DENVs displaying serotype-specific E protein domain I/II hinge epitopes show qualitative differences between serotype-specific neutralizing responses, while a Vero-cell based microneutralization assay shows certain discrimination between serotypes. Analyses using serotype-specific monoclonals show that important E protein DI/II hinge region epitopes are displayed on the vaccine viruses, including CYD2. Finally, prior clinical data suggest a relationship between vaccine potency (CCID50) and neutralization, as well as *in vivo* competition between serotypes in different formulations, and no differences in IFNγ responses between serotypes. Additional investigations, including Phase 3 efficacy study data anticipated in the second half of 2014, will deepen our understanding of the results observed in this Phase IIb efficacy trial.

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LARGE SCALE SAFETY AND IMMUNOGENICITY OF CYD DENGUE VACCINE; RESULTS FROM A RANDOMIZED, PLACEBO-CONTROLLED, MULTICENTER PHASE III EFFICACY TRIAL IN LATIN AMERICA

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In a phase III study of the efficacy of the recombinant, live, attenuated, CYD tetravalent dengue vaccine (TDV) against virologically-confirmed dengue fever in dengue-endemic areas of five Latin American countries: Brazil, Colombia, Honduras, Mexico, and Puerto Rico (N=20 875). Children and adolescents aged 9-16 were randomized 2:1 to receive 3 injections of CYD-TDV or placebo at study months 0, 6 and 12. Safety and immunogenicity were assessed as secondary objectives. Serious adverse events (SAEs) occurring at any time throughout the study in the whole study population were documented, and reactogencity was described in a representative subset of 2000 children (300-600 per country) who were randomly selected from among those enrolled during the first months of the study. SAEs were reviewed by an independent data monitoring committee. Reactogenicity data included solicited injection site reactions, solicited systemic reactions, and unsolicited adverse events, respectively collected for 7, 14, and 28 days after each vaccination. Immunogenicity was also assessed in this subset of 2000 children using Vero cell-based PRNT50 assays against the four dengue serotypes to test serum collected at enrolment, on D28 after the 2nd and 3rd injections, and at 13 months after the 3rd vaccination. Results of these analyses are expected in September/October and will be presented here for the first time. ClinicalTrials.gov: NCT01374516

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EFFICACY OF THE CYD DENGUE VACCINE; RESULTS OF A RANDOMIZED, PLACEBO-CONTROLLED, MULTICENTER PHASE III TRIAL IN LATIN AMERICA

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The protective efficacy afforded by vaccination with the recombinant, live, attenuated, CYD tetravalent dengue vaccine (TDV) against virologically-confirmed dengue fever was evaluated in a phase III trial in dengue-endemic areas of five Latin American countries: Brazil, Colombia, Honduras, Mexico, and Puerto Rico. (N=20 875). Children and adolescents aged 9-16 were enrolled over a 9 month period, randomized 2:1 to receive 3 injections of CYD-TDV or placebo at study months 0, 6 and 12, and were actively followed for febrile illness (temperature ≥38°C for ≥2days) until the 13th month after the 3rd injection; ie for 25 months after the 1st injection. The primary endpoint was efficacy against cases against symptomatic, virologically-confirmed dengue occurring at least 28 days after the 3rd injection, regardless of severity or serotype. Efficacy by serotype was evaluated as a secondary objective. Active surveillance consisted of weekly contacts via phone or home visits to remind parents to consult the trial center or health care center in the event of febrile illness, considered as a suspected dengue case. An acute sample was to be collected within 5 days of fever onset, and a convalescent sample 7-14 days later. Virological confirmation was by dengue qRT-PCR or dengue NS1 antigen ELISA and cases were subsequently serotyped by qRT-PCR. Together with a second phase III efficacy study in >10000 children aged 2-14 in 5 Asian countries conducted in parallel with a similar protocol, this study will provide pivotal data on the efficacy of the CYD-TDV vaccine in different populations and epidemiological settings. Results of our study are expected in September/October and will be presented here for the first time.

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A SINGLE DOSE OF LIVE ATTENUATED TETRAVALENT DENGUE VACCINE TV005 IS SAFE, IMMUNOGENIC AND HIGHLY INFECTIOUS IN HUMANS

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Dengue virus remains a public health burden in many tropical and subtropical areas of the world. The lack of a vaccine or antiviral therapy and the relative unsustainability of vector control together contribute to the ongoing emergence of dengue disease. The last decade has seen an increase in vaccine development efforts, with live attenuated vaccines making significant progress. Our laboratory at the NIAID has developed live attenuated dengue vaccine candidates shown to be both safe and immunogenic in monovalent and tetravalent studies in humans. These studies have enabled the down-selection of vaccine candidates to an optimal mixture of rDEN1Δ30, rDEN2/4Δ30, rDEN3Δ30/31, and rDEN4Δ30. In admixture TV003, each component is delivered at a potency of 1000 PFU and neutralizing antibody data collected 90 days post-vaccination in flavivirus-naïve adults showed seroconversion to DENV1, DENV2, DENV3, and DENV4 in 92%, 76%, 97%, and 100% of vaccinees, respectively, after a single subcutaneous dose. In this cohort, 74% of vaccinees achieved a tetravalent antibody response. When the potency of the DENV2 component of the vaccine was increased 10-fold in admixture TV005 and administered in the same manner as TV003, frequencies of seroconversion in vaccinees to the individual serotypes 1 - 4 reached a remarkable 92%, 97%, 97%, and 97%, respectively, after a single dose, with 90% of vaccinees achieving a tetravalent antibody response. In both studies, low level vaccine viremia was detected in 70 - 75% of vaccinees and mild asymptomatic vaccine-associated rash was observed in 55 - 68% of vaccinees. Following a second dose of vaccine given 180 days after the first dose, vaccine viremia, rash, or boosts in neutralizing antibody titers were not observed in any vaccinee, indicating that sterilizing immunity was elicited following the first dose. Importantly, the data suggest that admixtures such as TV005 can be administered as a single dose. This is unprecedented among dengue vaccines and has positive implications for vaccine safety, compliance, cost, and dose sparing.

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PHASE I CLINICAL TESTING OF A RECOMBINANT SUBUNIT VACCINE FOR DENGUE

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A recombinant subunit vaccine is being developed to prevent disease associated with dengue virus infection. The vaccine candidate comprises truncated dengue envelope proteins (DEN-80E) from all four serotypes produced in Drosophila S2 cells. The vaccine is being evaluated in a Phase I clinical trial in 98 healthy, flavivirus-naïve, adults in Australia. The study is a randomized, placebo-controlled, dose escalation study which evaluates 3 different dosage levels of the tetravalent DEN-80E. The formulations evaluated include non-adjuvanted, aluminum hydroxide adjuvanted, and two different dosage levels of ISCOMATRIX™ adjuvanted vaccine. Volunteers received three doses of vaccine at a 0, 1, 2 month schedule. Safety is being followed throughout the study and immunogenicity is

being assessed pre-dose, post-dose 1, post-dose 2, and 1, 6, and 12 months post-dose 3. The primary endpoint for immunogenicity is based on assessment of virus neutralizing antibody responses with analysis of seroconversion and geometric mean titers. Subjects have now completed their follow-up through the primary endpoint (1 month post-dose 3) and the immunogenicity and safety data through this time point will be presented. Subjects are continuing through the long term follow-up stage of the protocol.

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SAFETY AND IMMUNOGENICITY OF TAKEDA'S LIVE ATTENUATED DENGUE VACCINE CANDIDATE IN A PHASE I STUDY CONDUCTED IN FLAVIVIRUS-NAÏVE HUMAN VOLUNTEERS

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We conducted a phase I, randomized, double-blind dose-escalation study of two different formulations of Takeda's live attenuated dengue vaccine candidate in 72 healthy flavivirus-naïve adults at the Saint Louis University VTEU (NCT01110551). Volunteers received two doses of the tetravalent dengue vaccine candidate 90 days apart and were followed for safety and immunogenicity. Serum samples were collected after each dose to measure vaccine virus replication by qRT-PCR and virus isolation and to measure neutralizing antibodies to wild-type DENV. In this phase 1 study, the vaccine was well-tolerated and no serious vaccine-associated adverse events occurred. Low levels of vaccine viral RNA were detected after prime in 46% of individuals that received the lower dose formulation and in 79% of individuals that received the higher dose formulation. Both vaccine formulations induced seroconversion rates of 67-100% to each of the 4 serotypes of DENV, and more than 90% of subjects who received two doses of the high dose formulation seroconverted to three or more DENV (trivalent response). In summary, these findings highlight the good tolerability and immunogenicity of Takeda's live attenuated dengue vaccine in flavivirus-naïve healthy volunteers and support further development and clinical testing of the candidate dengue vaccine.

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BURDEN, RISK FACTORS AND CHARACTERIZATION OF ROTAVIRUS AMONG CHILDREN WITH MODERATE-TO-SEVERE DIARRHEA IN RURAL WESTERN KENYA–2008-2011: THE GLOBAL ENTERIC MULTICENTER STUDY (GEMS)

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Rotavirus is the most common cause of severe diarrhea among children worldwide. Data on risk factors for morbidity are limited. We analyzed data from children <5 years old seeking health care with moderate-to-severe diarrhea (MSD) and enrolled as cases in the Global Enteric Multicenter Study (GEMS) site in Nyanza Province, Kenya. An MSD case was defined as a child with a diarrheal illness <7 days duration comprising ≥3 loose stools in 24 hrs and ≥1 of the following: sunken eyes, skin tenting, dysentery, IV rehydration, or hospitalization. Rotavirus VP6 antigen was detected in stools by the ProSpecT ELISA rotavirus kit. Demographic and clinical information were collected at enrollment and during a ~60-day follow-up visit. We used logistic regression to evaluate factors associated with rotavirus among children with MSD compared with non-rotavirus MSD. From January 31, 2008 to January 29, 2011, 1,476 cases were enrolled; rotavirus was detected in 217 (14.7%); among cases aged 0-<6, 6-11, 12-23 and 24-59 months old, rotavirus was detected in 24.0% (59/246), 18.3% (78/427), 15.6% (64/410) and 4.1 % (16/393), respectively. Compared to cases without rotavirus, cases with rotavirus infection were more likely to: be 0-11 months old (65.6% vs. 44.7%, OR= 6.08, 95% confidence interval (CI) [3.80-9.72]), be female (48.7% vs. 41.4%, OR= 1.34, 95%CI [1.06-1.70]), and present with vomiting ≥3 times/24hrs (69.9% vs. 45.1%, OR= 2.83 95%CI [2.19-3.65]), sunken eyes (97.1% vs. 92.2%, OR= 2.85 95%CI [1.45-5.59]), lethargy (18.7% vs.11.3%, OR= 1.80 95%CI [1.31-2.47]) and restlessness (72.7% vs. 58.2%, OR= 1.92 95%CI [1.47-2.49]). Among children with MSD, rotavirus was more prevalent among infants and significantly associated with severe diarrheal illness in our study setting. Our findings support the Kenya Ministry of Health plan to introduce rotavirus vaccine.

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THE THRESHOLD OF ROTAVIRUS SPECIFIC PLASMA IGA AS A CORRELATE OF PROTECTION FROM ROTAVIRUS DIARRHEA IS AGE DEPENDENT

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Optimizing and interpreting immunogenicity data and correlates of protection may help to better identify children in whom rotavirus vaccination is failing in developing countries. Here we use the PROVIDE study to examine the ability of rotavirus specific plasma IgA to correlate with protection from future episodes of rotavirus diarrhea within the first year of life. At the icddr,b site in Mirpur, Dhaka, 700 children were enrolled at birth and followed for 24 months. Bi-weekly surveillance for diarrhea was performed and diarrheal specimens were evaluated for rotavirus by enzyme-linked immunoabsorbent assay (ELISA). Diarrheal disease severity was measured by a modified Vesikari-Ruuska score, with score ≥11 indicating severe diarrhea. Rotavirus plasma IgA was detected by ELISA at weeks 6, 18, and 24. Per published standards, children were considered seropositive if the plasma IgA was ≥20 U/ml. Five percent of children were seropositive at week 6 with a Geometric Mean Titer (GMT) of 1.5 (66.5 for seropositive). Irrespective of rotavirus vaccination status, at weeks 18 and 24, 26% and 37% of the children were seropositive with a GMT of 7.1 and 12.3 (114.1, 139.4 for seropositive) respectively. Seropositivity at 18 and 24 weeks of age was correlated with up to 85% and 87% protection from future episodes of rotavirus diarrhea, respectively. At six weeks, seropositivity as previously defined was not associated with protection against future episodes of diarrhea. Increasing the cut-off to 40 U/ml at six weeks increased the proportion protected to 86% however the sample size was small. Our data show that regardless of natural exposure or vaccine-induced responses, rotavirus specific plasma IgA levels at ≥20 U/mL at weeks 18 and 24 serves as a correlate of protection from rotavirus diarrhea in the first year of life. If measured at 6 weeks of age, higher IgA levels are needed to indicate protection from future episodes of rotavirus diarrhea. After unmasking, analysis will assess plasma IgA as an effective correlate of protection in rotavirus-vaccinated children.

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PATHOGEN-SPECIFIC MORTALITY AMONG INFANTS AND YOUNG CHILDREN WITH MODERATE-TO-SEVERE DIARRHEA - WESTERN KENYA, 2008-2011

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Diarrhea is a leading cause of childhood morbidity and mortality in sub-Saharan Africa; one in ten child deaths in the first 5 years of life is due to diarrheal disease. We assessed pathogen-specific mortality following an episode of moderate-to-severe diarrhea (MSD) in children enrolled in the Global Enteric Multicenter Study in western Kenya. We recruited children <5 years old presenting to sentinel health facilities with MSD. At enrollment each child was assessed clinically, and provided anthropometric data and a stool sample to identify enteropathogens. Survival status was determined at 60 days. We calculated unadjusted exact odds ratios (OR) and 95% confidence intervals (CI) using simple logistic regression models. From 2008 to 2011, 1,476 children with MSD were enrolled; 52 (3.5%) died. Nineteen (37%) children died at health facilities (7 at enrollment) and 33 (63%) died at home. Case-fatality rates by age stratum were 4.5% (<12 months), 3.1% (12-23 months), and 2.3% (24-59 months). Pathogens associated with increased risk of case-fatality were *Shigella dysenteriae* (OR: 7.2; CI: 1.3-27.8), non-typhoidal *Salmonella* (OR: 2.8; CI: 1.0-6.6), typical enteropathogenic *Escherichia coli* (OR: 2.6; CI: 1.1-5.5), and enterotoxigenic *E. coli* producing heat-stable toxin (OR: 2.5; CI: 1.1-5.2). Children who died were more likely to be underweight (weight-for-age z score < -2: OR 12.6; CI: 6.4-26.8), wasted (weight-for-length/height z score < -2: OR 8.2; CI: 4.4-15.1), and stunted (length/height-for-age z score < -2: OR 4.2; CI: 2.3-7.9) at enrollment. Malnutrition and four bacterial pathogens for which no vaccines are available were associated with increased risk of mortality from MSD. In the near-term, optimizing nutritional status and water, sanitation, and hygiene interventions are urgent priorities for childhood diarrheal mortality reduction in Kenya.

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PATHOGEN-SPECIFIC ETIOLOGY AND BURDEN OF COMMUNITY DIARRHEA IN THE FIRST TWO YEARS OF LIFE IN DEVELOPING COUNTRIES: RESULTS FROM THE MAL-ED MULTISITE COHORT STUDY

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Studies of diarrheal etiology in developing countries have historically focused on children presenting with severe symptoms to health centers and thus best describe pathogens associated with severe diarrhea. However, the etiologies of community diarrhea may be different. MAL-ED is a multisite birth cohort study with intensive community surveillance for diarrhea as well as collection of monthly asymptomatic stool specimens from eight sites in South America, Africa and Asia. A total of 7,068 diarrheal and 22,599 non-diarrheal control specimens from 2,073 children aged 0-24 months were comprehensively tested for a broad range of enteropathogens. In the first year of life, Campylobacter, rotavirus, ST-producing enterotoxigenic E.coli (ST-ETEC), Cryptosporidium and astrovirus were associated with the highest burdens of diarrhea in descending order of attributable fraction. In the second year of life, diarrhea attributable to Shigella was more prominent while diarrhea attributable to Campylobacter decreased. There was substantial site-to-site variation, such that 6 distinct pathogens had the highest burden of diarrhea for at least one combination of site and age. Cryptosporidium, rotavirus, ST-ETEC and Shigella were associated with more severe diarrheal episodes. This study reveals substantial heterogeneity in the pathogen-specific burden of community diarrhea including an unexpectedly high burden of disease associated with Campylobacter and astrovirus.

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CHANGES IN GUT MICROBIOME COMPOSITION DURING DIARRHEA EPISODES IN NICARAGUAN CHILDREN

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The gut microbiome, the collection of bacteria in the human gastrointestinal tract, plays an important role in human health. Understanding how the gut microbiome is affected by diarrhea episodes may help explain alterations in intestinal function among children in low-income settings. This study examined the gut microbiome of Nicaraguan children during diarrhea episodes and while free of diarrhea for at least 2 months. 16S amplicon sequencing was performed to determine changes in the gut bacterial microbiome during diarrhea episodes. Sequencing data analysis was done using Qiime (Caporaso, 2010). Rarefaction analysis and diversity estimates were carried out to compare the overall diversity of microbiota in diarrheal versus healthy stools. Principal Coordinate

Analysis of amplicon sequences (Hamady, 2010) was used to compare clustering by state (diarrheal vs. healthy). In all, 74 stools were provided by 27 children who were enrolled in a one-year cohort study of diarrhea etiologies. These children had a mean age of 21 months (range: 1 to 45 months), 49% were female, 63% were breastfed, and 10% had received an antibiotic during the diarrhea episode. A total of 593,509 sequences (an average of 7,707 reads per sample) were assigned to 7,880 operational taxonomic units at ≥97% similarity, clustering into 237 genera, 115 families, 54 orders, 28 classes, and 14 phyla. Diarrheal and healthy stools had statistically significant differences ($p<0.05$) for the phyla Firmicutes, Bacteroidetes, and Actinobacteria. Also, as compared to healthy stools, diarrheal stools had a greater relative abundance of the taxa Enterobacteriaceae (13.0% diarrheal vs. 7.9% healthy) and Streptophyta (9.4% vs. 3.3%), and a lower relative abundance of the taxa Ruminococcaceae (9.8% vs. 21.0 %) and Cyanobacteria YS2 (2.1% vs. 9.7%). Phylogenetic diversity did not differ significantly between diarrheal vs. healthy stools. Principal Coordinate Analysis showed clustering by state (diarrheal vs. healthy samples) indicating an overall perturbation of the microbiota in diarrheal stools.

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MARKERS OF OPV FAILURE: INFLAMMATORY AND NUTRITIONAL ENVIRONMENT CRITICAL MEDIATORS OF VACCINE RESPONSE

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The oral polio vaccine (OPV) shows reduced immunogenicity in low income countries; children often have lower antibody titers in response to the vaccine, but the biological cause is unknown. We are measuring OPV responses in infants in an urban slum of Dhaka, Bangladesh. While OPV2 had only a 1.2% failure rate, OPV1 and OPV3 failed in 6.5% and 9.9% respectively as measured by serum neutralizing antibody responses at 18 weeks of age. We hypothesized that nonpolio enterovirus infection at the time of immunization could interfere with OPV. We found that the presence of a nonpolio enterovirus at the time of the 14 week immunization was associated with failure of OPV1 and OPV3 at 18 weeks (p=.005 & .003). We further hypothesized that nonpolio enterovirus infection was interfering by inducing an innate antiviral response in the gut that prevented OPV replication. In support of this, up to a quarter of OPV failures were associated with the inability to culture OPV1 or OPV3 virus from stool samples 24 hours after the first OPV dose at 6 weeks of age (chi-square probability=.01 & .07). In addition to these analyses, blood, stool, and urine samples from the children have been tested for over 20 biomarkers of immunity, inflammation and nutritional status. We performed univariate linear regression analyses to evaluate the association of each biomarker with vaccine performance using serum antibody titers. Markers of poor intestinal health reg1B and mannitol (p=.0002 & .044) were negatively associated with vaccine performance. Serum ferritin and IL-5 (p =.028 & .029) levels, markers of systemic inflammation, were also negatively associated with vaccine performance. Serum zinc concentration and WAZ at 18 weeks of age (p=.034 & .006) positively correlated with increased antibody titers. These results indicate that competing nonpolio enterovirus infections and a chronic inflammatory response likely serve to hinder initial OPV replication and the ability to elicit an effective immune response. Further support for this hypothesis was obtained by measuring fecal excretion of vaccine virus after the final OPV dose as a measure of mucosal immunity. We found that high levels IL-5 positively trended with increased virus excretion (p = .065). We concluded that the majority of the children in the cohort exhibited high levels of inflammatory markers, resulting in an enteric environment of chronic inflammation and infection that left little room for an OPV response.

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IMMUNOGENICITY IN MICE OF A CHOLERA CONJUGATE VACCINE CONTAINING *VIBRIO CHOLERAE* O1 INABA O-SPECIFIC POLYSACCHARIDE (OSP) AND A RECOMBINANT TETANUS TOXIN HEAVY CHAIN FRAGMENT

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Immunity against cholera is serogroup-specific and serogroup specificity is determined by the O-specific polysaccharide (OSP) of Vibrio cholerae lipopolysaccharide (LPS). Here, we describe a cholera conjugate vaccine containing Inaba OSP from a well-characterized source strain isolated in 2007 from a patient with cholera in Bangladesh. The OSP was conjugated via its core oligosaccharide to a recombinant tetanus toxin heavy chain fragment (rTThc) using squaric acid chemistry. We administered the vaccine intramuscularly to mice in the presence and absence of immunoadjuvantative alum. We immunized mice at day 0, 21 and 42. Immunization with OSP:TThc induced detectable anti-OSP IgG responses after one immunization. There was a trend toward higher immune responses in the presence of alum. Although vibriocidal responses were detectable in mice receiving OSP:TThc, they were low-level compared to what has historically been reported following oral whole-cell cholera immunization. OSP:TTHc induced memory B cell responses targeting OSP as well as TT. Importantly, serum from mice immunized with OSP:TThc and alum protected against lethal challenge in mice orally challenged with wild-type V. cholerae. These results suggest that a cholera conjugate vaccine containing Inaba OSP can be highly immunogenic for inducing anti-OSP responses and that such anti-OSP-based immunity can be protective even in the absence of significant vibriocidal responses. These results suggest that OSP:TThc may warrant further development as a cholera conjugate vaccine.

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SALIVARY ANTIBODIES TO WB123 PROVIDE A NON-INVASIVE TOOL FOR ASSESSMENT OF ONGOING *WUCHERERIA BANCROFTI* TRANSMISSION

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Significant progress has been made toward the global goal to eliminate lymphatic filariasis (LF) by 2020 though the tools for monitoring control success and certification of transmission interruption need to be refined further. WHO guidelines for transmission assessment surveys (TAS) will guide decisions about stopping mass drug administration (MDA); the tools for post MDA surveillance, however, are likely to involve antibody testing --likely based on antibodies to Wb123, a *Wuchereria bancrofti* (Wb)-specific antigen that is expressed early in parasite development and has been shown to be a sensitive and specific marker of exposure to Wb infective stage larvae (L3). Although serum/plasma based IgG4 anti-W123 testing has already proved useful in assessing prevalences in target (6-7 year olds) populations, and a rapid diagnostic test (RTD) for these same IgG4 anti-Wb123 antibodies is currently under development for use with whole blood, both require at a minimum a finger prick. To development a

non-invasive methodology for the measurement of antibodies to Wb123, a commercially available salivary collection device was used to collect saliva from a group of 321 6-7 year olds from 5 villages in Mali 3 years after cessation of 7 annual rounds of MDA with albendazole and ivermectin. At the same time, dried blood spots were collected for antibody elution and whole blood was used for measurement of circulating filarial antigen by ICT. Prevalences of serum IgG4 anti-Wb123 was performed by ELISA and compared to salivary IgG and IgG4 anti-Wb123 performed by luciferase immunoprecipitation assay systems (LIPS). While the prevalence of CFA was 4.5% (16/321) and for IgG4 serum anti-Wb123 from dried blood spots was 4.0% (13/321), the salivary antibody prevalences were higher with IgG anti-Wb123 being 8.4% (27/321) and for the more specific IgG4 anti-Wb123 being 6.5% (21/321). Our data suggest that saliva is a rich source of specific antibody to Wb123 and may provide a convenient, sensitive and non-invasive alternative for antibody surveillance following cessation of MDA.

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A COMPARISON OF TWO RAPID TESTS FOR DETECTING FILARIAL ANTIGENEMIA IN LOW PREVALENCE SETTINGS IN SRI LANKA

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Filarial antigen tests are useful for mapping the distribution of *Wuchereria bancrofti* infections and for detecting areas with persistent infections following mass drug administration (MDA). Prior studies have shown that the new Alere™ Filariasis Test Strip (Strip) has better analytical sensitivity than the BinaxNOW® Filariasis card test (Card), and the Strip detected 26% more positives than the Card in field studies performed in a highly endemic area in Liberia. This study compared the performance of the Strip and Card in 2 areas in Southern Sri Lanka with low level persistence of filariasis some 7 years following that country's intensive MDA program. The study design called for testing ~400 people > 8 years of age in each site (cluster sampling of households). Card and Strip tests were performed in parallel in the field with finger prick blood samples; test results were scored at 10 and 30 min and at 12 hr. The Strip detected many more positives at 10 min than the Card (28/389 versus 13/390 in study site A and 50/462 versus 13/462 in study site B). With one exception, all blood samples positive by Card were also positive by Strip. Semi-quantitative test scores based on the intensity of "T" lines tended to be higher by Strip than by Card. Thus filarial antigenemia rates by Strip can be much higher than those by Card test in the post-MDA setting, when filarial antigen levels tend to be low, and this could affect the outcome of transmission assessment surveys. It was not uncommon for Strips to turn from negative to positive between 10 and 30 min (5.6 % of samples tested), and this rarely occurred with the Card (0.36%). However, many Cards that were negative at 30 min were positive at 12 hr (14.3%), and this was less common with Strip tests (4.0%). These results underline the importance of taking care to read filarial antigen test results at 10 minutes according to the manufacturer's instructions. The improved sensitivity, lower cost, and longer shelf life of the Test Strip favor its use over the Card test for filariasis elimination programs.

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INTEGRATED MAPPING OF LYMPHATIC FILARIASIS AND PODOCONIOSIS IN ETHIOPIA: RESULT OBTAINED FROM A NATIONWIDE SURVEY

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Lymphatic Filariasis (LF) is endemic in Ethiopia, while efforts to eliminate LF and management of podoconiosis are continuing; there is no complete map that details the geographical distribution of LF and podoconiosis in Ethiopia. The Federal Ministry of Health has recognized LF and podoconiosis among priorities for control in the recent national master plan for the control of NTDS, and indicated that a nationwide mapping is the loop-hole. Considering clinical similarities and both diseases have the same target group for mapping the survey integrated the two diseases. Thus, this survey aimed to generate a complete map of LF & podoconiosis distribution in Ethiopia. The study was conducted between June to September 2013, based on WHO guideline for mapping of LF. Equal number of male and female age ≥15 years were randomly selected. Data was collected using Smartphone and server was hosted by the Task Force for Global Health. 100 µl of blood was collected from each consented/assented individual and tested for circulating W. banchrofti antigen using Immunochromatographic tests (ICT).Differentiation between LF and Podoconiosis was made by clinical examination, ICT result and Wb123 antibody tests. A total of 130116 individuals aged between 15 and 100 from 660 unmapped districts were participated on the survey. Demographic data showed 1:1 Female to Male ratio. More than half of the study participants (58.4%) were illiterate. 32.2%study participants reported bed net Utilization. ICT results confirmed the presence of W. banchrofti antigen in 139 (0.1%) of the total study participants. Among 313 (0.24%) of the study participant who showed the development of hydrocoel, only one participant tested positive by ICT for LF infection. 1.8%and 0.6%of the total participant report hematuria and chyluria respectively. Lymphoedema was observed in 6083 (4.7%) of the total participants, of which 5.9% was of upper limbs, 90.7% was of lower limbs, 3.5% was of breast. Of the total lymphoedema of lower limb, 5253 was due to podoconiosis 20 was due to LF and the rest is due to various health problems. The current study revealed 76 districts with at least one ICT positive for the antigen of W. banchrofti. Districts with ≥1% of ICT positive result, considered new endemic districts. The study also revealed massive presence of Podoconosis. The available data will be a direct input for the national and global control and elimination of LF and management of Podoconiosis.

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CORRELATION BETWEEN HIGH *LOA LOA* MICROFILAREMIA AND LEVELS OF CIRCULATING FILARIAL ANTIGENS USED TO DETECT *WUCHERERIA BANCROFTI* INFECTION

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The methods of choice to detect infection with *Wuchereria bancrofti* are tests detecting circulating filarial antigens (CFA): the Og4C3 ELISA and the field-friendly immunochromatographic card test (ICT) which has been widely used to map lymphatic filariasis (LF) and assess the impact

of mass treatments. During a LF survey conducted in 26 villages in South Cameroon (1805 individuals tested), we noted that none of those 52 subjects who had a positive ICT presented *W. bancrofti* microfilariae (mf) in their night blood smear, whereas 91% had *Loa loa* mf. As blood smears were also prepared by day for all the individuals, we could analyze the association between ICT positivity and *Loa* microfilaremia. At village level, the prevalence of ICT positivity was strongly correlated with the prevalence of diurnal *Loa* microfilaremia (r=0.61, p=0.001). Among the ICT-positive subjects, 96% presented *Loa* mf by day (arithmetic mean: 36,977 mf/mL). The association between ICT positivity and *Loa* mf density was assessed using multivariate logistic regression adjusting on individual characteristics (age, sex, *Mansonella perstans* mf density). Five groups were defined: one including the amicrofilaremics (reference group) and four with increasing mf densities (divided into quartiles). Odds-ratios [95% confidence interval] associated with ICT positivity were 85 [18-294] and 315 [72-1373] in the two groups with the highest *Loa* microfilaremias (3061-12,120 mf/mL and >12,120 mf/mL). We also compared the results of Og4C3 ELISA tests, using dried blood spots, between ICT negative subjects with no *Loa* mf and ICT negatives with >20,000 *Loa* mf/mL. Optical density values obtained with samples from the latter group were higher than those obtained in the former (mean 0.44 vs 0.25, p<0.001). Our results suggest that high *Loa* mf density may cause false positivity with CFA detection tests (in that case LF mapping based on ICT would have to be redone in *Loa*-endemic areas) or is associated with amicrofilaremic *W. bancrofti* infection. Antibody detection tests will be applied on our samples to resolve the issue.

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ELIMINATION OF ONCHOCERCIASIS IN AFRICA: DO WE HAVE STRATEGIES AND TOOLS? UGANDA CASE ANALYSIS

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Onchocerciasis is a neglected parasitic disease that infects at least 37 million people in Africa. The first major attempt to control onchocerciasis was launched in West Africa in 1974 with vector control as the only strategy. However, the advent of Ivermectin (Mectizan®) in 1987, provided opportunities for other endemic African countries to join the war against onchocerciasis. Uganda has had a long history of onchocerciasis control and elimination dating back to the 1950s. Control of onchocerciasis using annual mass treatment with Ivermectin started in 1992. However, annual treatments alone were not able to interrupt transmission of onchocerciasis in some foci. The Ministry of Health adopted new strategies to address this challenge, hence the initiation of Simulium spp. vector control and semi-annual treatments. New sensitive M&E tools have been developed to delineate areas where transmission has been interrupted. Impact assessments conducted in 2004 revealed a reduction of microfilaria prevalence in most of the foci. Assessments were based on skin snip surveys; serology (Ov16), pool screening of flies using PCR and crab collections and examinations for infestation with immature stages of the vector. Based on the results in 2007, an elimination policy was adopted in some foci. Since then, interruption of onchocerciasis transmission has been achieved in a total of 7 foci, where the vectors have also been eliminated. This translates to 1,354,390 treatments halted with more than 1.2 million people living in areas where transmission has been interrupted. With a combination of strategies and available evaluation tools, Uganda has demonstrated that it is feasible to achieve onchocerciasis elimination in Africa.

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MONITORING TRANSMISSION OF *WUCHERERIA BANCROFTI* POST-MDA IN GHANA

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The WHO recommends using Transmission Assessment Surveys (TAS) as a primary tool for deciding when to stop Mass Drug Administration (MDA) and for post-MDA surveillance in lymphatic filariasis (LF) elimination programs. In Awutu Senya, Effutu, Agona East and West districts of Ghana, MDA was stopped in 2010 after 9 rounds, and TAS conducted. These areas have passed the TAS and thus the need for post-MDA surveillance to monitor recrudescence. The aim of this study is to monitor transmission of *Wuchereria bancrofti* Post-MDA in Ghana using periodic surveys. Two surveys were conducted, school-based and household surveys. A xenomonitoring study was also undertaken alongside the surveys using Indoor Residual Spraying (IRS) technique. A total of 1,708 children (aged 6-10 years) from selected schools and 1,214 (aged 11-60 years) community members participated in the school-based and household surveys respectively. Daytime finger-prick blood samples were collected from all consenting participants and tested using ICT kit and OG4C3 (ELISA). Mosquitoes were captured from 324 households, and LAMP assay performed to detect *W. bancrofti* parasites. Preliminary results show that prevalence of LF in humans is fairly stable [2010=0.13%, 2012=0%, 2013=0.06%] among 6-10 year old children and the general population [2010=1.17%, 2012=1.00%, 2013=0.08%]. A total of 3347 mosquitoes were captured; 2038 *Anopheles* spp. (2002 *An. gambiae*, 36 *An. funestus*), 1269 *Culex* spp., 33 *Aedes* spp. and 7 *Mansonia* spp. For each community, *Anopheles* spp. were pooled with an average pool size of 15. Forty-two (30%) out of 139 pools were found to be positive, (35 *An. gambiae* and 7 *An. funestus*). While the surveys in humans revealed very low prevalence of infection, xenomonitoring has proven to be much more sensitive showing a prevalence of 30% in known vectors, especially using the LAMP method in detecting the presence of *W. bancrofti*. Information from this study will provide recommendations on mechanisms for monitoring transmission of LF post-MDA which can be scaled-up both in Ghana and globally.

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FINANCIAL AND ECONOMIC COSTS FOR CONTROL, ELIMINATION AND ERADICATION OF RIVER BLINDNESS IN AFRICA

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Onchocerciasis, also known as river blindness, has been a serious public health problem in Africa; however, the transmission of the disease has been suppressed with successful mass drug administration (MDA) with ivermectin. Also, recent studies in Mali and Senegal proved the feasibility of elimination with ivermectin. These provided momentum for shifting the goal from control to elimination along with the global movement toward elimination of neglected tropical diseases. As part of an Eradication Investment Case for onchocerciasis, financial and economic costs of alternative scenarios of control, elimination, and eradication are estimated using a micro costing approach from a viewpoint of service providers (i.e. ministries of health, WHO, and NGOs). The costs are estimated up to 2045 considering regional elimination in Africa is predicted to be achievable by 2040 employing a micro simulation model for onchocerciasis transmission. Financial costs include costs for MDA and surveillance. Economic costs measure the opportunity costs for community drug

distributors (volunteers) and donated drugs. Unit cost per person living in endemic area is estimated at $1.43 with all costs included and $0.11 with only financial costs. Financial cost of staying in control mode is estimated at $527 million over 2013-2045. Scaling up MDA coverage and implementing regular surveillance to achieve elimination and eradication would cost $440 million -$453 million, the savings being due to decrease in the number of required treatments as MDA is stopped with elimination achieved. Economic costs are estimated to be significantly higher than financial costs, $6.3 billion for the control scenario and $3.1 billion - $3.3 billion for the scenarios of elimination and eradication over the same time horizon. The results suggest scaling up MDA coverage and implementing regular surveillance to achieve elimination and eradication, after initial resource-intensive efforts, would allow substantial financial and economic savings in the long term.

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EXPLORING THE RELATIONSHIP BETWEEN ACCESS TO WATER, SANITATION AND HYGIENE AND SOIL-TRANSMITTED HELMINTH INFECTION: A DEMONSTRATION OF TWO RECURSIVE PARTITIONING TOOLS

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Soil-transmitted helminths (STH) - a class of parasites that affect billions of people - can be mitigated using mass drug administration, though reinfection following treatment occurs within a few months. Improvements to water, sanitation and hygiene (WASH) likely provide sustained benefit, but few rigorous studies have evaluated the specific WASH components most influential in reducing infection. There is a need for alternative analytic approaches to help identify, characterize and further refine the WASH components that are most important to STH reinfection in order to improve WASH interventions for control of STH. In this paper, we introduce two recursive partitioning approaches: classification and regression trees (C&RT) and conditional inference trees (CIT). Utilizing data from a school-based randomized control trial in Kenya, we conduct an assessment of the school- and household-level WASH components and demographic indicators that contribute to reinfection of pupils 10 months following deworming treatment. We show how C&RT and CIT can be used to identify the WASH components most predictive of and associated with STH infection. In addition, we demonstrate how both tools can be used to identify complex interactions between WASH indicators and sub-populations that may be particularly susceptible to STH reinfection, both of which are difficult to identify using traditional epidemiological methods. We discuss the relative merits and weaknesses of each approach and make recommendations for their use as tools to enhance STH control programs.

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IMPACT OF WASH AND ALBENDAZOLE DISTRIBUTION ON INFECTION WITH SOIL-TRANSMITTED HELMINTHS IN TIMOR-LESTE: INITIAL RESULTS OF A CLUSTER RANDOMIZED TRIAL

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Soil-transmitted helminths (STH) infect more than two billion people worldwide, causing considerable morbidity, including malnutrition and anaemia. STH infections are most prevalent in communities where adequate water and sanitation are lacking and hygiene behaviour is poor. Deworming programmes with anthelminthic drugs are highly effective in reducing morbidity but rapid reinfection occurs if there is no change in the environment. Therefore, provision of water, sanitation and hygiene (WASH) programs is of critical importance in the sustainable control of STHs. "WASH for Worms" is a cluster randomised controlled trial assessing the impact of a community-based WASH intervention, implemented by WaterAid Australia, on infection with intestinal parasites following mass albendazole (ALB) chemotherapy in villages in Timor-Leste. In this trial, initiated in 2012, twelve intervention villages receive the WASH programme and ALB treatments every six months. Twelve control villages receive only the six-monthly ALB. All villages are followed-up for two years after the first ALB distribution. Infection prevalence and intensity is measured by a modified qPCR. The results for STH infection levels at baseline and after the first follow-up in the first 16 villages enrolled will be presented together with results of an anthelmintic efficacy trial using a single dose of ALB on STHs conducted in 8 villages. At baseline the prevalence of STHs in the first eight villages was high, with more than 90% of participants infected with at least one STH (assessed by PCR) mostly comprising Necator americanus (75.3%) followed by Ascaris lumbricoides (63.6%). At the first follow-up in 8 of the villages, it was possible to detect an additional benefit of the WASH intervention compared to deworming alone. This trial is the first reported RCT evaluating the impact of integrated WASH and deworming programmes on infection with STHs; and will provide essential evidence for scaling up integrated programmes for STH control.

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ASSOCIATION OF WATER QUALITY WITH SOIL-TRANSMITTED HELMINTHIASIS, DIARRHEA AND INFLUENZA-LIKE ILLNESS IN NUEVA SANTA ROSA, GUATEMALA -- 2010

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Improved water quality is essential in reducing diarrhea. Its impacts on influenza-like illness (ILI) and the soil-transmitted helminths (STH) *Ascaris* and *Trichuris* are less well described, though some data link them to water, sanitation, and hygiene. To assess their association with water quality, we conducted a cross-sectional survey in Nueva Santa Rosa, Guatemala among persons >1 year old in randomly selected households (HH). A stool sample was tested by Fecal Parasite Concentrator and Kato-Katz method for STH. Diarrhea and ILI were identified by self-reported symptoms in the past week. We explored associations between *Escherichia coli*-positive drinking water (water quality) and disease outcomes using exact logistic regression models. Median unbiased estimates (MUE) are reported when maximum likelihood estimates did not exist. We interviewed 920 persons from 204 HH. Water results were available for 778 persons; 84% (650/778) lived in HH with *E. coli*-positive water. Among persons in HH with water quality testing, 12.4% (76/611) tested positive for *Ascaris* and/or *Trichuris*, 9.4% (73/778) had diarrhea, and 13.5% (105/778) had ILI. Univariable analysis showed an association between water quality and STH (OR 8.9, CI 2.3–76.2), but not with diarrhea or ILI. There was no difference in water treatment practices between HH with and without diarrhea to explain the lack of association between water quality and diarrhea. In stratified analyses, *E. coli*-positive water remained associated with STH among persons ≥15 years (MUE 13.4, CI 2.9–infinite) and those living in densely populated areas (≥1,000 persons/km^2) (OR 15.1, CI 2.5–614.7). The lack of association between water quality and diarrhea was unexpected, as was the association between water quality and STH, since STH has been viewed primarily as a sanitation and hygiene issue. Waterborne transmission and effects of water treatment on STH should be investigated. If a causal relationship is found, practices such as household water treatment, might be useful adjuncts to sanitation, hygiene, and deworming in STH control programs.

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IMPROVEMENT OF WATER, SANITATION AND HYGIENE IN TWO URBAN SLUMS IN UGANDA THROUGH COMMUNITY PROACTIVE AND SUSTAINABLE INTERVENTIONS

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In urban slum settlements in Uganda, the major risk factor for water borne diseases such as diarrhea is contaminated drinking water due to poor latrine status, low safe water coverage, and poor domestic and personal hygiene practices. To address the challenges affecting these areas, a project aimed at improving the water, sanitation and hygiene (WASH) status in 2 urban slum communities in Uganda through community proactive and sustainable interventions was implemented. The 2 slums involved were in Kampala and Mukono in the central region of the country. To establish the WASH status of the communities before project implementation, a baseline survey was carried out among 213 households. The survey involved both quantitative and qualitative methods. Several interventions with full community participation were then implemented to improve the situation. The interventions included: community sensitization on WASH, promotion of hand washing using the tippy tap technology, supporting clean-up exercises in the community, providing advisory roles in WASH, supporting health clubs in primary schools, training community members in a short course in WASH, capacity building of youth in WASH, promoting drinking safe water through household chlorination and home improvement campaigns. After the project implementation period, a final evaluation survey was carried out among 300 households. This survey involved both quantitative and qualitative data collection methods. Latrine coverage improved from 86.0% to 98.7%. Piped water usage improved from 38.0% to 86.0% with a reduction in the use of unprotected sources from 30.0% to 2.3%. Treatment of drinking water improved from 95.3% to 99.7% with more households (96.0%) boiling their water from 94.0% who did so at the baseline. Indiscriminate disposal of solid waste reduced from 18.0% to 2.0% and satisfaction with solid waste management services increased from 40.0 to 91.8%. Drainage around homes improved from 57.0% to 86.0% while presence of soak pits at households increased from 2.3% to 10.0%. Improvements in the latrine statuses, environmental hygiene and waste management practices were also registered. There were significant improvements in the WASH status of these communities after the implementation of several multi-faceted interventions. Urban slums can benefit from WASH interventions when communities are fully involved and with a focus on capacity building.

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SOME CHILDREN WITH ACCESS TO IMPROVED WATER AND SANITATION DEMONSTRATE BETTER GROWTH: FINDINGS FROM THE YOUNG LIVES COHORT STUDY IN ETHIOPIA, INDIA, PERU AND VIETNAM

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Child undernutrition is widespread in developing countries and is perceived to have large costs over the lifecycle. We used data on 8,062 children from Ethiopia, India, Peru and Vietnam at ages 1, 5, and 8 yrs from the Young Lives cohort to estimate multivariate logit regressions for associations between household and community-level access to improved water and sanitation, and stunting and underweight at these three ages. Improved drinking water was associated with stunting only in Peru, where access at age 5 yrs decreased risks of stunting at age 5 yrs (OR: 0.68; $p<0.05$). In Ethiopia access to improved sanitation facilities (facilities) at age 1 yr was associated with decreased odds of stunting at 1 yr, 5 yrs and 8 yrs (OR: 0.59, 0.63, 0.64; $p<0.05$). In India and Peru, access to improved facilities at 1 yr was significantly associated with stunting at age 5 yrs only (OR: 0.61, 0.75, $p<0.05$). Improved facilities access when children were 5 yrs old was associated with stunting only at age 5 in Peru (OR: 0.71; $p<0.05$) and age 8 in Vietnam (OR: 0.64; $p<0.05$). Improved facilities at age 8 was not associated with stunting at age 8 in any country. Improved drinking water at 1 yr was significantly associated with underweight at age 8 in India (OR: 0.52; $p<0.05$); for all other time points and countries, improved drinking water was not significantly associated with underweight. Facilities quality at age 1 yr was associated with lower odds of being underweight in Peru at 1 yr (OR: 0.59, $p<0.05$) and in India at age 5 yrs (OR: 0.60, $p<0.05$). There were no other significant associations between facilities and underweight. The combination of significant associations with quality of sanitation facilities and reduced undernutrition and the numerous insignificant associations suggest the importance of further investigating mediating factors that affect whether interventions to improve sanitation reduce concurrent and long-term child malnutrition.

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INTEGRATING WATER TREATMENT INTO ANTENATAL CARE: IMPACT ON USE OF MATERNAL HEALTH SERVICES AND HOUSEHOLD WATER TREATMENT AMONG MOTHERS -- UGANDA, 2013

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In Uganda, high maternal and neonatal mortality rates reflect underutilization of reproductive health services; high diarrhea risk among children results from poor access to safe water. To incentivize household water treatment and reproductive health service use, water treatment kits (buckets and 30 sachets of flocculant-disinfectant powder) were provided at first antenatal visits. Refills of 30 sachets each were distributed at follow-up antenatal (ANC) visits, health facility deliveries, and postnatal check-ups. We evaluated this intervention through cross-sectional surveys of a random sample of women from participating health facilities who received reproductive health care in 2013 after project launch (intervention group) and in 2012 before project launch (pre-intervention/comparison group). We used the Chi-Square test statistic to compare groups. We surveyed 226 intervention group women and 207 comparison group women. A higher percentage of intervention than comparison group women reported treating drinking water on the day of the survey (31.7 vs 19.7%, P=0.01), and had detectable chlorine residual, an objective measure of treatment, in their stored drinking water (13.5% vs 3.4%, P<0.01). Of 226 intervention group women, 222 (98.2%) received water treatment kits. Although 96.8% of intervention group women had ≥2 ANC visits (median 4 visits, range 1-5), only 101 (45.5%) received one or more sachet refills. There was no difference in the percentage of women in intervention and comparison groups that reported ≥4 ANC visits (66.2 vs 68.5%, P=0.61) or health facility deliveries (67.8 vs 74.3, P=0.14). Intervention group women were more likely than comparison group women to treat their drinking water, but had similar low use of reproductive health services. Although water treatment kit coverage at first antenatal visit was high, inadequate distribution of, or low demand for, refill sachets may have contributed to limited program impact.

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IMPACT OF A SCHOOL-BASED WATER, SANITATION AND HYGIENE PROGRAM ON DIARRHEA, RESPIRATORY INFECTIONS AND ABSENTEEISM: A LONGITUDINAL EVALUATION

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School-based water, sanitation and hygiene (WASH) programs can lead to improvements on pupil health and attendance, but few rigorous studies are available. We conducted a quasi-experimental, longitudinal impact evaluation of a comprehensive school-based WASH program in Mali that provided schools with latrines, water points, drinking and handwashing stations, cleaning supplies, hygiene promotion, and training on management and governance. We randomly selected 100 primary schools participating in the WASH program and matched them to 100 control schools that were not participating in the program based on location, school population, and the presence of latrines and water points before the start of the program. Unannounced visits were conducted between February 2013 and June 2014, for a total of seven visits per school. At each visit we conducted a roll-call of all pupils in the school and asked 40 pupils to provide a one-week recall of absence, diarrhea, and symptoms of respiratory infections. We evaluated schools for adherence to the program. We will employ random effects longitudinal regression analysis with data clustered at the school level to examine the association between participation in the WASH program and roll-call absence, self-reported absence, self-reported diarrhea, and self-reported symptoms of respiratory infections. The study is powered to detect a 20% reduction in pupil absence and a 30% reduction in diarrhea. Data will be analyzed after the final round of data collection in June 2014.

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A CHIMERIC *PLASMODIUM VIVAX* CSP TAILORED TO ENHANCE THE CELLULAR IMMUNE RESPONSE

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Plasmodium vivax is the most widespread species of *Plasmodium* representing 50% of the malaria cases occurring outside sub-Saharan Africa. Existing control measures have significantly reduced the burden of malaria in the past 10 years. However, these measures are not effective against hypnozoites a dormant stage form responsible of *P. vivax* relapse infections. An effective vaccine is therefore essential to the control and eradication of *P. vivax*. A well characterized vaccine candidate is the circumsporozoite protein (CSP). Anti-CSP antibodies have the ability to inhibit the invasion of hepatocytes by *P. vivax* sporozoites *in vitro*. Cellular immune responses against CSP have also been correlated with protection. However, preclinical and clinical data of *P. vivax* CSP based vaccines have shown limited success in inducing cellular immune responses. Based on our reported data on the use of chimeric *P. yoelii* proteins to enhance cellular reactivity, we decided to design a recombinant chimeric protein based on the *P. vivax* CSP. In this study we tested the capacity of a recombinant protein chimera containing immunogenic domains that preserved the protein topology described for *P. yoelii*. The chimeric protein was expressed in soluble form with high yield. The proper configuration and antigenic integrity of the protein were defined by western blot analysis and ELISA. Groups of six different strains of mice were used to test immunogenicity. The chimeric protein was able to induce robust antibody responses in all the mice strains tested against the immunogen. Interestingly, synthetic peptides representing the allelic forms of the *P. vivax* CSP were also recognized to a similar extent regardless of the mouse strain. Cellular immune responses were investigated by IFN-y and IL-4 ELISPOT test as well as intracellular cytokine staining measured using flow cytometry. The immunization regimen resulted in robust production of IFN-y, IL-2 and TNF-a by CD4+ and CD8+ T cells. The fine specificity of the cellular immune responses induced by immunization with the chimeric *P. vivax* CSP will be discussed.

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INHIBITION OF *PLASMODIUM VIVAX* INVASION OF RED BLOOD CELLS USING ANTI-DUFFY BINDING PROTEIN ANTIBODIES

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It has long been established that the malaria parasite, *Plasmodium vivax*, depends on the interaction of its Duffy Binding Protein (DBP) with the erythrocyte Duffy antigen (either FyA, FyB) for host cell invasion. Recent evidence has indicated that patients who are FyAFyA homozygotes are far less likely to experience severe vivax malaria and that DBP protein has a lower binding affinity for this version of the Duffy receptor. As most vaccine resources and strategies concentrate on the parasite's use of the Duffy Binding Protein, it is important to determine if current studies of the FyB::DBP interaction should remain the sole interaction for vaccine development or if we need to explore the FyA::DBP more closely. The focus of this study was to examine the ability of *P. vivax* to invade red blood cells *in vitro* and to determine if inhibition of the parasite's invasion of FyAFyA red blood cells differs from FyBFyB cells. Reticulocyte enriched blood samples of varied Duffy-positive FyBFyB and FyAFyA homozygotes were exposed to different strains of *P. vivax* with genetic variations of DBP (*P. vivax* strains Nicaragua, Indonesia, and Thailand). New invasions were measured using flow cytometry and selective staining techniques, with and without anti-DBP blocking antibodies. The results showed that the blocking ability of the anti-DBP antibodies was highly dependent upon the parasite strains used. Anti-DBP antibodies directed against the DBP Sal 1 variant showed a 97.0% inhibition of invasion into FyAFyA red blood cells by *P. vivax* Indonesia, but only a 24.8% inhibition into FyBFyB red blood cells by the same strain. Conversely, anti-DBP antibodies showed 1.4% inhibition and 12.7% inhibition of *P. vivax* Nicaragua invasion into FyAFyA and FyBFyB red blood cells, respectively. Invasion by *P. vivax* Thailand was not inhibited in either Fy genotype. Also, anti-DBP antibodies showed highly varied efficacy against invasion by all strains into Duffy-negative (Fy0Fy0) red blood cells. These results demonstrate that differences in the parasite's DBP polymorphisms may play a large role in invasion success in the absence of other complex genetic factors between donors. This work indicates that both the genotype of the *P. vivax* and the Fy genotype of the host are potentially important considerations in the development of vaccine candidates.

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ANTIBODIES TO A HYPOTHETICAL FALCIPARUM MALARIA ANTIGEN (PF3D7_1134300) INHIBIT ERYTHROCYTE INVASION

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We previously screened a *Plasmodium falciparum* 3D7 strain blood stage cDNA phage display library using a differential approach and identified three antigens encoded by PF3D7_1335100 [MSP-7], PF3D7_1021800 [PfSEA-1] and PF3D7_1134300 that are uniquely recognized by antibodies in plasma from resistant Tanzanian children but not by those from

susceptible children. Resistance was defined by a median parasite density of zero (IQR, 25) documented in monthly blood smears obtained from children between 2 and 3.5 years. The current work characterizes the effector function of murine polyclonal antibodies directed against the immunorelevant portion of PF3D7_1134300. In silico analysis (PlasmoDB.org) predicts that the 6684 bp gene encodes a 263 kDa phospho-protein, contains no introns and has rodent syntenic orthologs. We cloned the immunorelevant region (nt 1,337,023-1,338,945) as well as three overlapping constituent fragments into a eukaryotic expression plasmid (VR2001) and immunized mice to generate antisera. To confirm that PF3D7_1134300 encodes a parasite protein, we probed *P. falciparum* 3D7 infected and uninfected erythrocytes with antisera, which recognized the relevant full length protein only in infected erythrocytes. We performed growth inhibition assays (GIA) by synchronizing 3D7 parasites three times with sorbitol and cultivating them to obtain mature trophozoites that were plated at 1.5% parasitemia (hematocrit 1.0%). Trophozoites were cultured in the presence of heat-inactivated and pre-adsorbed PF3D7_1134300 anti-sera or pre-immune mouse sera for 24 hours and ring stage parasites were enumerated. Anti-sera directed against the antigen of interest and its constituent fragments inhibited parasite invasion by 31-53% compared to controls (all $P < 0.01$). Based on the observation that extra-cellular merozoites appeared to aggregate in the presence of anti-sera, we are conducting studies to explore the mechanism of invasion arrest. Our data suggest that PF3D7_1134300 may be a novel vaccine candidate for pediatric falciparum malaria.

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FROM EXPERIMENTAL HUMAN MALARIA INFECTION TO A CHEMICALLY ATTENUATED *PLASMODIUM FALCIPARUM* WHOLE PARASITE BLOOD-STAGE VACCINE

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Malaria is a leading cause of morbidity and mortality attributable to infectious disease. The possibility of a malaria vaccine was first realized in the 1940s, yet a vaccine capable of inducing long lasting immunity remains elusive. We have shown that a chemically attenuated whole parasite blood-stage vaccine, consisting of ring stage malaria parasites attenuated with the Cyclopropylpyrroloindole analogue Tafuramycin-A, offers profound protection in rodent models. To evaluate this vaccine approach in humans, suitable reagents and systems were developed. Two malaria cell banks, consisting of human red blood cells infected with *Plasmodium falciparum* NF54 or 7G8, were manufactured by *in vitro* cultivation of the malaria parasites in a GMP compliant facility. Clinical studies were undertaken to assess the safety and infectivity of these cell banks in malaria naïve human volunteers. The cell banks were well tolerated, however differential infectivity was observed between the 2 cell banks, with a significantly higher dose of NF54 required to initiate a blood stage infection. Phenotypic analysis of the malaria parasites in the two cell banks prior to participant inoculation revealed a profound difference. Unlike the 7G8 parasites, the NF54 parasites were unable to adhere to CD36 and there was an absence of knobs on the parasitised erythrocyte surface. Interestingly, when infectivity of the NF54 cell bank was demonstrated, parasitised erythrocytes obtained *ex vivo* from the study participants were able to adhere to CD36 and knob expression was observed. Following these studies, the *P. falciparum* 7G8 cell bank was selected for the manufacture and evaluation of a chemically attenuated blood-stage malaria vaccine in humans. Malaria naïve volunteers will be inoculated with a single dose of 3×10^7 *P. falciparum* 7G8 parasitised erythrocytes, attenuated with Tafuramycin-A, to evaluate its' safety and immunogenicity in humans. The results from this study will be available in the second half of 2014 and will be presented. This is the first evaluation of a whole parasite blood-stage malaria vaccine approach in humans.

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HUMANIZED HLA MICE ARE PROTECTED AGAINST CHALLENGE WITH *PLASMODIUM FALCIPARUM* SPOROZOITES (PFSPZ) WHEN IMMUNIZED WITH IRRADIATED PFSPZ OR LIVE PFSPZ UNDER CHLOROQUINE COVER BUT NOT BY IMMUNIZATION WITH HUMAN ADENOVIRUS 5-VECTORED *PLASMODIUM FALCIPARUM* ENCODING AMA1 AND CSP

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Development of humanized mouse models that are able to act as surrogate human immune system is a highly pursued goal for testing human vaccine candidates, generating fully human monoclonal antibodies and studying the development of human immune system. Previously we have demonstrated that humanized mice expressing HLA molecules in NOD.RagKO.IL2RgcKO background (DRAG/DRAGA) that were infused with HLA matched human hematopoietic stem cells develop a functional human immune system and respond to vaccination. Humanized HLA mice also develop human hepatocytes, kupffer cells, liver endothelial cells and erythrocytes and sustain the vertebrate life cycle of *P. falciparum*. Herein we show that humanized mice are protected against liver stage *Plasmodium falciparum* infection upon immunization with irradiated Pfspz or live sporozoites under chloroquine cover. In contrast, humanized HLA mice were not protected by immunization with human adenovirus 5-vectored *P. falciparum* NMRC-M3V-Ad-PfCA encoding AMA1 and CSP constructs. Humanized HLA mice represent a new pre-clinical model for testing vaccines against malaria.

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IDENTIFICATION OF A CORE GROUP OF *PLASMODIUM FALCIPARUM* MHC CLASS I PEPTIDES SEQUENCED FROM INFECTED LIVER CELLS

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Sterile protection from *Plasmodium falciparum* (Pf) infection can be achieved by developing potent cell mediated immunity against malaria liver stages. Liver stage immunity has been established in human clinical trials using two different but similar approaches: 1. Immunization with radiation-attenuated-sporozoites (RAS) and 2. infection-treatment-vaccination (ITV). The model for RAS immunization suggests that attenuated sporozoites enter hepatocytes, initiate development, and then die leaving behind parasite material for degradation and presentation on MHC Class I receptors. During ITV human volunteers are challenged with live sporozoites under chloroquine coverage resulting in complete hepatocyte invasion and development. Both RAS and ITV immunization suggest that liver stage immunity is mediated by Class I presentation of Pf peptides by either aborted liver stage development or during successful merozoite development. A long standing goal in the malaria vaccine field is to identify the Pf antigens that induce liver stage immunity. Towards this goal we have worked to identify Pf Class I peptides presented from *in vitro* infected human hepatocytes by direct peptide sequencing. We have identified a core group of 12 Pf proteins sequenced from three genetically

distinct primary human hepatocyte sources infected with sporozoites. Among this core group of Pf proteins we have identified a subset that induces IFN-γ secretion from PBMCs of RAS-protected volunteers. In our future experiments we will test this core group of liver stage antigens as vaccine candidates in malaria challenge models.

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IMMUNODOMINANCE AND VACCINE DEVELOPMENT: NEW INSIGHTS FROM PROTEOME-WIDE PROFILING OF T CELL AND ANTIBODY RESPONSES TO MALARIA

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For many infectious diseases, the development of effective vaccines based on immunodominant antigens has thus far not been successful. Immunodominance is the phenomenon whereby pathogen-specific immune responses target only a small fraction of the full range of possible antigens and epitopes. It is a central feature of immunity but what distinguishes effective immune targets from less effective targets is not obvious. In experimental infections with simple organisms, immunodominance results in only a few specificities dominating the host's response. Large pathogens such as the *Plasmodium* parasite represent a far greater challenge because of the complexity and scale of potential immune targets. We have generated unique datasets of proteome-wide T cell responses and antibody responses to *Plasmodium* using omics-scale technology platforms, including protein microarrays and epitope prediction algorithms, with specimens from humans experimentally or naturally exposed to malaria. We have integrated these datasets to develop metrics of immunological, structural and genetic parameters associated with antigen immunodominance in the context of a complex parasite. We establish that the repertoire of T cell reactive antigens is largely distinct from the repertoire of antibody reactive antigens, and that T cell target antigens are more conserved as compared with antibody targets. We further establish that the immunodominance hierarchy for antibody responses is influenced by structural or functional properties that differ from those that underlie the hierarchy for T cells. By defining the degree to which immunodominance shapes immunity against *Plasmodium* and identifying antigens and epitopes that represent key targets of protective immunity in humans, our studies have direct outcomes for vaccine development. These also data further our understanding of host-pathogen interactions.

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PLASMODIUM FALCIPARUM ACTIVATION OF B-CATENIN MEDIATES THE DISRUPTION OF INTERENDOTHELIAL CELL JUNCTIONS: A ROLE IN CEREBRAL MALARIA

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A high proportion of deaths by *Plasmodium falciparum* is caused by cerebral malaria, the most profound syndrome of severe malaria that is fatal within 24-72 hours in 20% of the cases. The disruption of the blood brain barrier (BBB), characteristic of cerebral malaria, causes diffusion of blood cells and serum into the brain tissue leading to coma and damage to the nervous system. The mechanisms underlying this process are largely unknown but the need for adjunct therapy to rescue patients from death is mandatory due the high rate of mortality. Using monolayers of human brain microvascular endothelial cells (HBMECs), we have observed that incubation with erythrocytes infected with *Plasmodium falciparum* promotes the disruption of interendothelial cell junctions (IEJs) between these cells. This process is mediated by the delocalization of β-Catenin from the adherent junctions to the nuclei and the activation of the Tcf/LEF pathway, which leads to the detachment of the HBMECs. Infecting HBMECs with a lentivirus carrying a dominant negative mutant tcf4 vector, we were able to inhibit the detachment of HBMECs induced by *P. falciparum*. Compounds that inhibit the activation of the β-Catenin pathway also inhibit the disruption of IEJs and detachment of HBMECs induced by *P. falciparum* and have a protective effect against experimental cerebral malaria in mice. Our findings describe an unprecedented role for β-Catenin in the maintenance of the BBB integrity in the setting of cerebral malaria and open new perspectives for the treatment of the disease.

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MULTIPRONGED PROTEOMICS AND METABOLOMICS ANALYSIS OF *PLASMODIUM FALCIPARUM* AND *P. VIVAX* INDUCED ALTERATIONS IN HUMANS TO DECIPHER DISEASE PATHOGENESIS AND IDENTIFY SURROGATE MARKERS

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In this study serum samples from severe and non-severe falciparum and vivax malaria patients and healthy controls from different endemic regions of India were investigated using multiple quantitative proteomics approaches and results were validated in larger clinical cohorts by using WB, ELISA and surface plasmon resonance-based measurements. Additionally, metabolomics analysis was performed using LC-MS/MS to identify the altered metabolites and associated pathways in malaria. Specificity of the identified serum markers was evaluated by analysis of dengue fever and leptospirosis patients as febrile disease controls. In quantitative proteomic analysis, 67 and 82 differentially expressed ($p < 0.05$) serum proteins were identified in falciparum and vivax malaria respectively, and almost half of these proteins were commonly modulated in both of the plasmodial infections. Using LC-MS coupled with multivariate statistical data analysis approaches over 3000 serum metabolites were screened among which nearly 200 exhibited altered serum level in the malaria patients. Functional pathway analysis involving the identified proteins and metabolites revealed the modulation of different vital physiological pathways including acute phase response signaling, amino acid and lipid metabolism, chemokine and cytokine signaling, complement cascades and blood coagulation in malaria. Different hematological, liver and renal function parameters were also measured in malaria patients and controls. Hemoglobin level and platelet count found to be significantly lower ($p < 0.01$) in the malaria patients. ROC curve analysis demonstrated Serum amyloid A, Apolipoprotein E and Haptoglobin as efficient predictor proteins (AUC > 0.90) for malaria detection at an early stage of infection. Expression levels of these three serum proteins also exhibited good correlation with parasite count ($r > 0.5$; $p < 0.001$). Interestingly, analysis of longitudinal cohorts (early febrile, defervescence and convalescent stages) indicated cyclic alterations in the expression levels of Haptoglobin, Retinol binding protein, ApoE and Apo-A1 during the different stages of the infection, which could serve as indictors of the disease progression. Our findings may open up new

opportunities for the early detection and prognosis of malaria as well as could provide better understanding of disease pathogenesis and host responses in malaria.

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THE ROLE OF ENDOTHELIN-1 IN THE VASCULAR PATHOBIOLOGY OF CEREBRAL MALARIA

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Cerebral malaria (CM) is a serious complication of *Plasmodium falciparum* infection associated with cerebral vasculopathy, high mortality, and adverse neurological sequelae. The vasoactive peptide, endothelin-1 (ET-1), has been shown to mediate blood-brain barrier (BBB) permeability, inflammation, and vascular tone, and may be important in CM pathogenesis. We previously reported that ET-1 was important in regulating cerebral blood flow, brain microvascular hemorrhage and mortality in our experimental CM (ECM) model. These actions were mediated by ET-1 activation of the endothelin A (ET_A) receptor. To test the hypothesis that ET-1 is involved in the pathological process of ECM, we investigated ET_A receptor mediated signaling in mice infected with *P. berghei* ANKA (PbA). ET_A receptor blockers (ET_ARB) significantly improved survival in ECM mice. In addition, ET_ARB enhanced vascular integrity during PbA infection. BBB permeability, and protein levels of angiopoietin-2 and VCAM-1 were significantly lower in ECM mice treated with ET_ARB than in mice treated with saline. In addition, ET_ARB prevented the ECM-induced decrease in angiopoietin-1 in PbA-infected mice. CM is associated with astrogliosis in both human disease and in experimental models. Our preliminary data indicate that astrogliosis is associated with abnormal protein levels of connexin 43 (Cx43), a gap junction protein critical in gliosis and BBB integrity. ET_ARB prevented the PbA-induced dysregulation of Cx43. We hypothesized that ET-1 mediated vascular dysfunction in ECM potentially by regulating neuroinflammation and Cx43 expression. In this regard, we observed a reduction in the activation of JNK in the brains of mice with ECM. JNK, a downstream substrate of ET-1, has been demonstrated to regulate Cx43 expression and function, and is important in CM. Our data indicate that ET-1 may mediate the vascular pathology and neuroinflammation in ECM via regulation of JNK signaling and subsequent Cx43 dysregulation. The ET-1 pathway may thus be a potential therapeutic target as an adjunct in the treatment of human CM.

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PLASMODIUM FALCIPARUM IN AOTUS: A NOVEL MODEL FOR PLACENTAL MALARIA

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Pregnant women are infected with a distinct *Plasmodium falciparum* (Pf) phenotype that binds to chondroitin sulfate A (CSA) and grows to high density in the placenta. Placental sequestration is linked to severe pathologies in the mother and offspring, leading to tens or hundreds of thousands of deaths each year. CSA-binding parasites express VAR2CSA, a distinctly structured member of the Pf erythrocyte membrane protein 1 (PfEMP1) variant surface antigen family that is now the leading target for vaccine development. Further research progress is hindered in the absence of an animal model that recapitulates the features of human placental malaria. Previous work has shown that Pf can infect and sequester in brain, heart and spleen of non-pregnant Aotus, and we sought to establish a model of placental malaria using this parasite-monkey combination. We infected pregnant Aotus (n= 2) with CSA-binding Pf-CS2 during the 3rd trimester and observed a pronounced sequestration of parasites in placental intervillous spaces, with ~30-fold higher parasite density in placental versus peripheral blood. Most placental parasites were mature blood stages, while peripheral samples were exclusively young ring stages. Aotus (n= 1) infected with non-CSA-binding parasite lines (CAMP; FVO) before pregnancy developed recrudescences during subsequent pregnancy, similar to the experience of women. CS2 parasites collected from pregnant Aotus express VAR2CSA on the infected erythrocyte (IE) surface, and bind specifically to CSA. Similar to immune multigravid women, a monkey infected with Pf-CS2 parasites over successive pregnancies acquired antibodies against VAR2CSA, and purified antibodies blocked the binding of several maternal parasite isolates to CSA. In summary, Pf infections in pregnant Aotus monkeys recapitulate all the prominent features of malaria infection and immunity in pregnant women, and can be useful for basic mechanistic studies as well as preclinical studies to qualify candidate pregnancy malaria vaccines.

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SPLENIC PATHOLOGY SUPPORTS THE ACCELERATION OF SEVERE MALARIA THROUGH HIV INFECTION IN PEDIATRIC PATIENTS

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A number of cohort studies have demonstrated that HIV-1 infection correlates with severe *Plasmodium falciparum* malaria (higher parasitemia, more clinical malaria complications, and higher case fatality rate), and *in vitro* studies have shown that HIV-1 impairs phagocytosis of parasitized erythrocytes by macrophages. As macrophage-mediated clearance of parasites in the spleen plays an important role in the clearance of parasites during malaria infection, we hypothesized that histological analysis of the spleen might further elucidate the mechanisms by which HIV-malaria co-infection accelerates progression to severe malaria. As part of a post-mortem study in Blantyre, Malawi of parasitemic comatose children, we systematically surveyed malaria parasites across organs in patients with and without HIV. The overall HIV seropositivity rate in the cohort was 21%. Using a combination of histology, immunohistochemistry and qRT-PCR, we found that HIV-positive patients had significantly higher parasite loads in brain, heart, gut, spleen and skin. The difference was most pronounced in the spleen where the difference in the mean number of parasites was highly significant (320 ± 139 vs 81 ± 20 parasites in 10 high power fields, $p = 0.0018$). In a focused histological analysis of the spleen, we found that HIV-positive cases had higher levels of "free" parasitized erythrocytes, i.e. those not engulfed by macrophages, while phagocytosed parasites could be readily observed in HIV-negative cases. These HIV-specific effects were significant, even across a wide range of values for peripheral parasitemia, hematocrit, and final histologic diagnosis (cerebral malaria or non-malarial coma). These data suggest that HIV infection enhances parasite sequestration in many different organ systems, and specifically in the spleen, where phagocytosis of parasites is impaired. These findings bridge *in vitro* mechanistic data and *in vivo* association study data to further elucidate the process by which HIV accelerates progression to severe malaria.

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CHILDREN WITH CM MANIFEST MARKED DIFFERENCES IN GLOBAL STRESS RESPONSES WHICH ARE ASSOCIATED WITH CEREBRAL PARASITE SEQUESTRATION AND UNDERLYING PATHOPHYSIOLOGY

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Cerebral Malaria (CM), a severe complication of *Plasmodium falciparum* infection, is associated with a high rate of mortality and neurological sequelae. The WHO defines CM as a single clinical entity of coma and peripheral blood malaria parasitemia; however, disease heterogeneity has been suggested by the identification of brain sequestration (RET+) in some but not all patients with CM. RET+CM is associated with brain microvascular pathology, parasite sequestration, coagulopathy and heightened inflammation. To explore potential mechanistic diversity in factors resulting in CM, we carried out unsupervised clustering on whole blood transcriptomes from 98 children with CM and identified three transcriptional clusters. One cluster was significantly associated with hyperparasitemia ($p<0.0001$) and another cluster with lack of brain sequestered parasites (RET-) ($p=0.028$). To further define host features associated with parasite sequestration, we then compared transcriptional profiles between samples associated with brain sequestered parasites (RET+) to lack of brain sequestrated parasites (RET-) taking parasite load into account. Gene sets associated with RET+CM reflected heightened inflammation (cytokine activity GO.000512), changes in neutrophil (neutrophil chemotaxis GO.0030593) and platelet (alpha granules GO.0031093) biology and dysregulation of coagulation (blood coagulation GO.0007597). Elevated plasma inflammatory cytokines and neutrophil proteins were found in RET+ samples, consistent with the RNA data. Surprisingly, neutrophils isolated from RET+ patients at the time of infection displayed impaired chemotaxis towards IL-8. In contrast, the transcriptional profiles of children with RET-CM were associated with upregulation of non-inflammatory stress pathways (protein catabolism pathways: GO.0006511; DNA repair pathways: GO.0006270) and type I interferon. Our data suggests host response diversity despite similar clinical presentations of WHO defined CM. Irrespective of parasite load, we found that children with cerebral parasite sequestration demonstrate evidence of neutrophil activation and coagulopathy. Further study of how neutrophil and platelet biology is involved in sequestration and why children with RET-CM have distinct host profiles could reveal novel strategies to prevent brain sequestration in CM and improve clinical outcomes.

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PHENOTYPIC VARIATION IN THE ABILITY OF *PLASMODIUM KNOWLESI* LAB AND FIELD STRAINS FOR THE INVASION OF HUMAN RED BLOOD CELLS

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Plasmodium knowlesi, primarily a simian parasite, is now the major cause of human malaria in Malaysian Borneo. While the majority of the cases are uncomplicated, clinical profiles similar to that of severe *P. falciparum* cases occur in a small subset of infected patients, with high parasitemia as the only risk factor consistently associated with disease severity. However, there has been little investigation to understand what causes such disparity in parasitemia. Recently, we showed that *P. knowlesi* H strain, a primate adapted lab strain, preferentially invades very young human red blood cells (RBCs). This strain however can gradually adapt to proliferate at a much higher efficiency in human blood. To expand this observation to other *P. knowlesi* strains, we have established *in vitro* culture rhesus RBCs 3 additional *P. knowlesi* strains. RBC age-dependent invasion assays and proliferation assays demonstrate that these lab stains exhibit different capacity in invading human RBCs. We are currently carrying adaptation experiments for these additional lines. To determine whether variations observed in the lab are also found in the field, we investigated the human RBC tropism of 32 field strains isolated from patients admitted to Kapit Hospital, Sarawak, Malaysia. The *in vivo* preference determination of 26 of them shows that a majority of the strains have a skew for invading younger RBCs (18/26). Indeed, 5 isolates display a very strong preference in invading reticulocytes. *In vitro* invasion assays performed with 9 isolates (parasite density greater than 10,000/ul) confirm that field strains differ significantly in the range of human RBCs they can invade. Together, these results suggest a significant variation in the ability of *P. knowlesi* strains to invade human RBCs,underlying a possible mechanism for the virulence of the parasite in human infections.

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CHARACTERIZATION OF THE EXPANDED ACYL CO-A SYNTHETASE GENE FAMILY

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The plasticity of the *Plasmodium falciparum* genome allows this malaria parasite to adapt quickly to selective pressures encountered in the human host by the acquisition of single nucleotide polymorphisms, recombination or gene duplications. One example of such duplication events is the acyl Co-A synthetase (ACS) gene family where four conserved orthologs of ACS are predicted to perform classical ACS function while nine paralogs have expanded and diverged from the *Pf*ACS9 ortholog and their function remains unknown. ACSs activate fatty acids (FA), which can then be used for protein modification, phospholipid biosynthesis, FA elongation, and beta-oxidation. In *P. falciparum* the majority of FA are taken up from the host environment. Long-range haplotype analysis suggests that members of this gene family are under recent positive selection. To address the biological function of the individual members of the *Pf*ACS family, we tagged individual genes with an HA tag and a protein degradation domain resulting in inducible protein knockdowns. These modifications allowed us to identify different expression patterns and distinct cellular localization of individual ACSs that could be a result of neo-functionalization. PfACS5 and PfACS1a were exported to the host cell cytosol and membrane periphery while PfACS9 remained within the parasite. Comparison of growth rates for wildtype and PfACS1a, PfACS4, PfACS5 and PfACS9 knockdown parasites in complete media did not differ and we could not detect any changes in gene expression of any other family member by quantitative RT-PCR. However, growth defects could be detected for PfACS5 knock down parasites in media containing restricted glucose and solely palmitic and oleic acid. We hypothesize that the expansion and recent positive selection of the *Pf*ACS gene family are the consequence of metabolic pressures driving parasite evolution, and understanding FA metabolism will give us insight into key metabolic pathways that might serve as potential targets for novel antimalarials.

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MUTATIONAL STUDIES FOR FUNCTIONAL CHARACTERIZATION OF ATP SYNTHASE COMPLEX IN *PLASMODIUM FALCIPARUM*

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The energy-converting rotary nanomotor ATP synthase is a central player in the bioenergetics of most organisms. However, several studies have found that blood stages of *Plasmodium falciparum* rely on glycolysis as its major source of ATP rather than oxidative phosphorylation, suggesting dispensability of ATP synthase. Yet, we have been unable to disrupt the

genes encoding the β and γ subunits of the ATP synthase complex in *P. falciparum* blood stages, raising the possibility that non-enzymatic functions of the ATP synthase may be essential. To address this possibility, we aimed to design a catalytically inert but structurally intact ATP synthase complex. A conserved residue in the catalytic site of the β subunit was identified as an initial target for mutagenesis. A merodiploid line expressing a tagged mutated β subunit at an ectopic site was generated in Dd2attB and NF54attB parasites. In theory, this should reduce the overall ATP synthase catalytic activity by about 87.5%. Although the mutant subunit was correctly trafficked to the mitochondrion in both lines, the transgenic parasites grew at the same rate as the parental lines, indicating that the ectopically expressed mutant subunits did not have a dominant negative effect. Mitochondria from the NF54attB merodiploid line were isolated and initial blue native page results indicated the mutant form of the protein being incorporated into the complex. Given that no dominant negative effect was seen with the merodiploid lines, a synthetic gene to replace the endogenous gene with a mutated version through single allelic exchange has been designed and will be transfected into the sexually competent NF54 line. We are furthermore attempting to knockdown the mRNA of the β subunit with a ribozyme system. These studies will help determine the role of ATP synthase in erythrocytic and insect stages of *P. falciparum*.

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AN ALGORITHM FOR ASCERTAINING THE *VAR* GENE REPERTOIRE IN *PLASMODIUM FALCIPARUM* FIELD SAMPLES

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Plasmodium falciparum erythrocyte membrane protein-1 (PfEMP1) proteins are encoded by the *var* gene family, present in 40 to 60 copies per genome. PfEMP1 mediates tissue-specific cytoadherence of infected erythrocytes, allowing parasites to evade clearance by the spleen and cause pathologic effects via sequestration. An infected erythrocyte usually expresses only one PfEMP1 variant on its surface at a time. PfEMP1 molecules undergo clonal antigenic variation that is central to their ability to evade the host immune response. Next generation short-read sequencing data cannot be used to accurately and comprehensively characterize sequence variation of this large, repetitive and highly variable gene family. Each *var* gene consists of two exons, the first of which is longer (2,500 -10,500 bp) and much more variable in length than the second (1,000 - 1,500bp). We developed a novel algorithm to perform targeted assembly of *var* gene exons, based on a combination of Pacific BioSciences (PacBio) and Illumina data. The algorithm takes advantage of patterns of conservation at the boundaries of the single intron, and the different variation profiles of the two exons, to generate exon 1 and exon 2 sequences from a combination of *k*-mer walks using Illumina reads and information from PacBio-based contig assemblies. Our novel algorithm recovers a complement of exons which is similar in number and length distribution to those found in the reference 3D7 genome, suggesting that it captures most of the *var* exons in the genomes of field-derived samples. Using this algorithm, we have characterized the repertoire of *var* genes in 12 clinical samples from Mali. We are determining the constitutive domains and upstream promoters for identified *var*s in each sample and the sequence diversity present. We will compare these results to known *var* repertoires. This new tool will advance efforts to understand the role of PfEMP1 variation in pathogenesis and immune evasion and may aid in the design of diversity-covering malaria vaccines.

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SUBTLE CHANGES IN *PLASMODIUM FALCIPARUM* INFECTION COMPLEXITY FOLLOWING ENHANCED INTERVENTION IN MALAWI

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With support from the Global Fund, the United States President's Malaria Initiative (PMI) and other cooperating partners, Malawi is implementing a comprehensive malaria control programme involving indoor residual spraying in targeted districts, free distribution of insecticide-treated bed nets to children and pregnant women, and use of the highly effective artemisinin-based combination therapy, CoArtem, as the first-line treatment for malaria. We genotyped 24 genome-wide single nucleotide polymorphisms (SNPs) in *Plasmodium falciparum* infections (n=295) sampled from a single location in Malawi before and after the switch to CoArtem to evaluate the impact of this enhanced malaria control programme. We used the SNP data generated to examine temporal changes in the incidence of infections containing multiple parasite genotypes (MIs), mean number of heterozygous SNPs within MIs, parasite genetic diversity (He), multilocus linkage disequilibrium and effective population size (Ne). While the incidence of MIs, He, multilocus linkage disequilibrium and Ne were unchanged over time, the mean number of heterozygous SNPs in MIs decreased significantly (p=0.0120) from 9±1 in 2006 to 7±1 (95% CI) in 2012. These findings indicate that the genetic diversity of malaria parasites remains high in this area, suggesting that only subtle gains, if any, have been made in reducing malaria transmission. Continued surveillance is required to evaluate the impact of malaria control interventions in this area and the rest of Malawi, and to better target interventions.

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COMPARATIVE ANALYSIS OF FIELD ISOLATE AND MONKEY-ADAPTED *PLASMODIUM VIVAX* GENOMES

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Due to the difficulty to culture *Plasmodium vivax in vitro*, significant insights into this important malaria parasite is based upon a history of successful adaptations of human infections to non-human primates. *P. vivax* strains grown in monkeys serve as a renewable source of parasites for studying relapse characteristics, mosquito species compatibilities, drug susceptibility profiles or immune response characteristics for potential vaccine development. However, little is known as to how the adaptation to a new host influences the parasite's genome and, consequently, its biology. Additionally, despite the consistent observation of complexity of infections in clinical isolates, these monkey-adapted strains are typically assumed to consist of a clonal population of a single strain. We describe here the comparative analysis of genome sequences from seven *P. vivax* parasites that have been adapted to monkey hosts with sequences obtained from six field isolate genomes. Our results reveal that the adaptation of parasites into monkey hosts is unlikely to result in any systematic modification of the genome. We also show that monkey-adapted strains are not always

homogenous and that they can still consist of a mixture of strains. Additionally, we describe the analysis of six blood samples collected during the generation of the Mauritania-I and Mauritania-II strains and show that, starting from a single complex infection, different strains became dominant in different monkeys. Overall, our study highlights some of the complications associated with studying monkey-adapted strains but also provide a solid framework for developing better, more controlled studies of this important resource for understanding vivax malaria.

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ASSESSMENT OF POPULATION STRATIFICATION AND COMPLEXITY OF INFECTION IN CAMBODIAN *PLASMODIUM VIVAX* BY HIGH-THROUGHPUT SEQUENCING

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In Cambodia, malaria is one of the foremost public health problems and its control is a high priority for the government and NGOs. Among the estimated 13.6 million Cambodians, 2.5 million individuals live in forested areas where malaria transmission is highest. With the implementation of extensive control efforts, the number of reported malaria cases has globally decreased since 1997, due to a decrease in falciparum malaria. By contrast, in the same period, the number and proportion of cases attributed specifically to *Plasmodium vivax* has significantly increased. In order to design more efficient elimination and control strategies in this region, it is critical to first understand the dynamics and organization of the *P. vivax* population. Here, we described a novel sequencing assay that enables robust genotyping of 130 single nucleotide polymorphisms (SNPs) in a high-throughput and cost-efficient manner. We applied this assay to 401 *P. vivax*-infected patients recruited from 9 study sites throughout Cambodia between 2004 and 2013. Our analysis provides a thorough perspective on the diversity, organization and dynamics of the Cambodian *P. vivax* population as well as a first assessment of the factors influencing complexity of infections. Overall, our study provides a foundation to design better control strategies against vivax malaria in Cambodia and to limit the spread of antimalarial drug resistance.

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SPATIAL DISTRIBUTION OF THE MICRONEMAL PROTEIN CELTOS DURING MOSQUITO MIDGUT TRAVERSAL

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Malaria remains a major burden on the health of global populations. Transmission of the malaria parasite through the Anopheline vector has emerged as a major focus of interest with a view to developing strategies to block mosquito infection, or reinfection of the human host. To date, whilst greater detail is emerging, there are still very few molecular details of any of the stages of mosquito or host re-infection. One protein that functions in both of these stages is CelTOS (cell traversal in ookinetes and sporozoites protein) a secreted soluble micronemal antigen that plays a role in both the colonisation of the mosquito midgut by the ookinete (traversing the epithelium towards oocyst formation) and infection of the liver by the sporozoite (traversing liver cells towards hepatocyte infection). Whilst knockout of this protein has already definitively demonstrated its function in these two distinct processes we still do not know what CelTOS does or its spatial localisation during these key stages. Here we present an update on our work using a tagged line of CelTOS that we have developed along with a suit of imaging reagents and microscopy approaches to make steps towards the functional dissection of CelTOS during mosquito transmission. Given its biphasic role, further understanding of CelTOS is hoped to lay the foundations for developing this key transmission protein into a future multi-stage anti-malarial vaccine.

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GENOTYPIC DIFFERENCES IN DENV NEUTRALIZATION ARE EXPLAINED BY A SINGLE AMINO ACID MUTATION THAT MODULATES VIRUS "BREATHING"

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Flaviviruses explore multiple conformations via dynamic motion of virus structural proteins. This introduces complexity to the antigenic surface, as virus "breathing" varies epitopes available for antibody (Ab) binding. A recent study explored the structural basis for genotypic differences in the neutralization potency of a DENV-1 specific mAb (E111) that binds a poorly exposed domain III epitope on the envelope (E) protein, as reported previously. Studies with DENV strains WP and 16007 indicated that ~100-fold difference in neutralization sensitivity could not be explained by differences in the affinity of mAb E111 for each strain. Instead, we hypothesized that the ensemble of structures sampled by these two viruses was distinct. To investigate the basis for differences in the "breathing" of these two strains, we generated a panel of DENV RVP variants expressing E protein chimeras or single amino acid differences on both WP and 16007 backgrounds. By assessing E111 potency against the E protein chimera RVPs, the difference in neutralization was mapped to three consecutive residues in domain II that differed between 16007 and WP. Further use of single amino acid mutant RVPs demonstrated that residue 204, but not neighboring residues 202 and 203, was responsible for the difference in neutralization. That residue 204 is located at a distance from the E111 epitope suggested that this amino acid dictates changes in the conformational ensembles sampled by the virus. In support of this, differences in neutralization by a panel of mAbs representing epitopes distinct from E111 were all significantly modulated by the same residue. Our results demonstrate that neutralization susceptibility can be altered in an epitope-independent manner by subtle mutations that alter the overall structural ensemble. That different conformational ensembles of flaviviruses may affect the landscape available for Ab binding has important implications for vaccine development and antibody mapping studies.

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CONSTRUCTION AND CHARACTERIZATION OF CHIMERIC DENGUE VIRUSES CONTAINING ANTIBODY EPITOPES FROM TWO SEROTYPES

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Dengue is the most prevalent mosquito-borne viral disease of humans and a major public health problem worldwide, with approximately one-half of the world's population at risk of infection by the four dengue virus (DENV) serotypes. Dengue vaccine development has been slow to date, as achieving a balanced and robust immune response against all four serotypes has proven elusive. The reasons for imbalanced immunity elicited by candidate vaccines are not fully understood, but are thought to include variable immunogenicity and competition between the live-attenuated viruses in the vaccine formulations. However, as the precise determinants of DENV immunity are incompletely understood, improving vaccine

formulations is difficult. Here we describe the results of investigations into the role of epitopes within the DENV envelope (E) domain I/II hinge region in DENV immunity. We transplanted DENV-3 EDI/II residues that constitute the epitope footprint of the DENV-3 specific monoclonal antibody (MAb) 5J7 into a DENV1 infectious clone, making the recombinant virus rDENV-1/3. We then transplanted the DENV-1 EDI/II residues that constitute the epitope footprint of the DENV-1 specific MAb 1F4 into a DENV-3 infectious clone, making the clone rDENV-3/1. Both rDENV-1/3 and rDENV-3/1 can be neutralized *in vitro* via the MAb corresponding to the transplanted residues, demonstrating that MAb epitopes can be transplanted between DENV serotypes. To test how epitope transplant affected rDENV-1/3 and rDENV-3/1 neutralization by human sera, these viruses were tested against a panel of DENV-1 and DENV-3 1° immune sera. Strikingly, both recombinant viruses are neutralized by 1° DENV-1 and 1° DENV-3 human immune sera, suggesting that the recombinant viruses displays epitopes recognized by both DENV-1 and DENV-3 neutralizing Abs in polyclonal immune sera, giving them "bivalent" qualities. To test the immunogenicity of these viruses *in vivo*, we have subsequently challenged groups of rhesus macaques with both recombinant viruses and here present the results of the macaque neutralizing antibody response following infection.

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CHARACTERIZATION OF NEUTRALIZING ANTIBODY RESPONSES FOLLOWING NATURAL SECONDARY DENGUE VIRUS INFECTIONS

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Dengue Virus (DENV) is an arthropod-borne flavivirus and the causative agent of dengue fever and dengue hemorrhagic fever. The goal of this study is to characterize the human neutralizing antibody response that develops after secondary DENV exposure. As there are 4 serotypes of DENV, people can be infected multiple times, each time with a new serotype. Based on recent studies, the dengue field has learned a lot about the properties of neutralizing antibodies generated in people exposed to dengue for the first time (primary infections) but little is known concerning the response generated during a repeat infection with a new serotype (secondary infection). Following primary infections, people develop lifelong protective immunity against the serotype of infection. Primary infections stimulate serotype-cross reactive and serotype-specific (to the serotype of infection) antibodies. However, only a small fraction of serotype-specific antibodies that bind to quaternary structure epitopes at the hinge region between domains I and II of the viral envelope protein are responsible for DENV neutralization. In the present study we investigated the properties of neutralizing antibodies produced during a secondary infection. Following a secondary infection, people develop neutralizing/protective antibodies to multiple serotypes including serotypes that have not infected the individual. An antibody depletion technique using beads coated with purified virus was used to measure levels of serotype specific and cross reactive antibodies and their relative contribution to neutralization. We observed two types of responses: in some sera, dengue virus neutralization was dominated by cross reactive antibodies, whereas in other sera both type-specific and cross-reactive antibodies contributed to neutralization. Thus, unlike primary sera, secondary sera contain antibodies that cross neutralize and presumably cross-protect from DENVs. Ongoing studies with recombinant DENVs and human monoclonal antibodies indicate these secondary infection antibodies recognize novel conserved epitopes that are distinct from the E protein domain I/II hinge epitopes targeted after primary exposure.

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TRANSPLANTATION OF A COMPLEX QUATERNARY SEROTYPE-SPECIFIC NEUTRALIZING ANTIBODY EPITOPE BETWEEN DENGUE 3 AND 4 REVEALS DETERMINANTS OF POLYCLONAL NEUTRALIZATION RESPONSES

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Dengue virus (DENV) is the most significant human arboviral disease worldwide with upwards of 300 million infections annually; however the determinants of human immune responses to DENV infection remain largely unknown. Thus we set out to develop tools with which to characterize antibody (Ab) responses to DENV infection in humans. Using reverse genetics we developed infectious clones (IC) for all 4 DENV serotypes which allow us to study Ab-virus interactions. Characterization of a panel of monoclonal Abs (mAb) identified a strongly type-specific neutralizing Ab of DENV3. Using a structure-guided approach a 12Å region of the envelope (E) protein domain I/II (EDI/II) hinge region encompassing mutations that led to escape of neutralization was identified and transplanted from DENV4 into DENV3 (rDENV3/4) to assess the contribution of this epitope to the polyclonal immune response in humans. Interestingly, this rDENV3/4 gained full sensitivity to neutralization by human DENV4 immune sera while becoming resistant to DENV3 sera, indicating that this EDI/II hinge region contains major determinants of type-specific neutralization responses. When the reciprocal transplant was made into DENV4, mAb binding was not retained and there was not a significant shift in neutralization profiles, indicating that the adjacent residues in the recipient DENV serotype play a role in epitope presentation on the virion surface. The addition of 5 amino acid residues from DENV3 into DENV4 was able to restore mAb binding and neutralization, however polyclonal serum neutralization remained largely unchanged. Finally, we moved a complex quaternary epitope encompassing residues spanning multiple E dimers into DENV4 (rDENV4/3). This rDENV4/3 was viable and grew to high infectious titers and exhibited sensitivity to DENV3 immune sera, while neutralization responses to DENV4 remained largely unchanged. These results provide insights into the determinants of type specific neutralization responses that could guide future development of rationally designed DENV vaccine platforms.

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GENERATION OF RECOMBINANT DENGUE VIRUSES ENCODING VARIANT ENVELOPE GENES FROM INTRASEROTYPIC GENOGROUPS REVEALS BREADTH OF TYPE-SPECIFIC NEUTRALIZATION RESPONSES

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It is estimated that over 300 million individuals are infected with dengue virus (DENV) each year, yet a detailed understanding of the humoral response to infection is poorly understood. Recent clinical trials of tetravalent DENV vaccine platforms have shown that eliciting a balanced and robust immune response against all 4 DENV serotypes has proven difficult. Within each DENV serotype exist multiple genogroups; however the breadth of serotype-specific immune responses across these genogroups is not well characterized. Using reverse genetics, we have developed infectious clone (IC) systems for all 4 DENV serotypes allowing for the generation of recombinant DENV (rDENV) for use as novel reagents for studying determinants of DENV-specific immune

responses. Using this technology we have created chimeric rDENV that express envelope (E) genes derived from variant genogroups within DENV serotypes 3 and 4 as well as a sylvatic DENV4 strain within the context of our DENV IC backbones . We examined immune responses from naturally infected persons and experimentally inoculated non-human primates (NHP) using these rDENV strains to characterize neutralization breadth across genogroups. Additionally, we have also identified critical epitopes localizing to the E domain I/II (EDI/II) hinge region that are critical components of serotype-specific neutralization responses. rDENV were generated by transplanting these epitopes between serotypes, and NHP were inoculated to examine immune responses elicited. These data showed NHPs had high serotype-specific antibody titers to the EDI/II hinge epitope; however the breadth of this response was unknown. Using our intraserotypic E chimeras, we characterized the extent to which these NHP sera neutralized other genogroups within each serotype. We show here these results detailing intergenotypic neutralization by naturally infected humans and rDENV vaccinated NHP. These results provide insight towards the rational design of DENV vaccine candidates capable of eliciting broad protection against multiple genotypes within each serotype.

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LONG-TERM MAINTENANCE OF DENGUE VIRUS-SPECIFIC CELL MEDIATED IMMUNE RESPONSES

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All 4 serotypes of dengue virus (DENV) can cause dengue fever (DF) and severe dengue (dengue hemorrhagic fever/dengue shock syndrome, DHF/DSS), mediated by cross-reactive T cells. Infection with any of the 4 DENV viruses is thought to confer life-long immunity to re-infection with that same serotype only, and heterotypic secondary infection is associated with development of severe disease. Our understanding of how memory T-cells may contribute to anti-DENV immunity is incomplete, and the duration of DENV-specific T-cell-mediated immune responses is not known. We enrolled volunteers in Hawaii who recalled experiencing a dengue-like illness in 1943, and following an outbreak of DENV-1 in 2001, the first known autochthonous transmission since 1943. Dengue is not endemic in Hawaii and in the absence of repeated exposures we were able to assess the duration, memory phenotype, and effector function of DENV-specific T cell-mediated immune responses in volunteers who experienced a single infection 3, 9, or 60+ years previously. DENV-specific proliferation was observed in all previously infected subjects, but not in any Control (seronegative) subject. Both CD4+ and CD8+ T-cells proliferated in response to stimulation with DENV-1 however there were marked differences between the two subsets with respect to longevity of responses. CD4+ responses were maintained at similar levels for the 3yr, 9yr and 60+yr groups, whereas CD8+ T-cell memory declined with time, with substantially diminshed responses apparent at 9yrs; in one subject this decline was already apparent 6 yrs after infection. B*3501/NS3500 tetramer analysis in age-matched 3yr and 60+yr subjects showed that the frequency of polyfunctional IFN-γ+/TNF-α + DENV-specific CD8+ T-cells declined significantly between 3yr and 6yrs after infection, reaching values similar to those seen at 60+yrs, and that IFN-γ production declined while TNF-α was maintained. We demonstrate the long-term persistence of dengue-specific T-cell immunity and show that the CD4+ and CD8+ memory pools are regulated independently.

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INTERACTION OF A DENGUE-SPECIFIC CD8+ T CELL NS1 EPITOPE WITH KIR3DL1 ON NK CELLS REVEALS AN UNDERAPPRECIATED ROLE FOR NK CELLS IN IMPACTING DENGUE DISEASE SEVERITY

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Killer immunoglobulin-like receptors (KIRs) interact with HLA class I ligands and play a central role in the regulation and activation of natural killer **(**NK**)** cells. During our investigation of CD8 T cell responses to a highly conserved HLA-B57 restricted DENV epitope, we observed substantial binding of a tetramer (B57-$NS1_{26-34}$ TET) to an NK enriched population. Since HLA-B57 is a known binding partner to KIR3DL1, we hypothesized that the B57-$NS1_{26-34}$ TET bound KIR3DL1 on NK cells. Staining of a KIR3DL1 transfectant cell line confirmed that B57-$NS1_{26-34}$ TET bound KIR3DL1. Consistent with the function of an inhibitory KIR, incubation of PBMC with HLA B57 expressing $NS1_{26-34}$ pulsed target cells suppressed the degranulation of only KIR3DL1+ NK cells. Furthermore, staining of PBMC from our clinical cohort revealed marked activation of NK enriched cells only in HLA B57+ patients who developed severe dengue disease DHF. These observations reveal a previously unappreciated role for a dengue T cell epitope in modulating NK cell function and have important implications for the pathogenesis of severe dengue disease.

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A MARKED REDUCTION IN MORTALITY AMONG PARTICIPANTS IN A CLINICAL TRIAL THAT REMOVED BARRIERS TO CARE AND IMPLEMENTED NATIONAL CASE MANAGEMENT GUIDELINES

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KEMRI/CDC in Kisumu Kenya is an evaluation site for the RTS,S/AS01 vaccine trial to measure efficacy in children vaccinated at age 5-17 months or 6-12 weeks. Participants at this site appeared to have low mortality rates compared with non-enrolled children in the KEMRI/CDC demographic surveillance system (DSS) where the trial was conducted. Although clinical and severe malaria were prevented, RTS,S did not show protection against mortality at 18 months follow-up. The comparator vaccines, meningC and rabies, prevent rare diseases, and thus were also unlikely to reduce mortality. We conducted a case control study to quantify the reduction in mortality among children enrolled in the malaria vaccine trial (cases) vs. children not enrolled, but living in the KEMRI/CDC DSS (controls). Participants in the malaria vaccine trial were provided transport reimbursement of 150 KSh (1.80 USD) for clinic visits. Study clinicians followed national case management guidelines. Severely ill children were transported to the district hospital, where adequate staffing, supplies and equipment were made available through the trial to implement the Kenyan National Pediatric Protocol for inpatient care. Outpatient and hospital care were provided free of charge. Microbiology capacity (including blood culture) was instituted. Cases and controls were matched

1:3 by date of birth (within 14 days), proximity (control within 3 km of a case), and gender. Cox regression analysis was used to calculate hazard ratio (HR). In multivariable analysis, we controlled for distance from the clinic. In total, 1618 cases were matched to 3541 controls. Half of cases and controls were female and no difference in socio-economic status was detected (p=0.12). Mean distance to the clinic was 1.9 km for cases and 1.8 km for controls (p<0.01). In all, 229 deaths occurred, 31 among cases (1.9%) and 198 among controls (5.6%). Cases contributed 2719 person years (py) and controls 5335 py of observation. Mortality rates were 0.01 and 0.04 deaths/py among cases and controls, respectively; the adjusted HR was 0.30 (95% CI: 0.19, 0.47) corresponding to a 70% (95%CI: 53, 81) reduction in mortality. Children enrolled in the clinical trial at KEMRI/CDC experienced a marked reduction in all-cause mortality. These data suggest that considerable reduction in child mortality could be achieved by reducing barriers to health care and providing quality care according to national guidelines.

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REACTIVE VACCINATION IN THE PRESENCE OF DISEASE HOTSPOTS

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Reactive vaccination has recently been adopted as an outbreak response tool for cholera and other infectious diseases. A global revolving stockpile of cholera vaccine was recently established for emergency use, but there is only enough vaccine globally - within and outside the stockpile - to vaccinate approximately one million people. When responding to outbreaks, public health officials must quickly decide who and where to distribute limited vaccine. Transmission hotspots have been discussed as one potential mechanism to efficiently allocate vaccine, however the effectiveness of this approach is likely to be context dependent. We compared strategies for allocating vaccine across multiple areas with heterogeneous transmission efficiency. We constructed meta-population models of a cholera-like disease and compared simulated epidemics where: vaccine is targeted at areas of high or low transmission efficiency, where vaccine is distributed equitably across the population, and where no vaccine is used. We find that connectivity between populations, transmission efficiency, vaccination timing, and the amount of vaccine available all shape the performance of different allocation strategies. In highly connected settings, like cities, when vaccinating proactively or early in the epidemic, targeting limited vaccine at transmission hotspots (i.e. areas with high transmission efficiency) is often optimal. However, once vaccination is delayed, targeting the hotspot is rarely optimal, and strategies that either spread vaccine between areas or those that focus on non-hotspots will avert more cases. Although transmission hotspots may seem like an intuitive target for outbreak control, we show that in many situations the hotspot epidemic may proceed so fast that hotspot-targeted vaccination will prevent relatively few cases, and vaccination shared across areas where transmission can be sustained is often the best approach. Our results suggest a general rule of thumb for vaccination in cities: reactive distribution of vaccine across areas that can independently maintain transmission is generally preferred to targeting any particular transmission hotspot, and hotspots should only be targeted when they are thought to be necessary drivers of transmission. These results provide new insights on how to efficiently vaccinate in response to an epidemic when vaccine is limited.

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ASSESSMENT OF REASONS FOR POLIO VACCINE REFUSALS IN NORTHERN NIGERIA - OCTOBER, 2012

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Oral polio vaccine (OPV) refusals during supplemental immunization activities (SIAs) contribute to the spread of polio virus in Nigeria. In September 2012, the Expert Review Committee (ERC) on Polio Eradication and Routine Immunization in Nigeria recommended that social research be conducted to better understand the reasons for polio vaccination refusals among caregivers in Northern Nigeria.Following the OPV campaign in October,2012, we conducted a cross-sectional study using semi-structured questionnaires to assess polio risk perception, reasons for refusals and perception of OPV campaigns. We interviewed caregivers who refused OPV for their children in two purposively selected high refusal districts (one rural and one urban) in five north-western Nigeria states. Median age of the 148 study participants was 39.5years, 82.4% were male, all were Muslims and 28% had primary level education or higher. Polio risk perception was low (77%), 89% of participants believed that the polio vaccine was neither necessary nor helpful, and possibly harmful. Most participants (75%) had an unfavourable or indifferent view of the polio campaigns. Religious belief was reported as an important driver of the participants' understanding of health and disease (70%). Most (85%) study participants indicated they were more concerned about other health issues such as malaria. Caregivers refuse OPV largely due to poor polio risk perception, unmet felt-needs and religious beliefs. Partners have therefore adopted strategies including; communication schemes aimed at increasing awareness of polio as a health threat; educating communities about the safety of the vaccine; engagement of religious leaders, polio survivor groups and polio/pro-OPV Community Development Volunteers, comprehensive health camps and provision of basic amenities like water boreholes to address identified issues. The adoption and implementation of the recommendations of this study contributed to the 59% reduction in the incidence of polio in Nigeria between week 52 of 2012 and week 52 of 2013.

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CHARACTERISTICS ASSOCIATED WITH THE REGULAR CONSUMPTION OF MILK OR SOLIDS IN BREASTFEEDING INFANTS IN MAL-ED, AN EIGHT SITE COHORT STUDY

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The WHO recommends exclusive breastfeeding for the first 6 months of life in order to maximize nutrient intake, increase immune function, and decrease exposure to pathogens. We expect that mothers choose to stop exclusive breastfeeding or introduce milks or solids based on multiple infant (size, illness, behavior), maternal (maternal age, education, number of live births) and environmental (food security, resources) factors. Using data from an 8-site birth cohort study, we explored the relationship between child, maternal and environmental characteristics and the timing

of regular consumption of milks or solids during the first six months of life. Overall, 1,967 children were included in this analysis, approximately 200 per study site. The median age of first regular consumption of milk or solids ranged from 41 days (Pakistan site) to 176 days (Bangladesh site). We used Cox proportional hazards models with time (in days) to regular consumption of milk or solid as the outcome, including both fixed (sex, maternal age, parity, etc.) and time varying (weight, height, illness, etc.) exposures. Higher weight-for-length (WLZ) and length-for-age Z-scores (LAZ) were associated with later introduction of milk or solids. Food insecurity, as well as illness (diarrhea, cough in the past 30 days), were also associated with later consumption of milks and solids. Factors related to breastfeeding initiation (colostrum, prelacteal feeding), maternal education, and parity were not strongly associated with timing of regular consumption of milks and solids. We included interactions between time and WLZ, LAZ, and cough and found that the protective effect of higher values of WLZ, LAZ, and cough decreased over time. These preliminary results indicate that the diet of the breastfed child may be altered according to the child's size or health status. These data will improve our understanding of the site-specific and overall timing of the regular consumption of milk and solids as it relates to child and maternal factors, and will allow us to make recommendations regarding how to incorporate breastfeeding in future analyses in order to avoid the potential for reverse causality. In addition, we can explore the factors associated with early regular consumption of milk and solids in these populations in order to understand and appropriately evaluate their impact on growth and overall health.

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HEMOGLOBIN CONCENTRATION DURING PREGNANCY AND INFANT COGNITIVE AND MOTOR DEVELOPMENT: A PROSPECTIVE COHORT STUDY

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There have been several studies on the consequences of anemia during pregnancy but to date, very little is known about the impact of prenatal anemia on infant neurocognitive function. The objective of this study was to assess the impact of anemia during pregnancy on the cognitive and motor functions of one-year-old children in Benin. Our prospective cohort study included one-year-old children born to women enrolled at their first antenatal care (ANC) visit, before 29 weeks of pregnancy, within the MiPPAD trial comparing sulfadoxine-pyrimethamine and mefloquine. Hemoglobin (Hb) concentrations of pregnant women were determined from venous blood samples collected at first and second ANC visits of at least, one-month interval and at delivery. Women were prescribed oral iron, folic acid and anthelminthics as part of the ANC package in Benin. A total of 635 children (76.7% of eligible children) were assessed for cognitive and motor functions, using the Mullen Scales of Early Learning (MSEL), at twelve months of age by trained research nurses. Prevalence of anemia decreased from 67.8% at first ANC visit to 40.1% at delivery. Children of mothers who were anemic at second ANC visit had better gross motor function (an estimated mean increase of 2.4 points, 95% Confidence Interval, CI, 0.1 to 4.7). We observed a significant negative quadratic relationship between infant gross motor function and Hb concentration at first ANC visit for women of gestational age greater 22 weeks (p=0.025). Thus, infant gross motor scores increased with increasing maternal Hb concentration until 88 g/L where it plateaued and began to decline at 110 g/l. We found no significant association between Early Learning Composite scores and maternal Hb concentration. There appears to be an optimal range Hb concentration (88-110 g/l) between 22 and 32 weeks of gestation that may be beneficial to infant gross motor function at age one year. These may reflect physiological hemodilution, which peaks between 22 and 32 weeks of gestation. Further studies are required to corroborate our findings.

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FOOD INSECURITY AND INDIRECT COSTS OF MEDICALLY-ATTENDED GASTROENTERITIS IN CHILDREN YOUNGER THAN FIVE YEARS OF AGE IN A "POST-ROTAVIRUS" SETTING

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Several vaccines are in development against norovirus (NV), the leading cause of pediatric diarrhea after rotavirus. In order to analyze the cost-effectiveness of potential vaccines, the direct and indirect costs of diarrhea must be ascertained. Since indirect costs have the potential to be high, accurate estimations of these costs are vital. In order to estimate the economic impacts of NV diarrhea, this study was designed to estimate the indirect and out-of pocket direct costs of a single diarrheal episode in a population with 90% rotavirus vaccine coverage. Children younger than five years of age presenting with diarrhea to the national children's hospital in Lima, Peru (Instituto Nacional de Salud del Niño) were screened for diarrhea according to the World Health Organization's case definition. Children meeting the case definition whose caregivers agreed to participate were actively followed with daily phone calls from the time of presentation until the conclusion of the diarrhea episode. The caregiver was then interviewed by a health care provider about the indirect costs of the diarrhea episode using a survey adapted from the WHO's indirect costs of diarrhea survey tool and the United States Department of Agriculture food security screening tool. Minimum wage was used to estimate the cost of lost productivity. Costs were converted to US dollars. The caregivers of 30 children younger than five years of age with diarrhea completed the interview. The median monthly household income was $306 (IQR: 252-360). The median estimated total indirect cost of the diarrheal episode was $75 (IQR: 54-117). The median number of hours of housework lost was 5 (IQR: 4-18). Thirty three percent of caregivers worked outside the house. Of those, the median number of workdays lost was 3 (IQR: 1.5-4.5). The median total out of pocket expense for one diarrheal episode was $85 (IQR: 65-126), including $22 (IQR: 14-41) for drugs and medical supplies. As a result of time and money lost due to the diarrheal episode, 70% of caregivers reported being worried about not having enough money to buy food, and 17% reported not having money to buy food. Significant indirect costs are incurred from a single episode of acute pediatric diarrhea, and these costs contributed to food insecurity in a segment of this population. Indirect costs should be considered in vaccine cost-effectiveness estimates and guide decisions about the introduction of new childhood vaccines.

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CLINICAL ASSESSMENT OF CHILDREN WITH FEBRILE ILLNESS AT HEALTH FACILITIES IN THREE DISTRICTS IN SOUTHERN ZAMBIA

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Given the decline in malaria prevalence in many areas in sub-Saharan Africa, fever in children can no longer be treated presumptively as malaria. The purpose of this evaluation was to examine health system capacity and clinicians' ability to accurately diagnose and treat pediatric febrile disease in three districts in Zambia with varying malaria endemicity. We conducted health facility surveys, health worker (HW) interviews, and observed patient-provider interactions for children under the age of 5 who presented with history of fever in the previous 48 hours. Quality of the clinical evaluation was assessed using the Zambian Ministry of Health IMCI guidelines. We observed 161 patient-provider interactions and interviewed 53 HWs at 24 health facilities with regard to their assessment of danger signs. Inability to feed was the most commonly assessed (53%) danger sign, followed by persistent vomiting (47%), convulsions (31%) and lethargy (29%). All four danger signs were assessed in just 8% (13/161) of children. None of the HWs conducted all, and less than 15% performed half of, the physical examination and history taking items as prescribed by IMCI guidelines. All facilities had capacity to test for malaria with either RDT or microscopy, but only 57% of children presenting with fever were tested. Clinical diagnosis of pneumonia was also incomplete. Less than one third of children with cough or difficulty breathing had their respiratory rate counted. Adherence to the malaria treatment protocol was high, as all patients who were RDT positive received ACT, while only 1 of 85 RDT-negative received ACT. However, none of the pneumonia-diagnosed children received the recommended treatment (amoxicillin), and 48% of those with fast breathing (WHO-defined, non-severe pneumonia) did not receive appropriate treatment. Identification of danger signs, diagnosis and treatment of febrile illness were not conducted in accordance with IMCI guidelines. There is an urgent need for interventions to improve management of pediatric febrile illness at the health facility level in Zambia.

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THE TRANSCRIPTOMIC ANALYSIS OF EARLY ADULT *ECHINOCOCCUS GRANULOSUS*

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Despite the substantial efforts have been made to control echinococcosis there is a clear need for new tools in its prevention. Dogs are pivotal in *Echinococcus granulosus* transmission and we contend that interruption of the parasite life cycle in the definitive host stage provides a very practical and cost-effective vaccination strategy. Specific or differential genes expressed in the scolex of adult worms are likely the vaccine targets for dogs against *E. granulosus*. To identify the genes, adult *E. granulosus* worms aged two weeks were collected from experimentally infected dogs. The worms were fixed and stored in ethanol. Each of the worms was cut into two parts, head and neck region. mRNA were extracted from the two parts respectively and RNA-seq technique was used to precisely quantify transcript levels in the two parts. A real time PCR was used to confirm the gene expression in the tissues. We identified 953 genes specifically or differentially expressed in the scolex of *E. granulosus*. Most of the up-regulated genes are novel, indicating these genes may compose a network in regulating specific function of worm head, and they may play an important role in settlement of *E. granulosus* in dog intestinal surface. Known genes include calcium-transport, dynein light chain, early growth response protein 2-like isoform 4, myosin heavy chain and transmembrane protein 144. Interestingly, neuropeptide f was highly expressed in the head of *E. granulosus*. The peptide acts as a neurotransmitter in the central nervous system(CNS), indicating the peptides may involve in the early CNS of the parasite. In addition, lanine aminotransferase was also up-regulated in the head, which may involve in nervous development in the early stage of adult worm. As scolex is an important worm part for attachment, the specific and differential genes are likely the targets for drug and vaccine development against adult *E. granulosus*.

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A HIGH SENSITIVITY DOT-BLOT FOR THE DIAGNOSIS OF HEPATIC CYSTIC ECHINOCOCCOSIS USING WHOLE BLOOD SAMPLE

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The diagnosis of hepatic cystic echinococcosis (CE) is based on ultrasonography (US) and confirmed by serology. However, a high proportion of inactive cysts, as well as early CE1 cysts, remain seronegative with conventional serodiagnostic tests, which poses diagnostic problems where no pathognomonic signs of CE are present on US examination. We present preliminary results of the performance of a whole blood dot-blot with human hydatid cyst fluid (HCF) especially in patients with hepatic CE who are seronegative with routine diagnostic test. HCF was collected from a CE patient after percutaneous aspiration of an active CE1 liver cyst. Dot-blot membranes were prepared with 50ng of total proteins, and whole blood samples were diluted 1:2000. We tested a total of 34 whole blood samples, 24 of which from CE patients with active, transitional and inactive cysts and 10 from volunteers and patients with non-parasitic cysts (control group). All CE patients with different cyst stages recognized the proteins present in the HCF, while all samples in the control group did not

recognize any proteins.These preliminary results are encouraging and we plan to evaluate our test on a higher number of CE patients and patients with other helminthic infections. This diagnostic test could be useful for the development of a rapid finger prick test for the serodiagnosis of CE in remote endemic areas. Further identification of the different antigens recognized will support the development of diagnostic tools that could improve the sensitivity of CE diagnosis.

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LONG-TERM FOLLOW-UP OF PATIENTS WITH ALVEOLAR ECHINOCOCCOSIS IN GERMANY

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From 1992 to 2011, 312 patients with alveolar echinococcosis (AE) were diagnosed and treated at the specialized outpatient clinic of the Ulm University. Demographic and clinical data were assessed and updated from the patients' first visits. At that time, 74.7% of the patients were alive, 12.8% had died, and 12.5% were lost to follow-up. Patients were treated either by surgery with subsequent benzimidazole (BZM) prophylaxis for at least 2 years (n=133), or continuous BZM treatment in case of inoperability (n=157). At first diagnosis, 17 patients had inactive lesions. Imaging and treatment schemes changed during the 20 years of observation. AE was diagnosed more often by chance in patients from 2000 onwards (48.0%) than before 2000 (28.7%). Since 2000, the disease was detected more frequently with lesions at PNM stages I and II (27.0% vs. 15.8%) according to WHO classification; as a consequence, radical resections were feasible in more patients (57.7% vs. 20.0%). Surgical resections were less frequent since 2000 (38.2% vs. 50.5%). Since 1993, PET-CT-scans with 18F-FDG were used routinely to visualize larval activity at time of diagnosis. For follow-up, the rationale for performing a PET-CT every other year was to monitor the effect of continuous BZM treatment, and to detect relapses after surgery. At the end of follow-up, medical treatment had been interrupted for 25.3% of the patients. Of 56 patients with R0 resection, 42 had stopped medical treatment. At their last visit, the disease status of 73.1% of the patients was judged as stable, in 5.1% as progressive while under medical treatment. AE was considered as being cured in 15.7% of the patients.The 5- and 10-years' survival rates in this cohort were 96.8% and 90.5%. Data analysis of two decades' experience in the management of AE showed that best care can be provided to the patients when they present at an early stage of the disease. As the disease is rare, expertise is best acquired in a single specialized institution; a benefit for the patients results from strict adherence to the WHO treatment recommendations.

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NEW CT-CLASSIFICATION OF HEPATIC ALVEOLAR ECHINOCOCCOSIS

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Computed tomography, mostly combined with PET, provides one of the most important diagnostic tools in suspected alveolar echinococcosis. Aim of the study was to establish a new CT-classification based on a large patient collective with confirmed hepatic alveolar echinococcosis. In 224 patients, CT-morphology of liver lesions due to an alveolar echinococcosis was retrospectively examined. The findings were grouped into the new classification scheme. Within the classification a lesion was dedicated to a "primary morphology" as well as to a "pattern of calcification". The primary morphology distinguishes following types: I. Diffuse infiltrating (with / without cystoid portion), II. Primarily circumscribed tumor-like (with / without cystoid portion and with / without offshoot at the edge), IIIa. Primarily cystoid, intermediate (with / without more solid portions at the edge), IIIb. Primarily cystoid widespread (with / without more solid portions at the edge), IV. Small-cystoid / metastatic* and V. Mainly calcified. Except for the "primary morphology" type V., following patterns of calcification were attributed additionally: without calcifications; with feathery calcifications; with focal (p.r.n. central - just possible with*) calcifications; with diffuse calcifications; with calcifications primarily at the edge. The various classification patterns are demonstrated by image examples. The proposed CT-morphological classification shall facilitate the interpretation of lesions due to a hepatic alveolar echinococcosis. This could help to interpret different clinical courses better and shall assist in the context of scientific studies to improve the comparability of CT findings.

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COMPARING GROUP MEANS AND INDIVIDUAL RESPONSES TO TREATMENT OF SOIL-TRANSMITTED NEMATODES WITH BENZIMIDAZOLE DRUGS: A POOLED ANALYSIS OF INDIVIDUAL PATIENT DATA

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Monitoring anthelmintic treatment efficacy is important to detect suboptimal response where drug pressure is high. The recommended method is egg reduction rate (ERR): percentage reduction in the mean number of eggs per gram (EPG) in excreta post- vs pre-treatment calculated using arithmetic rather than geometric mean (ERRam, ERRgm). We compared these group mean outcomes to individual patient outcomes: centile distribution of individual ERR (ERRic) from which we derived: proportion cured (cure rate, CR), or with reduced EPG (rEPG) or no change/ increased EPG (nEPG); and median ERR (mERR). We analysed trials which treated Ascaris lumbricoides (AL), Trichuris trichiura (TT) or hookworms (HW) with benzimidazoles (1832 subjects): four trials of albendazole (ABZ: n= 613; AL: n=121, TT: n=297, HW: n=195); two of mebendazole (MBZ: n=1219, AL: n=174, TT: n=513, HW: n=532). Both drugs were very effective on AL: CR were 90.9% with ABZ and 94.2% with MBZ. Treating TT with ABZ gave CR=15.4%, rEPG=48.8%, nEPG=35.7% and mERR=39%; and MBZ gave CR=22.4%, rEPG=58.1%, nEPG=19.4%; mERR=76%. Treating HW with ABZ gave CR=69.2%, rEPG=23.1%, nEPG=7.7, mERR=100%; MBZ gave CR=14.1%, rEPG=51.7%, nEPG=34.1%, and mERR=56%. Efficacy estimates expressed as ERRgm were systematically higher the ERRam for both drugs. As opposed to group mean estimates ERRam/gm, ERRic within one analysis, describes better the distribution of individual responses (centile distribution) and calculates proportions cured, with partial or no response, and median ERR; it should be tested on larger datasets to pinpoint changes in patterns of response and poor responders.

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WATER AND SANITATION TARGET DIFFERENT SOIL TRANSMITTED HELMINTHES ACCORDING TO THEIR ROUTE OF INFECTION

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Soil transmitted helminthes (STH) are a group of parasites of public health relevance due to its burden of disease and prevalence. The global strategy of the World Health Organization for the control of the morbidity related to STH includes preventive chemotherapy but also highlights the need for developmental improvements in the living conditions of affected populations. As part of a project for the development of strategies for the control of STH in northwestern Argentina, a survey was performed in randomly selected families of Wichii aboriginal communities of Tartagal with known high endemic prevalence of STH; socio-demographic information originating in the primary care routine sanitary data forms was incorporated into the database. The surveys were designed to include a representative randomized sample that included whole family groups. STH were surveyed through examination of single fresh stool specimens with 5 methods and an ELISA assay for Strongyloides stercoralis based on NIE antigen. In a community of 2914 individuals, 229 stool samples and 255 serum samples were evaluated and revealed an overall prevalence of STH of 54% with species specific prevalences of 49% for hookworms, 41% for S. stercoralis, 1% for Ascaris lumbricoides and 0.5% for Trichuris trichiura. The sanitary conditions of this communities revealed improved drinking water availability in 98% of the individuals which contrasts with a mere 2.3% with improved sanitation (unimproved pit latrines 94%, open defecation 1.4%, no data 1.9%). These results highlight the distinctly contrasting prevalence between STH with life cycles that infect through skin penetration of filariform larvae (hookworms and S. stercoralis) versus those that infect through the oral ingestion of embryonated eggs (A. lumbricoides and T. trichiura) according to environmental conditions. In the communities described in this report the lack of adequate sanitation favored skin penetrators but the availability of improved water blocked those transmitted trough oral ingestion. Life cycles determine risk factors and the interplay of these elements should guide control measures.

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DETECTION AND CHARACTERIZATION OF AN IMMUNODOMINANT ANTIGEN PRESENT ON THE SURFACE OF *ASCARIS* L3 LARVAE

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The human roundworm *Ascaris lumbricoides* is estimated to infect over 800 million people and is a significant public health problem. The closely related pig parasite *A. suum* plays and important role in veterinary medicine and represents a suitable model for *A. lumbricoides*. A continued exposure to *Ascaris* induces immunity at the level of the gut in pigs, protecting the host against the migrating larvae. The objective of this project was to identify and characterize parasite antigens targeted by this immune response that may be crucial for parasite invasion and survival. Pigs were immunized by trickle infection (100 A. suum eggs 5 times per week) for 30 weeks, challenged with 1,000 eggs at week 32 and euthanized two weeks after challenge. At necropsy, there was a 100% reduction in L4s recovered from the intestine and a 97.2% reduction in white spots on the liver in comparison with challenged non-immune controls. Antibodies purified from the intestinal mucus of immune pigs were subsequently used to probe L3 larval extracts resulting in a strong specific recognition of a 12kDa antigen (As12). This antigen is present on the surface of infective L3 larvae and is shed actively. As12 appears to be a glycolipid that contains phosphorylcholine, and it cannot be visualized by protein staining. Furthermore, As12 is highly resistant to different enzymatic and chemical treatments. This molecule could be of significant importance to the survival of the parasite during the initial stages of infection. Further studies are needed to investigate its molecular nature and its role at the parasite-host interface.

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FINE-SCALE SPATIAL AND TEMPORAL EVOLUTION OF WEST NILE VIRUS IN CALIFORNIA

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West Nile virus (WNV) is an arbovirus that was first reported in North America in 1999 and, by 2003, had spread nearly 3,000 miles from New York to southern California. The spread of WNV across the U.S. has been shown to be dependent on long-range movements, though sporadic sampling and an ascertainment bias from selecting isolates not associated with disease in humans may have affected these analyses. In addition, methods of viral overwintering and viral genetics associated with viral spread are not well understood. Herein, we report sequences for more than 125 WNV isolates collected from 15 counties of California. These isolates were made from mosquito pools collected as part of routine surveillance by the California Vectorborne Disease Surveillance System from 2003 - 2012. This unique dataset allows inference of fine-scale spatial and temporal movements of WNV. Using the Bayesian MCMC approach implemented in BEAST, we performed phylogeographic analyses and demonstrate that 3 independent introductions of WNV (2 WN02 strains and 1 SW03 strain) occurred in California between 2002 and 2003 via the Coachella and Sacramento Valleys. The two genotypes of WNV have remained in co-circulation in California from 2003 to 2012, though SW03 viruses were primarily restricted to southern California. An association between WNV genotype and WNV neuroinvasive disease (WNND) in humans has not been consistent, as both WNV genotypes have circulated in counties with high and low WNND incidence. Multiple examples of short-range movements of WNV across a few hundred miles, as well as geographic constraint of WNV strains within a single region for up to 8 years, suggest viral transmission has been driven by resident, rather than migratory, birds. In addition, while most mosquito pools containing infectious virus were collected in summer months, 2 viruses from mosquitoes collected during winter months were phylogenetically consistent with continued viral transmission as an overwintering mechanism for WNV. These data show that dense sampling across space and time can help to explain basic biological and ecological properties of WNV transmission.

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AVIAN TROPISM-ASSOCIATED PATHOGENESIS OF WEST NILE VIRUS: CHARACTERIZING THE ROLE OF AVIAN LEUKOCYTE INFECTION

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West Nile virus (WNV) replicates in a wide variety of avian species, which serve as amplification hosts. In particular, WNV generates high titers and elicits severe pathology in American crows (AMCRs; *Corvus brachyrhynchos*), a species that has been used as a sentinel for WNV circulation. Based on preliminary time-course studies, as well as a

comparison with WNV tropism in mammals, we hypothesized that early WNV replication in AMCRs is driven specifically by replication in circulating monocytes. Therefore, peripheral blood mononuclear cells (PBMC) were isolated from AMCRs and infected with WNV in an *ex vivo* culture system. The degree of replication attained by various WNV strains and mutants in this *ex vivo* system recapitulated their relative viremia and pathogenicity *in vivo* in AMCRs. Flow cytometric analysis suggested that the cells infected in *ex vivo* PBMC culture were predominantly monocytes. A WNV virus engineered to express target sequences for miR223, a miRNA found specifically in myeloid lineage cells, was generated to assess the specific role of infectivity of these cells for modulating *in vivo* avian virulence phenotypes. This cell-restricted recombinant virus failed to replicate in AMCR *ex vivo* PBMC cultures, demonstrating the myeloid lineage as the source of viral replication in the leukocyte preparations. Furthermore, the myeloid-restricted virus exhibited peak viremias in AMCRs 200-fold lower than the parental WNV strain or a control virus expressing a mosquito miRNA target sequence. Additionally, mortality of the miR223 target sequence WNV was 50% with an average survival time of approximately ten days, while the wild type virus yielded 100% mortality with birds succumbing within approximately five days. Thus, several lines of evidence point to the importance of leukocytes, including monocytes, in WNV infection of AMCRs. The *ex vivo* PBMC culture system may be a useful model for pathologic assessment of WNV strains, and can be used to further elucidate the mechanism of action of viral mutations that affect WNV host competence in avians.

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ROLE OF TNF-α RECEPTOR 2 IN WEST NILE VIRUS INFECTION

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West Nile virus (WNV) is the most common cause of arboviral encephalitis in the USA. Several lines of evidence suggest that production and release of TNF-α is one of the early cellular events in WNV infection. While the role of TNF-α receptor 1 (TNF-R1) in WNV infection is controversial, there are no studies that have evaluated the role of receptor 2 (TNF-R2) in WNV-associated neuropathogenesis. We *hypothesized* that TNF-α acting through TNF-R2 promotes survival of WNV-infected neurons and immune cells by counteracting WNV-induced apoptosis and enhancing regulatory T cells (Tregs) functions. Therefore, TNF-R2 knock out (KO) mice infected with WNV will have more severe disease and higher mortality than the wild type (WT) mice. The *objective* of this study was to examine the role of TNF-R2 signaling in protecting WNV-infected mice. Age- and gender-matched 8-12 weeks old, KO and WT (C57Bl/6) mice were inoculated with 100 or 10 plaque forming units (pfu) of WNV NY-99 strain and monitored daily for 3 weeks. Mice survival was analyzed using Log-rank (Mantel-Cox) tests and viremia was quantitated by quantitative reverse transcriptase real-time PCR (qRT-PCR). When infected with 100 pfu of WNV, 97% of KO mice succumbed to death whereas 31% WNV-infected WT mice survived (N=29 in each group, Chi-square 14.38, df 1, p= 0.0001). While WT mice had lower viremia ($7\pm3x10^3$ vs. $4\pm7x10^4$ pfu equ/mL) and slightly longer median survival time (11 days vs. 10 days), these differences were not significant. Interestingly, when infected with 10 pfu of WNV, there was no difference in the survival between WT and KO mice suggesting the role of TNF-R2 while is critical in heavy infection, it may not have any role in low dose WNV-infection. Therefore, because of limited distribution of TNF-R2 in immune, hematopoietic and neuronal cells that are primary targets of WNV, specifically augmenting the TNF-R2 could be a better and safer therapeutic strategy in heavy WNV infection.

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RESTRICTION OF 2'-O METHYLATION DEFICIENT WEST NILE VIRUS IN INSECTS

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Interferon (IFN) induced proteins with tetratricopeptide repeats (IFITs) were recently identified as mammalian sensor molecules that recognize non-self viral RNA lacking 2'-O methylation at their 5' end and selectively block translation. Similar to mammals, the host mRNA of insects is methylated at both the N-7 and 2'-O positions yet insects lack any known IFN response pathway or apparent IFIT gene orthologs. Here, we show that a mutant West Nile virus (WNV) lacking 2'-O methyltransferase activity (WNV-NS5-E218A) is attenuated in mosquito cells and intact mosquitoes. Wild type WNV (WNV-WT) grows to approximately 2 logs higher titer than WNV-NS5-E218A in both C6/36 and AAG2 mosquito cells and WNV-WT had an increased infection rate and higher titer of infection in Culex tarsalis mosquitoes at day 3 and 5 post infection in comparison to WNV-NS5-E218A. These results suggest that WNV-NS5-E218A is restricted by a novel effector pathway that recognizes the loss of 2'-O methylation, analogous to our observations in mammals; alternatively, insects use 2'-O methylation for efficient translation and thus, restrict non-self RNA lacking this modification. To identify the genes and pathways responsible for restricting WNV-NS5-E218A, a genome-wide RNAi screen that targets 12,870 Drosophila genes was performed. We identified 140 genes within the primary screen that increased infection of WNV-NS5-E218A when silenced. Of these 140 genes we are currently pursuing four genes with mammalian homologs that increase WNV-NS5-E218A infection to a greater extent than WNV-WT. We will discuss how these genes preferentially restrict WNV lacking 2'-O methylation in insect cells.

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SUBSTITUTION OF THE PREMEMBRANE AND ENVELOPE PROTEIN GENES OF MODOC VIRUS WITH THE HOMOLOGOUS SEQUENCES OF WEST NILE VIRUS GENERATES A CHIMERIC VIRUS THAT REPLICATES IN VERTEBRATE BUT NOT MOSQUITO CELLS

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Most known flaviviruses, including West Nile virus (WNV), are maintained in natural transmission cycles between hematophagous arthropods and vertebrate hosts. Other flaviviruses such as Modoc virus (MODV) and Culex flavivirus (CxFV) have host ranges restricted to vertebrates and insects, respectively. The genetic elements that condition the differential host ranges and transmission cycles of these viruses have not been identified. In this study, fusion-PCR was used to replace the capsid (C), premembrane (prM) and envelope (E) genes and the prM-E genes of a full-length MODV infectious cDNA clone with the corresponding regions of WNV and CxFV. Fusion products were directly transfected into baby hamster kidney-derived cells that stably express T7 RNA polymerase. At 4 days post-transfection, aliquots of each supernatant were inoculated onto vertebrate (BHK-21 and Vero) and mosquito (C6/36) cells which were then assayed for evidence of viral infection by RT-PCR, Western blot and plaque assay. Chimeric virus was recovered in cells transfected with the fusion product containing the prM-E genes of WNV. The virus could infect vertebrate but not mosquito cells. The *in vitro* replication kinetics and yields of the chimeric virus were similar to MODV but the chimeric virus produced larger plaques. Chimeric virus was not recovered in cells transfected with any of the other fusion

products. In conclusion, our data indicate that genetic elements outside of the prM-E gene region of MODV condition its vertebrate-specific phenotype.

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THE YELLOW FEVER ICEBERG: THE PROBABILITIES OF MILD, SEVERE AND FATAL DISEASE FOR PEOPLE INFECTED WITH YELLOW FEVER VIRUS

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Yellow fever virus, like other pathogens, only causes disease in a proportion of individuals it infects. Severe illness and death represent the tip of the iceberg relative to asymptomatic and mild infections, which is more critical for determining the overall disease burden and prevalence of infection. We compiled data on asymptomatic infections, mild disease, severe disease, and fatalities from eleven outbreaks affecting hundreds to thousands of people in Africa and South America between 1969 and 2011. We defined severe disease as fever with jaundice or hemorrhagic symptoms and mild disease as fever or other reported illness not meeting the severe disease criteria. Using a Bayesian model, we estimated the probabilities and 95% credible intervals (CI) for asymptomatic infection, mild disease, and severe disease in infected individuals. The average probability of being asymptomatic was 0.55 (95% CI: 0.37-0.74). The probability of infection resulting in mild disease was 0.33 (95% CI: 0.13-0.52) and severe disease was 0.12 (95% CI: 0.05-0.26). We also estimated the case fatality rate for individuals experiencing severe disease; the probability of fatality for severe cases was 0.47 (95% CI: 0.31-0.62). For unknown outbreaks, the uncertainty and variability between studies indicates that for every severe case of yellow fever, there may be 1 to 70 additional infections, representing asymptomatic or mild infections. The large range in all of these estimates reflects the scarcity of data, intrinsic variability, and variation between outbreaks, possibly due to study design or differences in environmental, host, vector, or virus characteristics. Nonetheless, the results are generally inline with previous estimates, which are derived only from individual studies. As it is generally only the most severe cases that are recognized and reported, these estimates will help improve the understanding of the burden of disease and the estimation of the potential risk of spread during YF outbreaks.

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FLAVIVIRUS EXPOSURE IN NORTHEASTERN KENYA

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Flaviviruses are transmitted throughout the world, though their burden is still largely underestimated in Africa. The objective of this study was to assess the seroprevalence of flavivirus exposure in northeastern Kenya and to link previous exposure to risk factors and reported historical symptoms associated with prior febrile illness. Questionnaires on demographics, reported symptoms, and mosquito exposures were administered to 891 participants among 6 northeastern Kenyan villages in February and March of 2011. Visual acuity tests and physical exams were also performed. Sera were tested via standardized ELISA protocols for anti-dengue virus IgG. Risk factors associated with seropositivity were determined using Fisher's exact test and logistic regression models. Forty-three (4.9%; 95% CI 3.6-6.5%) participants were flavivirus seropositive (aged 3-85 years). Prevalence differed significantly among villages ($p<0.001$) with higher risk in Sabenale (19%), Golabele (10%) and Tumtish (9%), and lower risk in Gedilun (4%), Korahindi (2%) and Matarba (1%). Children (<16 years old) were less likely to be seropositive than adults ($p<0.001$). Women tended to be less at risk than men ($p=0.057$). Seropositivity was associated with poor measured visual acuity ($p=0.026$) and the following reported lifetime historical symptoms: red eyes ($p=0.005$), poor vision ($p=0.038$), eye pain ($p=0.005$), photophobia ($p<0.001$), backache ($p<0.001$), malaise ($p<0.001$), and sleepiness ($p=0.045$). Seropositivity was also associated with home flooding ($p=0.0215$). Flavivirus exposure is rare in northeastern Kenya, but more common among adults than children. Differences in seroprevalence between residents of nearby villages with similar ecology may be due to varied mosquito control practices or economic factors. Flavivirus exposure is associated with many historical visual symptoms and poor measured visual acuity. Despite low prevalence and no reported outbreaks, ongoing interepidemic transmission is demonstrated by documentation of seropositive children.

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MALARIA IMPORTATION AND ELIMINATION IN SWAZILAND

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Achieving country-wide elimination of malaria requires the cessation of local transmission caused by imported malaria. Swaziland, a sub-Saharan country that has worked towards malaria elimination for over a decade, has brought local transmission down to extremely low levels. As Swaziland makes further progress towards elimination, the methods and metrics typically used to assess the risk of local transmission initiated from imported malaria become inadequate. Additionally, independent of method or metric, accurate accounting of both local and imported cases becomes ever more critical as the relative weight carried by each individual case increases. To both assess transmission dynamics in a low-transmission setting such as Swaziland, as well as evaluate surveillance efforts, we developed algorithms to estimate malaria transmission chains. Using comprehensive case data from 2010 through 2013, these estimated chains - based on identifying likely causal links between successive cases through the use of spatio-temporal kernels - provided insight on the frequency and length of chains of local transmission. By identifying secondary cases that appear to have no likely primary case we catalogue likely gaps in surveillance. Our analysis suggests than most locally acquired cases were caused by a few imported cases and that most imported cases resulted in no extended local transmission. Further, we highlight regions in space where local transmission chains appear to be longest. Our results suggest these regions would benefit from more systematic control and surveillance efforts to identify and curtail extended local transmission in the future.

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MAPPING SEASONAL INTERACTIONS BETWEEN POPULATION MOVEMENTS AND MALARIA TRANSMISSION FOR STRATEGIC MALARIA ELIMINATION PLANNING

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In most countries that are planning for malaria elimination a strong seasonality in malaria transmission can be observed. This often drives the timing of intervention and surveillance efforts, and the seasonal patterns can potentially be exploited to optimally target resources

for achieving elimination. Factors that have received lesser attention in designing elimination strategies are population movements, their seasonal patterns and their demographic composition. As a country or area transitions towards malaria elimination, imported cases make up an increasingly larger proportion of those seen, and the importance of accounting for population movements rises. Throughout the world, the volumes and major routes of population movements tend to follow seasonal patterns, with certain times of year showing significantly greater amounts of movement than others, and this varying by demographic groupings. These movements can impact substantially on the dispersal of parasites in a region, depending on how the timings and routes of seasonal movements interact with the seasonality of malaria transmission. Here, we demonstrate how the simple combination of national malaria surveillance system data, case investigation information and population mobility metrics derived from mobile phone records can inform on these seasonal interactions, using Namibia as an example. Using anonymized cellphone call detail records to determine the mobility patterns of nearly 2 million residents, we show that the timing, duration of stay and magnitude of movements vary substantially across time and space, with significant movement peaks in December and January aligning with peak malaria transmission. Further, case investigation data from more than 100 malaria patients in the region with highest transmission (Zambezi) enables validation of these mobility patterns and provides valuable additional demographic and epidemiological insights into risk groups and contact patterns. The approaches presented can be updated rapidly and used to identify which regions would benefit from coordinating efforts at certain times of year and how spatially progressive elimination plans can be designed to account for the interacting seasonality in transmission and mobility.

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A MONITORING AND EVALUATION TOOL FOR REACTIVE CASE DETECTION: PILOT EXPERIENCE IN ACEH PROVINCE, INDONESIA

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Active case detection (ACD) strategies are recommended by the World Health Organization and are implemented in many low transmission settings worldwide as a critical part of malaria elimination programs. ACD strategies include determining the origin of infection, case investigation, and responding to locally acquired cases of malaria, known as reactive case detection (RACD). Effectively implementing RACD requires substantial programmatic and human resources. Aceh Province has a goal to eliminate malaria by 2015. Between June and September 2013, a pilot was conducted to evaluate RACD activities and identify best practices to inform RACD efforts in Aceh Province. Using a standardized monitoring and evaluation (M&E) tool, a sample of 5 districts including 34 health facilities were evaluated to measure RACD indicators, in addition to staff interviews regarding RACD procedures. To measure case investigation and RACD rates and timeliness, quantitative data was extracted from the district health offices and health facility registers and measured against defined indicators. Questionnaires were administered to 59 health facility staff involved in conducting RACD. From January to December 2012, a total of 289 cases (range 0 to 153) were reported at health facilities with a completeness of 92% when compared with the district health office records. Mean case investigation rates were 78% (range from 62 to 100). Screening by microscopy was conducted on a total of 931 community individuals (16.3 individuals per index case) with 3 new infections identified. Mean timeliness of RACD conducted within 7 days was 82% (range from 43 to 100). These findings indicate the need for improved patient data collection upon presentation at the health facility, and for staff to visit index cases after normal work hours to prevent loss due to follow up during case investigations. Rollout of the M&E tool to additional districts in Aceh Province and the monitoring of currently sampled districts will improve timeliness and reporting completeness of facilities and optimize RACD program effectiveness.

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SUSTAINABLE MALARIA ELIMINATION ON ANEITYUM ISLAND, VANUATU, 1991 -2014

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Weekly mass drug administration (MDA) of chloroquine, pyrimethamine/sulfadoxine, and primaquine was carried out on the entire population of 718 inhabitants of Aneityum island for 9 weeks in 1991 before the onset of the rainy season. Simultaneously insecticide-treated bednets (ITNs) were distributed to the entire population. Microscopy showed the immediate disappearance of *Plasmodium falciparum*, whereas *P. vivax* disappeared from 1996 onwards until new cases were reported in January 2002. In July 2002 *P. vivax* infections were detected by microscopy in 22 of 759 individuals: 20/298 born after 1991, 2/126 born between 1991-82, and 0/335 born before 1982. Subsequent PCR increased the total to 77 (36, 21, and 20 in respective age groups). The age distribution was similar to those before elimination and on other islands. In November a similar age pattern was found but with fewer (39) infections. In December 2002, the 2nd MDA of weekly chloroquine for four weeks and daily primaquine for 14 days was carried out as a containment measure on the population born after 1982, in concert with re-strengthening of the community-based provision of ITNs. Population-wide mass blood surveys detected by PCR no cases in December 2002 (n=436, only those born after 1982), immediately after the MDA, but 26 in 2003 (730), 20 in 2004 (732), 34 in 2005 (836), and 15 in 2007 (719). No positive cases were detected in 2010 (950) and 2013 (1093). The age distribution of 2003-2007 positive cases was different from those before elimination and on other islands: i.e. a substantially lower prevalence was observed in the population born after 2002 (0.8%) than those between 2002-1992 (3.7%), between 1991-1982 (5.3%), and before 1982 (2.1%), suggesting that these submicroscopic infections mainly reflected relapses from liver stages. Sero-epidemiological monitoring suggested that the persistence of antibodies against *P. vivax* may partially explain the lower parasite prevalence in the oldest age group. On Aneityum, indigenous *P. falciparum* transmission has never re-established after the 1st MDA in 1991, despite surveillance by community microscopists that showed continuous parasite importation from other islands. A high degree of community engagement to prevent resurgence, in addition to high ITN coverage (1.05 net/person) and usage (95%, 2014), sustains malaria freedom on this island.

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GENETIC CORRELATES OF DECLINING TRANSMISSION: *PLASMODIUM VIVAX* IN SRI LANKA

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We examined whether the recent dramatic decline in malaria transmission in Sri Lanka led to a major bottleneck in the local *P. vivax* population, with a substantial decrease in the effective population size. To this end, we

typed 14 highly polymorphic microsatellite markers in 185 *P. vivax* patient isolates collected from 13 districts in Sri Lanka over a period of five years (2003-2007). Overall, we found a high degree of polymorphism, with 184 unique haplotypes (12-46 alleles per locus) and average genetic diversity (expected heterozygosity) of 0.8744. A marginal decline in Allelic diversity (15.4 to 10.9) as well as heterozygosity (0.87 to 0.82) was observed from the 2003-4 to 2006-7 period. Almost 69% (n=127) isolates had multiple-clone infections with no major changes observed over time. Significant spatial and temporal differentiation (F_{ST}=0.04 - 0.25; p≤0.0009) between populations was observed. The effective population size was relatively high but showed a decline from 2003-4 to 2006-7 periods (estimated as 45,661 to 22,896 or 10,513 to 7,057, depending on the underlying model used). We used three approaches - namely, mode-shift in allele frequency distribution, detection of heterozygote excess and the *M*-ratio statistics - to test for evidence of a recent population bottleneck but only the low values of *M*-ratio statistics (ranging between 0.15-0.33, mean 0.26) were suggestive of such a bottleneck. The persistence of high genetic diversity and high proportion of multiple-clone infections, with little change in effective population size, despite the collapse in demographic population size of *P. vivax* in Sri Lanka indicates the importance of maintaining stringent control and surveillance measures to prevent resurgence. It also appears that more samples (over decades) might be required to detect the genetic signatures of shrinking malaria transmission.

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EVALUATION OF TOPICAL REPELLENTS AS ADDITIONAL VECTOR CONTROL MEASURES TO CONTROL RESIDUAL TRANSMISSION IN MALARIA PRE-ELIMINATION AREAS

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In Southeast Asia a substantial decrease of malaria has been achieved during the last decade and elimination is now becoming a realistic goal. However residual transmission due to outdoor and/ or early biting vectors is not tackled by wide coverage of insecticide treated nets (ITNs). This may compromise the elimination efforts. For this purpose we set up a study to evaluate the public health value of mass use of topical repellent in addition to ITNs. A randomized community based design has been adopted covering a population of 40,000 inhabitants in the province of Ratanakiri in Cambodia. The 98 clusters were randomly divided in two arms after a pre-trial survey: one intervention arm (ITN and repellent) and one control arm (only ITNs). Comparing to a randomized household trial, present design has the advantage to avoid the risk of the repellent aversion effect and the exchange of products between the households. The principal indicators of effectiveness is the prevalence of parasite carriers measured by PCR techniques using a mobile molecular lab in the field, and the measurement of malaria antibodies. While parasite-prevalence provides a snapshot of the exposure to malaria at a certain moment, serological indicators provide a picture of the "force of malaria infection" over a prolonged period. Moreover passive case detection provides a measurement of malaria disease incidence in both arms. We expect a community protection against residual transmission when a high adherence in the use of repellents is achieved. To address this working hypothesis of mass effect of repellents on the vector population entomological surveys are carried out in both arms. The effectiveness of the intervention is dependent on the efficacy of the repellents against vector bites and the effective use of repellents by the population and both are addressed in the study design. First results will be presented. The outcome of this study will be crucial in the development of new strategies to control not only the indoor transmission during sleeping time (ITN) but also the increasing proportion of residual transmission which occurs mainly outdoors, and before and after sleeping time.

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A CLUSTER-RANDOMIZED TRIAL OF TARGETED CONTROL TO ELIMINATE MALARIA IN CENTRAL SENEGAL: STUDY DESIGN AND ACCEPTABILITY OF THE INTERVENTIONS

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The purpose of this trial is to evaluate the extent to which a targetted malaria control strategy combining vector control with indoor residual spraying (IRS) and chemotherapy, delivered by district health staff to villages reporting clinical cases, can reduce the transmission of malaria, in a region where scaling up of control measures has been effective in reducing the incidence of malaria. The trial will also determine whether, as part of this strategy, chemotherapy should be delivered to all members of targetted communities (MDA, Mass Drug Administration) or only those who have been tested and are known to be infected (MSAT, Mass Screening and Treatment). Methods: 45 health posts, each serves about 10,000 people, were randomized in 40 clusters; 15 to receive IRS and MSAT, 15 to receive IRS and MDA, and 10 to serve as controls. In intervention clusters, villages with evidence of transmission (hotspots) were identified on the basis of confirmed malaria cases reported the previous year. Interventions are delivered over two years, the primary outcomes are the incidence of malaria and the prevalence of parasitaemia just after the main peak period of transmission in the second year. To assess effects on transmission, incidence in non-targetted areas in each cluster will be compared. In 30 clusters, all households in hotspot villages were targetted to receive IRS with Actellic 300CS in July, followed in 15 of the clusters by MDA with dihydroartemisinin-piperaquine (DHA-PQ) administered to all persons in the household in September and again in October, and in the other 15 clusters, instead of MDA, all persons in the household were screened using a malaria RDT and those who test positive treated with DHA-PQ. In all trial clusters, LLIN coverage was topped-up by providing persons diagnosed with malaria with a LLIN. Results and conclusion: Preliminary results after the first year of intervention will be presented, including description of the logistics of MDA and MSAT delivery, the coverage of the interventions, the sensitivity of RDTs for detecting infections, and the acceptability of both interventions by the community.

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ZOONOTIC RISKS OF NON-TUBERCULOUS MYCOBACTERIA BETWEEN HUMANS AND SMALL MAMMALS (POTENTIAL TRANSMISSION OF BURULI ULCER) IN COTE D'IVOIRE AND GHANA

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The prevalence of Buruli ulcer, caused by the non-tuberculous Mycobacterium ulcerans (MU), ranges from 1-150/100,000 infected individuals in Ghana and Côte d'Ivoire, making it the second most important mycobacterial disease. The mode of transmission is unknown and compounding this is the fact that mycolactone, the major virulence factor, has been found in other environmental mycobacteria species (MPMs) which cause disease in some animals. Although there are no

reports of human disease caused by these novel species, they share similar ecological niches in the endemic aquatic environments. Our work is based on the One Health concept and suggests that overlapping environmental habitats of the pathogen, animals and humans directly influence transmission. Using (i) active case surveillance of human disease burden; (ii) molecular characterization of NTMs; (iii) and zoonotic risk analysis, we are conducting a study in 6 communities of Côte d'Ivoire and 4 communities in Ghana to understand transmission. Comparative analysis of our socio- anthropological data suggests that individuals in Ghana have a higher level of Buruli ulcer awareness than in Côte d'Ivoire. In both countries, association with dirty water is a risk factor and affected people are generally poor. Molecular analysis of our environmental samples indicate a high presence of mycobacterial DNA including MPMs. Almost all of our suspected human cases also type positive for MU DNA. Using Variable Nucleotide Tandem Repeat (VNTR) typing, we have been able to profile and identify one common genotype that is present in both humans and the environment within the same community in both countries. Interestingly we have identified mycobacterial DNA with MPM profiles in lesions and swellings from trapped small mammals within our study sites. Most of the animals were rats, with the majority from homes close to water bodies in both countries. We intend to develop a model of infection dynamics, and identify key risk factors that will ultimately improve control and surveillance strategies for National Control Program.

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LINKED HUMAN AND LIVESTOCK STUDY ON SEROPREVALENCE AND RISK FACTORS FOR BRUCELLOSIS IN KENYA, 2012

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Brucellosis is an endemic zoonotic disease in Kenya with dual burden with significant economic losses among livestock and illness and disability in humans. Brucellosis studies in Kenya have previously focused on either animals or humans. We conducted a cross-sectional survey that examined humans and livestock (cattle, sheep, bovine, camels) in the same households. We assessed the seroprevalence and risk factors for human brucellosis, animal brucellosis and their association. The study was conducted in three counties with different livestock production systems; Random households were selected with a two-stage cluster sampling method by sub-location and then household. Three persons were enrolled and livestock species proportional to the size of the herd were sampled per household. A questionnaire was administered to household heads and the persons sampled. Human sera were tested for Brucella IgG antibodies using competitive ELISA and animal sera were tested using an indirect ELISA. Risk factors were assessed using multivariate logistic regression. A total of 1,099 households, 2,811 persons and 11,039 livestock were enrolled. Overall, 14% (95%CI 12-16) of households had at least one Brucella seropositive person and 15% (95%CI 12-18) of herds had at least one seropositive animal. Among humans sampled, 6.7% (95%CI 5.6 -7.8) were seropositive. Risk factors for human seropositivity included taking unboiled milk (aOR=3.9, 95%CI 2.0-7.6), exposure to goats [herding, milking, feeding] (aOR=2.5, 95% CI 1.6-4.0) and handling hides (aOR=3.9, 95%CI 2.5-6.2). Animal seropositivity was associated with intermingling with wildlife (aOR=4.3, 95%CI 2.3-8.1) and keeping goats (aOR=2.7, 95%CI 1.4-5.2). The odds of human seropositivity given a seropositive animal in the same household was 5.3 (95% CI 3.2 -8.8). This linked survey shows that human and animal brucellosis seropositivity is associated with factors that increase exposure to seropositive animals. The survey contributes to understanding the burden of brucellosis in Kenya and targeting of health education programs.

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Q FEVER OUTBREAK ON A LARGE U.S. GOAT AND CATTLE DAIRY: A ONE HEALTH INVESTIGATION

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Q fever, caused by *Coxiella burnetii*, is an under-recognized and under-reported zoonotic disease transmitted to humans primarily via infected livestock. Between June-November, 2013, 12 cases of Q fever linked to a Missouri community that operates a large goat and cattle dairy, Community A, were reported to the state health department. This compared to an average of 3 (range 1-5) cases reported annually in Missouri over the past 5 years. In December, 2013, we performed a coordinated human, animal and environmental investigation in Community A to determine the extent and epidemiology of *C. burnetii* infection. Community members were offered *C. burnetii* serologic testing and a standardized interview. A case was defined as a *C. burnetii* phase II IgG titer ≥1:128 in a person linked to Community A since June 1, 2013. Representative milk samples from the goat and cattle herds, vaginal swabs from peri-parturient animals, and environmental samples were tested by *C. burnetii* PCR. Of 135 persons interviewed and tested, 47 (35%) human Q fever cases were identified. Both cattle and goat samples were *C. burnetii* PCR positive, although goat samples were more frequently positive (17% vs. 2% of milk samples, and 26% vs. 7% vaginal swabs, respectively). Of environmental samples, *C. burnetii* was detected at highest levels in the goat birthing areas. The risk of Q fever was 2.7 times greater (95% CI: 1.3-5.3) for persons with livestock or manure contact compared to those without. Among persons without livestock exposure, having a household member with regular livestock contact was associated with 4.8 times greater Q fever risk (95% CI: 1.1-20.7). This is the largest human Q fever outbreak reported to date in the U.S. Contact with or proximity to goats and cattle was a significant risk factor for infection, and the possibility of fomite transmission to household members warrants further evaluation. A One Health approach incorporating education and modifications to husbandry practices was recommended to reduce potential morbidity and mortality and prevent future *C. burnetii* transmission.

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RISK FACTORS ASSOCIATED WITH HUMAN MONKEYPOX IN THE DEMOCRATIC REPUBLIC OF CONGO

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Monkeypox (MPX), a zoonotic orthopoxvirus causes a serious, smallpox-like illness in humans. Much remains unknown about the risk factors associated with this disease; to-date, no large-scale case-control studies have been conducted. Between 2007 and 2009, two studies were conducted concurrently in the Sankuru District of the Democratic Republic of the Congo (DRC). The first study aimed to collect data through a population-based active disease surveillance program to improve disease

surveillance activities under the Ministry of Health and gain insight into transmission dynamics associated with MPX disease. The second study, a population-based serological survey, collected health behavior information from healthy participants in the same geographic region. Both studies collected information on demographic, health, and animal exposure data as well as tissue samples from all active cases and blood samples from healthy participants. We subsequently preformed a matched case-control analysis using these two similarly collected datasets to identify potential risk factors for human MPX in the DRC. Samples were collected from 595 suspected MPX patients identified through active surveillance and from 2,345 healthy persons enrolled in the population-based study. Suspected cases of human MPX were investigated based on the presence of monkeypox-like lesions and confirmed with real time Polymerase Chain Reaction (PCR) using vesicle fluid or scabs; controls were chosen based on the absence of both skin lesions and specific MPX antibodies in serum by laboratory diagnosis. 390 MPX-positive cases were included in the analysis, stratified by the presumed source of infection (animal (n=252) or human (n=138) source), and were matched to a total of 653 MPX-negative controls based on sex and age categories. Initial findings from univariate and multivariate analysis suggest individuals with evidence of smallpox vaccination are at a reduced risk for MPX infection (adjusted OR (aOR): 0.12, 95% C.I: 0.03, 0.56) while exposures to animals including Gambian rats (aOR: 2.58, 95% C.I: 1.63, 4.07), large terrestrial rodents (aOR: 1.80, 95% C.I: 1.08, 2.98), prosimiens (aOR: 1.85, 95% C.I: 1.24, 2.75), and non-human primates (aOR: 2.66, 95% C.I: 1.44, 4.90), may be associated with an increased risk of MPX infection, particularly in unvaccinated populations.

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SOUTH AMERICAN EASTERN EQUINE ENCEPHALITIS: POSSIBLE ENZOOTIC HOSTS, HUMAN EPIDEMIOLOGY AND ASSOCIATIONS WITH LAND USE

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South American eastern equine encephalitis virus (SA EEEV, recently reclassified into the species *Madariaga virus*) had not been associated with significant human disease, unlike its North American counterpart, until a 2010 Panama outbreak. SA EEEV is an alphavirus (family *Togaviridae*) transmitted by *Culex* mosquitoes (subgenus *Melanoconion*). Since transmission and epidemiology of SA EEEV is not well understood, we conducted a serosurvey of small mammals and birds, coupled with a human serosurvey. We hypothesized that SA EEEV seroprevalence would be higher in agricultural areas compared to forested areas, mediated by habitat preferences of generalist species likely to serve as enzootic hosts. From January to December 2012, rodents and marsupials were trapped in 42 locations (village, cultivated fields, pasture, shrub, forest; 12,005 trap nights, n=556 rodents; n=20 marsupials). Mist nets were also placed at night for bat trapping (n=32), and bird samples obtained during a prior field effort were tested (n=162). During this period, 776 people from 3 population centers were surveyed. Sera were tested for SA EEEV and the closely related and endemic Venezuelan equine encephalitis virus (VEEV), by IgG ELISA and plaque reduction neutralization tests (PRNT), and mammal spleens were tested for virus by RT-PCR. We found that the short-tailed cane mouse (*Zygodontomys brevicauda*), a generalist species, had the highest overall EEEV seroprevalence (8.3%), and was the most numerous rodent species trapped (n=229). The abundance of this species was highly associated with cultivated fields and pasture. The abundance of EEEV-positive animals was greater for sites with high rodent diversity (using Shannon-Wiener and Simpson's indices). The overall human SA EEEV seroprevalence was 4.8%, with no age trend, suggesting that SA EEEV exposure may be recent, consistent with a lack of seroprevalence in past Panamanian surveys. The sites with highest abundance of EEE positive animals did not coincide, however, with highest seroprevalence in humans. Since human VEE seropositivity in these sites was as high as 78%, we hypothesize that this geographical discrepancy may be due to cross-protective immunity. Future studies to further clarify the impact of land use change on EEEV transmission are necessary to guide public health policy in Central and South American countries.

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CANINE RABIES VACCINATION TO PREVENT HUMAN RABIES IN RURAL TANZANIA

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The annual mortality rate of human rabies in rural Africa is 3.6 deaths per 100,000 individuals. Rabies can be prevented by prompt post-exposure prophylaxis, but this is costly and often inaccessible in rural Africa. As 99% of human exposures occur through rabid dogs, canine vaccination also prevents transmission of rabies to humans. We evaluate the cost-effectiveness of rabies control through annual canine vaccination campaigns in two districts of rural Tanzania, Ngorongoro and Serengeti, using a dynamic model of transmission in both dogs and wildlife and incorporating empirical uncertainty in the biological parameters to make probability-based evaluations of cost-effectiveness. We find that annual canine vaccination campaigns are very cost-effective in both districts compared with no canine vaccination. In Serengeti, annual campaigns up to 70% coverage are cost-saving. Across a wide-range of parameter assumptions and levels of societal willingness-to-pay for life-years, vaccination campaigns are always cost-effective and life-saving, and therefore preferred.

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CROSS-SPECIES TRANSMISSION OF TAXONOMICALLY DIVERSE PATHOGENS IN A COMMUNITY OF WILD PRIMATES

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Viruses are considered likely to "jump" species barriers due to their high genetic variability and adaptive potential, whereas more genomically complex pathogens are considered less likely to cross species barriers. We evaluated this hypothesis by examining a taxonomically diverse set of pathogens in a community of wild primates in Uganda. Metagenomic analyses of blood plasma identified approximately 12 novel RNA viruses of the families Retroviridae, Arteriviridae, and Flaviviridae. These viruses were highly diverse within primate species (up to approximately 13% sequence divergence) and displayed high levels of intra-host genetic variation (up

to approximately 2% nucleotide diversity), but all were restricted to hosts of a single species. Apicomplexan parasites of the genus *Hepatocystis*, related to *Plasmodium*, were detected using microscopy and PCR. "Deep sequencing" of the parasite cytochrome *b* gene showed one *Hepatocystis* lineage to be transmitted frequently between red colobus monkeys (*Procolobus rufomitratus*) and olive baboons (*Papio anubis*), whereas the remaining five lineages infected hosts of a single species. Analyses of fecal samples revealed helminth parasites of the genera *Oesophogostomum* (nodule worms) and *Trichuris* (whipworms), which were further characterized by PCR and sequencing of the internal transcribed spacer region of the ribosomal DNA complex. Both parasite genera contained multiple cryptic lineages, some representing putative novel taxa, and several infecting primates of multiple species, including humans. Collectively, these results suggest that pathogens with highly variable and mutable genomes (e.g. RNA viruses) are not necessarily more likely to cross species barriers than more genomically complex pathogens (e.g. protozoa and helminths), in certain ecological settings. Efforts to prevent zoonotic transmission in such settings should focus on common transmission pathways inferred from empirical studies of a range of pathogen taxa, rather than on specific classes of pathogens assumed to have high cross-species transmission potential.

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SAFETY AND IMMUNOGENICITY OF RTS,S/AS01 MALARIA VACCINE CANDIDATE IN HIV INFECTED INFANTS AND CHILDREN: A PHASE III RANDOMIZED, DOUBLE-BLIND, CONTROLLED TRIAL

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Malaria and HIV remain important global health problems. The RTS,S/AS01 malaria vaccine candidate, which showed protection against malaria with a favorable safety and immunogenicity profile during trials in sub-Saharan Africa, had previously not been evaluated specifically in HIV-infected participants. We measured safety and immunogenicity in children with WHO stage 1 or 2 HIV disease at two centers in Kenya (NCT01148459). Children aged 6 weeks to 17 months were randomized 1:1 to receive 3 doses of RTS,S/AS01 or rabies vaccine, administered monthly. The primary objective was occurrence of serious adverse events (SAEs) from first vaccination until 14 months post-dose 1. A secondary objective, incident clinical malaria, was captured by passive case detection. Baseline characteristics were similar between study arms. Out of 200 children enrolled, 177 (89%) completed follow-up. The frequency of SAEs was 41.4% (95%CI:31.6-51.8) among RTS,S/AS01 recipients and 36.6% (95%CI:27.3-46.8) among rabies vaccine recipients. At least one SAE was reported within 30 days of vaccination in 20.2% (95%CI:12.8-29.5) of RTS,S/AS01 recipients and 11.9% (95%CI:6.3-19.8) of rabies vaccine recipients. At least one episode of pneumonia occurred in 13/99 (13.1%) RTS,S/AS01 recipients compared to 5/101 (5.0%) rabies vaccine recipients within 30 days post-vaccination. Fatal SAEs occurred in 5.1% (95%CI:1.7-11.4) of RTS,S/AS01 recipients and 4.0% (95%CI:1.1-9.8) of rabies vaccine recipients. One related SAE was reported: a febrile convulsion in an RTS,S/AS01 recipient. Solicited adverse event frequency 7 days post-vaccination was: injection site pain 18.1% and 6%, fever 41.7% and 18.8%, irritability 25.3% and 10.7%, and loss of appetite 17.7% and 8.7% in the RTS,S/AS01 and rabies vaccine group respectively. No evidence of differential HIV disease progression (CD4+, HIV viral load and WHO HIV Clinical Staging) was seen between study arms. In the RTS,S arm, anti-CS antibody geometric mean titer (EU/mL) was 0.3 (95%CI:0.3-0.4), 329.2 (95%CI:260.6-415.8) and 18.4 (95%CI:13.3-25.5) at baseline, one and 12 months post-dose 3. Vaccine efficacy against first episode of clinical malaria was 31% (95%CI:-19-60). RTS,S/AS01 showed no serious safety concerns and was immunogenic in HIV-infected children. When considered with efficacy data, HIV-infected children need not be excluded from potential future vaccination programs with RTS,S/AS01.

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MATHEMATICAL MODELING OF THE SITE-SPECIFIC IMPACT OF THE RTS,S VACCINE UNDER MULTIPLE ROLL-OUT SCENARIOS

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Vaccines against malaria are one promising avenue for reducing the global burden of the disease beyond what has been achieved with current interventions. The pre-erythrocytic vaccine RTS,S is the candidate furthest along the development pipeline, and as such there is great interest in the potential impact of a large-scale roll-out. Here, we addressed this question by using the previously developed EMOD model of malaria to simulate the outcome of such a campaign. The central model is a mathematical description of the vector life cycle coupled to within-host parasite and immune dynamics. To study RTS,S, we incorporated a representation of the vaccine, characterized by an initial efficacy against infection and a half-life of protection. An iterative stochastic approach was used to optimize the modeled vaccine properties by sampling parameter space and evaluating a likelihood function that assesses the fit between the simulations and clinical trial data. Having obtained a calibrated model of RTS,S, we subsequently explored a variety of different roll-out scenarios to test the effects of policy choices (e.g., age of first administration, booster inclusion) and deployment setting characteristics (e.g., demographics, seasonality). Additionally, sensitivity analyses were performed on other axes, such as coverage level and treatment-seeking behavior, quantities that are at once difficult to measure and highly relevant to the success of a campaign. Simulated clinical and severe incidences were incorporated into a cost analysis in order to establish the incremental impact of the vaccine over other interventions, such as insecticide-treated nets (ITNs). We concluded that the cost effectiveness of population-wide infant vaccination is greatest in regions of moderate endemicity, though the specifics depend on a number of factors, including pre-existing ITN coverage and local anopheline feeding preferences.

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COMPARISON OF ANTI-PFS25 ANTIBODY RESPONSES FOLLOWING VACCINATION WITH PFS25-EPA/ALHYDROGEL® IN A MALARIA NAÏVE AND MALARIA EXPOSED POPULATION

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A vaccine to interrupt malaria transmission would be a valuable tool for local elimination or eradication of malaria. Phase 1 clinical trials to assess the safety and immunogenicity of the *Plasmodium falciparum* transmission blocking vaccine candidate Pfs25-EPA/Alhydrogel® have been conducted in malaria naïve adults in the US and in malaria-exposed adults in Mali. The US Phase 1 study was an open label dose escalating study in which five volunteers (safety dose of 8 µg Pfs25-EPA/Alhydrogel®) received 2 doses, five volunteers (low dose of 16 µg Pfs25-EPA/Alhydrogel®) received 2 doses (Days 0, 56) and 20 volunteers (high dose of 47 µg Pfs25-EPA/Alhydrogel®) received 4 doses (Days 0, 56, 120, 300). The Mali Phase 1 study enrolled 120 volunteers in the double blind randomized control study: 20 volunteers (low dose of 16µg Pfs25-EPA/Alhydrogel® or control) received 2 doses (Days 0, 56) and 100 volunteers (high dose of 47µg Pfs25-EPA/Alhydrogel® or control) have thus far received 3 doses (Days 0, 56, 112) with a scheduled fourth vaccination on Day 480. Vaccinations were well tolerated in both populations. Specific anti-Pfs25 antibodies were detected by ELISA in sera from subjects receiving two or three doses of Pfs25-EPA/Alhydrogel®. The anti-Pfs25 antibody results obtained following three vaccinations in the high dose (47 µg) Pfs25-EPA/Alhydrogel® group in the US are comparable to the results seen following three vaccinations in the high dose (47 µg) Pfs25-EPA/Alhydrogel® group in Mali. The antibody response at each vaccine dose increased with each subsequent dose of vaccine given but diminished quickly following vaccination. The percentage of responders with anti-Pfs25 antibody responses at levels ≥546 units, which was the anti-Pfs25 antibody level that had previously been seen to have functional activity in the US study, was the same between the two studies following the second and third vaccination in the high dose groups. Anti-Pfs25 antibody response results following the fourth vaccination in the Mali study and associated functional activity will be available in October 2014. Overall, Pfs25-EPA/Alhydrogel® transmission blocking vaccine has been well tolerated and produced significant antibody responses in a malaria naïve and malaria exposed adult populations.

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ASSESSMENT OF SAFETY AND IMMUNOGENICITY OF INTRAVENOUS IMMUNIZATION WITH RADIATION ATTENUATED *PLASMODIUM FALCIPARUM* NF54 SPOROZOITES (PFSPZ VACCINE) IN HEALTHY AFRICAN ADULTS

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For decades it has been known that humans can be protected against malaria by repeated immunization with radiation-attenuated *Plasmodium falciparum* (Pf) sporozoites (SPZ). Traditionally, those PfSPZ have been administered by exposing vaccinees to the bites of >1,000 PfSPZ-infected, irradiated mosquitoes. Recently, a process for manufacturing aseptic, purified, radiation attenuated cryopreserved PfSPZ has been developed. When administered by IV injection, this product, Sanaria® PfSPZ Vaccine, induced sterile protection against controlled human malaria infection in 6/6 malaria naïve adults who received the highest dosage. To begin the process of assessing the vaccine in Africa, we are conducting a double blind, randomized, controlled Phase 1 clinical trial to assess PfSPZ Vaccine's safety, immunogenicity, and protective efficacy against naturally occurring malaria infection in healthy, malaria exposed, 18-35 years old Malians. Among the 105 volunteers vaccinated by direct venous inoculation (DVI), 12 volunteers (pilot safety group) have received 2 doses of PfSPZ Vaccine (Day 0: $1.35x10^5$ and Day 14: $2.7x10^5$ PfSPZ) and 93 volunteers will have received 5 doses of $2.7x10^5$ PfSPZ or normal saline placebo (Day 0, 28, 56, 84, 140; total dosage: $13.5x10^5$ PfSPZ) by the end of July 2014. Nine subjects in the pilot group will join the larger group to receive an additional 3 vaccinations (total dosage: $12.15x10^5$ PfSPZ). The incidence and severity of local and systemic adverse events occurring within 7 days after dose are being recorded. During the malaria transmission season all volunteers will be examined every 14 days and as clinically indicated for blood-stage parasitemia by microscopy. All volunteers have been immunized twice. Immunizations have been well tolerated with no local reactogenicity, minimal mild to moderate systemic reactogenicity, no serious adverse events, and no patent parasitemia. These early results show PfSPZ Vaccine administered via DVI is safe and well tolerated in healthy malaria-exposed, African adults. Results of all immunizations will be presented.

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ASSESSING EFFICACY OF THE PFSPZ VACCINE BY CONTROLLED HUMAN MALARIA INFECTION IN TANZANIA

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The rich potential for African scientists to spearhead phase 1 studies that have a controlled protection component has not been tapped because controlled human malaria infections (CHMI) have been available only at centers in the USA and Europe with access to infected mosquitoes. Sanaria has developed aseptic, purified, cryopreserved infectious *Plasmodium falciparum* (Pf) sporozoites (SPZ) (PfSPZ Challenge) manufactured in compliance with cGMPs, suitable for parenteral injection and shippable to any location. PfSPZ Challenge has been tested in the Netherlands, US, UK, Tanzania, Kenya, Germany and Spain using different dosages and inoculation routes. 3,200 PfSPZ administered by direct venous inoculation (DVI) reproduces the 100% infection rate and 11-11.5 day pre-patent period of 5 mosquito bite CHMI. In the present, first of its kind study, we will use PfSPZ Challenge to test the efficacy of Sanaria's radiation attenuated, non-replicating PfSPZ Vaccine against CHMI administered by DVI. PfSPZ Vaccine showed 100% efficacy in a recent study by the Vaccine Research Center (VRC), NIAID, NIH in the study group receiving the highest dose tested (5 doses of 1.35x10^5 PfSPZ by DVI). The Bagamoyo Clinical Trial Unit, Ifakara Health Institute, is now comparing the same regimen shown to be 100% protective at VRC, with a regimen that delivers twice the dose (2.7x10^5 PfSPZ) at each of the 5 time points, in minimally malaria-exposed Tanzanian adults. Both groups will be assessed for protection against CHMI using PfSPZ Challenge administered by DVI at 3 and 24 weeks after the 5th dose (40 immunized and 8 control subjects). An additional 6 volunteers will receive the higher dose and undergo CHMI only at 24 weeks. We will present safety, tolerability, and preliminary immunogenicity data for this unique study that is opening an exciting pathway for malaria vaccine and drug research and development in Africa and the world.

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POTENT CELLULAR AND HUMORAL IMMUNOGENICITY OF CHAD63 MVA ME-TRAP IN AFRICAN INFANTS AND CHILDREN

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Vaccination is one of the most cost-effective health care interventions available and an effective malaria vaccine against *Plasmodium falciparum* could save more than half a million lives each year. We developed a prime-boost immunisation approach employing the viral vectors ChAd63 and MVA, both encoding the pre-erythrocytic malaria antigen TRAP. Previous studies with ChAd63 MVA ME-TRAP have shown excellent immunogenicity and significant efficacy in adults with protection correlated with frequency of mono-functional $CD8^+$ T cells secreting IFNγ. We report here T cell phenotypes and antibody titres measured in 138 children vaccinated during three Phase I dose-finding and age de-escalation studies to assess safety and immunogenicity of the ChAd63 MVA ME-TRAP vaccine in malaria-exposed children in The Gambia and Burkina Faso. Age groups at first immunisation were 2-6 years, 5-12 months and 10 weeks in The Gambia and 5-17 months in Burkina Faso. IFNγ ELISPOT responses to TRAP were significantly lower in 2-6 year old and 5-12 month old children in The Gambia and 5-17 month old children in Burkina Faso than in malaria-naïve UK adults receiving the same vaccines. Immunogenicity in 10 week-old infants in The Gambia was high and comparable to that in adults. Flow cytometry revealed IFNγ secretion from both $CD4^+$ and $CD8^+$ T cells with IL-2 and TNFα also detected from $CD8^+$ T cells. Anti-TRAP IgG responses varied by age group and dose, with significantly higher titres detected after boosting in vaccinees primed with a higher dose of ChAd63 ME-TRAP. Titres were also significantly higher in 5-12 month and 10 week old children than 2-6 year-old children in The Gambia that received the same dose. IgG titres in 5-17 month-old children in Burkina Faso were comparable to those in 5-12 month old children in The Gambia. IgG responses were predominantly composed of IgG1 and IgG3 isotypes and we also detected IgA and IgM responses. We demonstrate excellent cellular and humoral immunogenicity of a pre-erythrocytic malaria vaccine in key target populations for vaccine deployment.

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SAFETY AND IMMUNOGENICITY OF THE BLOOD-STAGE *PLASMODIUM VIVAX* VACCINE CHAD63-MVA PVDBP

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There has been comparatively little research into vaccines for *Plasmodium vivax* in the past, with only two antigens previously reaching Phase Ia

clinical trials. More recently, the importance of a vaccine for *P. vivax* has been recognised and included in the 2013 update to the Malaria Vaccine Technology Roadmap. Here we report on the first blood-stage *P. vivax* vaccine Phase Ia clinical trial. This has been carried out in Oxford using recombinant simian adenovirus ChAd63 and the poxvirus MVA encoding the *P. vivax* antigen PvDBP (Duffy-binding protein region II) in a heterologous prime-boost regimen. The *P. vivax* parasite requires an interaction between the Duffy-binding protein ligand and its host receptor, the Duffy antigen receptor for chemokines (DARC), in order to invade reticulocytes, making PvDBP a promising antigen for vaccine development. Twenty-three healthy volunteers have been vaccinated and followed up in this Phase Ia dose escalation study of ChAd63-MVA PvDBP. The ChAd63 PvDBP priming vaccine was initially given alone to 4 volunteers at a dose of 5×10^9 vp before being increased to 5×10^{10} vp following a planned safety review. MVA PvDBP has been given to 15 volunteers 8 weeks after ChAd63 PvDBP prime at doses of 1×10^8 pfu – 2×10^8 pfu. The primary objective of the study was safety, and the vaccines have been well tolerated with no serious adverse events. Follow-up of volunteers will be complete at the end of June 2014. The secondary objective was humoral and cellular immunogenicity which has been assessed using assays including PvDBP IFN-γ T cell ELISPOT, B cell ELISPOT, PvDBP IgG antibody ELISA and functional antibody analysis. The vaccine regimen is immunogenic, with increases in the cellular and humoral responses seen after MVA PvDBP boost (compared with ChAd63 PvDBP alone). This is the first vaccine against PvDBP to be assessed in humans, and has shown a favourable safety profile and promising levels of immunogenicity which support onward clinical development towards proof-of-concept efficacy testing.

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ELQ-300 PRODRUGS FOR ENHANCED DELIVERY AND EFFICACY AGAINST MALARIA

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ELQ-300 is a preclinical candidate of the Medicines for Malaria Venture that targets the liver and blood stages of malaria, as well as the forms that are crucial to transmission of falciparum malaria: gametocytes, zygotes, and ookinetes. In mouse models of the disease, a single oral dose of 0.03 mg/kg prevented sporozoite induced infections while 4 daily doses of 1 mg/kg achieved complete cures of patent infections. A significant obstacle to the clinical development of ELQ-300 relates to its physical-chemical properties. Its relatively poor water solubility and high crystallinity limits absorption to the degree that only low bloodstream concentrations can be achieved by oral dosing. While these low bloodstream concentrations are sufficient for therapy the levels are too low to establish an acceptable safety margin required by regulatory agencies for clinical development. One way to address the challenging physical-chemical properties of ELQ-300 is through the development of prodrugs. In this presentation we will profile a series of ELQ-300 prodrugs, focusing primarily on the bioreversible nature of ELQ-337. At the equivalent dose of 3 mg/kg the delivery of ELQ-300 from ELQ-337 is enhanced by 4-5-fold, reaching a C_{max} of 6.9 micromolar by 4 hrs after oral administration. The superior *in vivo* efficacy of this compound will be discussed. Apart from highlighting the outstanding *in vivo* efficacy of ELQ-337, this data demonstrates that the prodrug strategy represents a viable approach to overcome the physical-chemical limitations of ELQ-300 to deliver the active drug to the bloodstream at high enough concentrations sufficient for safety and toxicology studies as well as achieving single dose cures.

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UNDERSTANDING THE MECHANISM FOR EFFICACY AND TOXICITY OF 8-AMINOQUINOLINE ANTIMALARIALS: *IN VITRO* AND *IN VIVO* STUDIES WITH HYDROXYLATED METABOLITES OF PRIMAQUINE

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Hydroxylated metabolites are presumed to be responsible for toxicity and/or efficacy of primaquine and other 8-aminoquinoline antimalarials. However, no definitive data are available on pharmacokinetics, pharmacodynamics and *in vivo* plasma/tissue profiles of the hydroxylated metabolites. Recent *in vitro* studies with primary human hepatocytes and application of stable isotope (13C) labeled PQ provided evidences for generation of hydroxylated metabolites presumably through CYP mediated pathways. Previous studies by us have shown that 5-hydroxyprimaquine (5-HPQ) and 6-Methoxy 8-N-Hydroxy aminoquinoline (MHQ) generated robust methemoglobin formation and oxidative stress in normal and G6PD deficient human erythrocytes. Both 5-HPQ and MHQ produced selective depletion of GSH in G6PD deficient erythrocytes. The studies were extended to include 2-, 3-, 4- and 8-N-hydroxyprimaquine (8-NHPQ). Both 2-HPQ and 4-HPQ are relatively stable analogs. 2-HPQ and 4-HPQ did not generate significant methemoglobin and only marginal ROS in normal and G6PDD RBCs with & without microsomes. Neither metabolite showed activity *in vivo* in the blood stage *Plasmodium berghei* mouse malaria model (i.v. route of administration). Until recently, no definite evidences have been available regarding generation of 3-HPQ and/or 8-NHPQ *in vivo* & *in vitro*. 3-HPQ can be further metabolized with pooled human liver microsomes to generate more toxic/reactive species. 8-NHPQ is a reactive metabolite, which generates significant methemoglobin and oxidative stress. Among 2-, 3-, 4- HPQ and 8-NHPQ, none affected GSH levels in normal or G6PD-deficient human erythrocytes. Formation of 2- and 3-HPQ metabolites was confirmed *in vitro* on incubation of PQ with recombinant human CYP2D6; another HPQ, perhaps 4-HPQ is also formed. However, 2-HPQ, 3-HPQ and 4-HPQ metabolites appear not to be responsible for toxicity/efficacy of PQ.

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A PHASE I/IB STUDY TO INVESTIGATE THE SAFETY, TOLERABILITY AND PHARMACOKINETIC PROFILE OF DSM265 IN HEALTHY SUBJECTS AND THEN ITS ANTIMALARIAL ACTIVITY IN INDUCED BLOOD STAGE *PLASMODIUM FALCIPARUM* INFECTION

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DSM265 is a new antimalarial that targets the malaria parasite's pyrimidine biosynthetic enzyme dihydrootate dehydrogenase (DHODH). A Phase I/Ib study was designed to collect safety, PK and efficacy data. It was undertaken in two parts: the first comprised a single ascending dose (SAD) design to assess safety, pharmacokinetics, and the maximum tolerated dose. Embedded within this first part was an assessment of food effect. The second part comprised exploration of the antimalarial activity of

DSM265 in a single-dose cohort using the induced blood-stage malaria (IBSM) system at a dose guided by data from preclinical efficacy prediction and emerging PK data. To date, 5 cohorts comprising 49 volunteers have participated in the SAD up to 400mg, study of food effect, or the IBSM phase. To date, no significant drug-related clinical or laboratory toxicity have been observed. Fitting of a population PK model to the data indicates mono-exponential disposition, with a blood clearance/f of 0.45 L/hr (95%CI: 0.41-0.50), and an elimination t1/2 of 91 hrs. DSM265 shows dose proportionality, both in terms of Cmax and AUCinf. No food effect was observed. A dose of 150 mg was selected for the IBSM phase by combining the MPC predicted from preclinical data (0.5-1 µg/mL) with the human PK, aiming to observe parasite clearance kinetics, including recrudescence. When tested in the IBSM system in 7 healthy volunteers, the drug showed encouraging antimalarial activity with 99.9% parasite clearance by 130 hrs, with recrudescent infection observed in 5/7 subjects between 10 and 21 days after drug administration. No mutations in the DHODH gene were observed in recrudescent parasites. Fitting of a population PK/PD model to the parasitemia data led to an estimate of the MPC of 954 ng/mL (95%CI: 678-1267). Population estimates of PRR and parasitemia t1/2 were 2.2 (95%CI: 2.0-2.7) and 6.6 hrs (95%CI: 5.4 - 7.2) respectively. These results demonstrate that it is possible to obtain well characterised efficacy data early in the clinical development of an anti-malarial. The combination of early safety, PK, and efficacy data with the PK/PD model mean that future clinical trials of DSM265 will benefit by being smaller and focused towards confirming the observed efficacy. The observed duration of action of DSM265 suggests its promise as a component of a new combination single-dose treatment for malaria.

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TARGETING DRUG RESISTANCE: HARNESSING EVOLUTIONARY FITNESS IN *PLASMODIUM FALCIPARUM* FOR DRUG DISCOVERY

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Management of drug resistance with combination therapies is key to prolonging the effectiveness of new antimalarials. Typical combination therapies pair drugs that are each effective as monotherapies and employ differing binding sites. Pairing drugs that target independent sites presents the opportunity for the parasite to individually develop resistance to each drug, rendering the combination ineffective. Here we explore new kinds of antimalarial combination therapies where the changes giving rise to resistance become the target for the second drug. This approach incorporates built-in protection for the partner drug from development of resistance. In the wild-type organism the partner drug has little or no effect and the organism is not subject to selective pressure to develop resistance. When resistant mutants to the first drug occur then, and only then, does the second drug act, greatly diminishing the population subject to selection. In the event that mutations occur conveying resistance to the second drug, those mutations will drive the organism back towards sensitivity to the first drug. We have taken a systematic approach in the study of resistant organisms and their unique susceptibilities. We screened the Malaria Box against a panel of mutant parasites with well-defined resistance mechanisms to identify compounds with differential activity between mutant and wild-type parasites. We show that a significant percentage of the Malaria Box targets one of the pathways tested. In particular, we find a number of compounds with negative cross-resistance, representing promising leads in pursuing inhibitor pairs as described above. Importantly, multiple chemotypes inhibit each target, indicating there are overlapping modes of action in the parasite despite broad chemotypic diversity in the compound set. This suggests the parasite has a limited number of chokepoints that can be exploited in drug development making it all the more critical to develop a means of protecting the long-term efficacy of compounds in the pipeline with combination strategies as discussed.

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NON-INFERIORITY CLINICAL TRIAL COMPARING FIXED-DOSE COMBINATION AND CHLOROQUINE FOR *PLASMODIUM VIVAX* UNCOMPLICATED INFECTION IN THE BRAZILIAN AMAZON

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In Latin America, *Plasmodium vivax* is responsible for around 80% of malaria episodes. As Chloroquine (CQ) efficacy decreases, alternative therapies such as artemisinin-based combinations recommended by WHO must be evaluated. This phase III, noninferiority randomized trial, compared the antischizontocidal efficacy and safety of a 3 days supervised treatment of the fixed-dose artesunate-amodiaquine Winthrop® (ASAQ) versus CQ for the treatment of uncomplicated P.vivax infection in children above 6 months-old and adults. Primary endpoint was the efficacy rate at day 28. Patients were followed-up until day 42. 380 patients were included in Manaus, Brazil. Baseline characteristics and dropout rates were similar between arms. In Per-Protocol analysis, adequate clinical and parasitological response (ACPR) at day 28 was achieved in 100% and 92.4% of patients in the ASAQ and CQ arm (93.7% versus 89.5% in the Intention-To-Treat analysis). Non-inferiority was achieved in both populations and subsequent bilateral tests concluded in superiority of ASAQ (p=0.001% and < 0.001% in ITT and PP analysis respectively). Clearance of parasite from D1 to D3 was observed in significantly more patients (p<0.001) and significantly more patients were afebrile at D1 (p=0.001) in the ASAQ group. A significant reduction in the number of gametocyte carriers was also observed in the ASAQ group versus CQ group during follow-up. A significant difference in the proportion of recurrence during the 42 days of follow-up was also observed (26.5% versus 3.9% in the CQ versus ASAQ groups, p<0.001). 79% of recurrences in the CQ arm, and all in the ASAQ, occurred after D28. The occurrence of emergent adverse events (AEs) was similar in both arms (CQ=115; ASAQ=110), with no serious AE in the CQ and 5 (related to 3 patients) in the ASAQ arms. ASAQ is a safe and efficacious alternative to treat uncomplicated *P. vivax* infection. 42-days-follow-up enables better assessment of CQ efficacy. Microsatellite genotyping correction and CQ levels results will be presented.

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QTC PROLONGATION AND DRUG SAFETY OF AN INCREASED DOSE OF DIHYDROARTEMISININ-PIPERAQUINE IN YOUNG CHILDREN 5-24 KG IN MALAWI

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Dihydroartemisinin-piperaquine (DHA-PPQ) is recommended for the treatment of uncomplicated *falciparum* malaria and is being explored as mass drug administration to reduce transmission. Recent pharmacokinetic (PK) studies and a pooled meta-analysis of individual patient efficacy data by the WorldWide Antimalarial Resistance Network have raised concerns

about potential under-dosing of young children with current weight-based dose recommendations and advocated higher dosing regimens in this vulnerable population. In contrast, the European Medicines Agency has called for more data to substantiate the cardiac safety of DHA-PPQ and its effect on QTc intervals. We conducted an open-label dose optimization study aimed at describing the population PK profile of PPQ and the tolerability of a higher dose regimen of DHA-PPQ in children of 5–24 kg with uncomplicated *falciparum* malaria [PACTR201303000506302]. In the first step of the study, 100 children received a dosing regimen based on supervised treatment with doses of 1.7–3.8 mg/kg DHA and 13.6–30.0 mg/kg PPQ given once daily over three days, using whole or half-tablets (20/160 mg and 40/320 mg DHA/PPQ tablets); children were followed-up for 63 days. In the second step 100 children received a more pragmatic regimen using whole tablets with wider ranging doses of 2.0–4.0 mg/kg DHA and 16.2–32.0 mg/kg PPQ. QTc was measured 4–6 h after the last DHA-PPQ dose and compared to baseline and Day 28 using digital 12-lead electrocardiograms and the vertical caliper on median overlapped template technique. We will present findings from the first step. Findings from the second step will be presented, dependent on study progress. Optimising the weight-based dose regimen for DHA-PPQ in the main high-risk group of young children ahead of its roll-out into control programmes in sub-Saharan Africa will help inform the translation of dosing recommendations into programmatically feasible, user-friendly, safe weight- and age-based dosing regimens.

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SCHEDULED SCREENING VERSUS PREVENTIVE TREATMENT FOR THE CONTROL OF MALARIA IN PREGNANCY IN MALAWI: A RANDOMIZED CONTROLLED TRIAL

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The decline in effectiveness of intermittent preventive treatment in pregnancy with SP (IPTp-SP) due to high levels of sulphadoxine-pyrimethamine (SP) resistance by *Plasmodium falciparum* malaria parasites necessitates urgent alternative approaches for malaria control in pregnancy. The objectives of this study is to determine whether scheduled intermittent screening with a malaria rapid diagnostic test (mRDT) and treatment of mRDT-positive women with Dihydroartemesinin-Piperaquine (ISTp-DP) from the second trimesters is more efficacious than IPTp-SP in reducing adverse birth outcomes and malaria infection at term among HIV-sero-negative women protected by insecticide-treated bed nets in a malaria endemic setting. This two arm, open label, multicentre, randomized controlled superiority trial was designed to assess a 25% or greater reduction in adverse birth outcome in primi- and secundigravidae and a 25% or greater reduction in placental malaria in multigravidae. Between July, 2011 and March, 2013, a total of 1,155 and 717 women in their first and second pregnancy and third to fifth pregnancy respectively were recruited from 3 sites of perennial malaria transmission with high grade SP resistance and near saturation of the dihydropteroate synthase/dihydrofolate reductase quintuple haplotype. Of 3,214 women screened 1,872 (58.2%) were enrolled. 92% of participants were retained to delivery and 90.5% to study completion.

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ECOLOGICAL GENOMICS AND PLASTICITY OF GENETIC REGULATION FOR SALTWATER TOLERANCE

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Evolution of osmoregulatory systems is a key factor in the transition of species between fresh- and saltwater habitats, including anopheline mosquitoes known for their role as disease vectors. Here we use RNA-Seq to investigate gene expression differences between an obligate freshwater (*Anopheles coluzzii*) and euryhaline malaria vector (*An. merus*). After rearing in freshwater (FW), both young and old larval instars of each species were briefly (6 h) exposed to either saltwater (SW) or FW conditions to test the impact of water salinity on mRNA levels. We aimed to describe the transcriptomic response of anophelines to water salinity, and how the response differs between *An. merus* and *An. coluzzii*. We also tested how the transcriptomic response to water salinity differs with age, particularly for the tolerant species *An. merus*. Our results are congruent with the ability of gene induction to mediate SW tolerance, with the intolerant *An. coluzzii* exhibiting little difference in gene expression (<2% of the transcriptome), in contrast to greater plasticity by *An. merus.* In the latter, >16% of the 11,025 genes assayed responded to saltwater exposure, with similar levels of up- and down-regulation. The impact of age at exposure was less dramatic than species identity, with 567 genes significantly differentially expressed in response to water type between young and old *An. merus*. Besides effector genes with putative roles in ion transport (e.g., Na^+/K^+-ATPase), we also report differential expression in response to water salinity by genes involved in general stress responses such as heat shock proteins, and potential cross-talk between the immune response and osmoregulation. Additionally, we report on a network of 115 co-expressed genes associated with SW tolerance. Finally, we complement our investigation of gene expression with QTL mapping from a backcross of *An. merus* and *An. coluzzii*. We report regions of sequence divergence associated with SW tolerance, and discuss the presence of differentially expressed genes within these regions.

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A COMPARATIVE APPROACH TO IDENTIFY PATHWAYS REGULATING FEMALE REPRODUCTIVE BIOLOGY IN ANOPHELINE MALARIA VECTORS

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Female *Anopheles gambiae* mosquitoes exhibit major behavioral and physiological changes in the first 24 hours after mating, including egg laying and refractoriness to further mating. These post-mating responses are in part mediated by transfer of seminal secretions contained in the mating plug, a complex of proteins, lipids and hormones, formed in the male, and deposited in the female atrium (uterus) during mating. However, not all Anophelines produce and transfer a plug, with this structure absent in the Nyssorhynchus mosquito series (*Anopheles albimanus*). This raises the question of how female post-mating changes are induced in absence of a plug? To address this question, we compared the molecular processes underpinning the mating responses of *An. gambiae* and *An. albimanus* females via RNAseq analysis of the atrium. A significantly larger response was observed in *An. gambiae* after mating (2235 genes, $p<0.05$) than in *An. albimanus* (212). Comparison of seminal secretions from the two species indicates that this >10-fold increase in *An. gambiae* response may be due to the transfer of potent transcriptional regulators. Enrichment analysis (DAVID) identified pathways of epithelial transport and molecule trafficking regulated in both species, suggesting the exchange of material across the atrium epithelium. Among the genes exclusively modulated in *An. gambiae,* were proteins associated with mating plug processing and energy production, suggestive of high-energy demands in mated females,

while in *An. albimanus* enriched processes included chitin metabolism, which may indicate structural changes to the uterus after mating, as well as carboxylesterase activity suggestive of xenobiotic metabolism. This comparison provides important insight into the different pathways shaping female post-mating behavior in *An. gambiae* and *An. albimanus*. Ultimately, a better understanding of Anopheline reproductive biology will aid vector control efforts aimed at reducing mosquito fertility, and may highlight conserved reproductive pathways that could be targeted in all malaria vectors worldwide.

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PFS47 ROLE IN THE ADAPTATION OF *PLASMODIUM FALCIPARUM* TO NEW WORLD ANOPHELINES

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The Pfs47 gene, a member of the 6-cys protein family, is required by *Plasmodium falciparum* to evade the *Anopheles gambiae* immune system. This led us to propose that Pfs47 may be important for the malaria parasite adaptation to different Anopheline mosquitos around the world. Pfs47 is a polymorphic gene with signatures of diversifying selection and a strong geographic genetic structure. The highest genetic diversity of Pfs47 was detected in Africa, and the lowest diversity was detected in the New World with one haplotype. Phylogenetic analysis of Pfs47 haplotype sequences present a geographic structure with most African haplotypes forming a clade separate from most of the Asian alleles. The haplotype found in the New World appears to be more closely related to the African clade. Infection of the New World malaria vector *A. albimanus* with *P. falciparum* was found to be highly dependent on the geographic origin of the parasite strain. The *A. albimanus* mosquito immune system was found to be responsible for the low infectivity found in African *P. falciparum* NF54 infections. Allele replacement showed that a Pfs47 haplotype from the New World can rescue the NF54 parasite infection of *A. albimanus*. This provides evidence that the mosquito immune system can be an important barrier for adaptation of *P. falciparum* to new vectors and that selection of particular Pfs47 haplotypes may be required to overcome this barrier.

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DISCOVERY OF NOVEL LONG NON-CODING RNAS AND PROTEIN-CODING GENES IN *ANOPHELES GAMBIAE* USING DEEP RNA SEQUENCING

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Recently, an expanding class of long non-coding RNAs (lncRNAs) that function in epigenetic regulation, the regulation of gene expression transcriptionally and post-transcriptionally, and the regulation of genomic stability have been described in mammals, Drosophila melanogaster and Caenorhabditis elegans. We have analyzed the transcriptome of the malaria vector *Anopheles gambiae*, based on deep-read RNA sequencing technology that generated over 500,000,000 reads from first and third instar larvae and adult females and males. Our de novo transcriptome assembly, and comparisons with gene models defined in VectorBase annotation Release 3.7, revealed over 1,100 novel lncRNAs and more than 200 previously unannotated putative protein-coding genes. The lncRNAs exhibit differential expression across life stages of the mosquito and display an increased rate of divergence over evolutionary time across the genus *Anopheles* when compared to the newly discovered protein-coding genes and previously annotated protein-coding genes. This initial description of lncRNAs in An. gambiae offers the first [large-scale] insights into non-coding RNAs in this mosquito and defines another potential set of targets for the development of vector-based interventions that may curb the malaria burden in disease-endemic countries.

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THE EXPRESSION OF THE PIRNA PATHWAY GENES, *PIWI, AUBERGINE* AND *ARGONAUT 3*, DURING HEAT AND COLD STRESS RESPONSE IN THE MALARIA MOSQUITO, *ANOPHELES STEPHENSI*

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Stress-induced mobilization of transposons is well-documented in many organisms. An RNA-interference pathway, designated the piRNA pathway, is responsible for repressing transposon mobilization in germ-line tissues of the fruit fly, *Drosophila melanogaster*. The genes encoding the components of the piRNA pathway, *Piwi, Aubergine* (*Aub*) and *Argonaut 3* (*Ago3*), were identified and characterized in the malaria vector mosquito, *Anopheles stephensi*. Preliminary experiments show that they are induced in embryos following heat or cold stress. Current experiments in adult female mosquitoes are designed to assay the effects of heat and cold stress on the expression levels of the mosquito orthologs of the heat-shock protein genes, *hsp70* and *hsp90*, as well as *Piwi, Aub* and *Ago3* and endogenous transposase genes. Additionally, mosquitoes mutant for *Piwi, Aub* and *Ago3* are being generated and will be tested for a phenotype affecting the heat-shock response. The results of this work are expected to inform the development of transposon-based gene-drive systems for introgressing beneficial traits into vector mosquitoes.

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THE ROLE OF INSECT INNATE IMMUNITY IN CONTROLLING *RICKETTSIA TYPHI* INFECTION

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Rickettsia typhi, the causative agent of murine typhus, is an obligate intracytosolic bacterium that is transmitted by the flea. Upon infection, *R. typhi* systemically infects fleas. However, infection level is kept manageable so that flea fitness is unaffected, presumably by the flea innate immune system. Two main arthropod innate immune signaling pathways, the Imd and Toll pathways, respond to Gram-negative bacteria and Gram-positive bacteria/fungi, respectively. Upon activation by the Imd or Toll pathway, Rel/NF-KB transcription factors Relish and Dorsal/DIF increase transcription of antimicrobial peptides. The immune pathways that control *R. typhi* infection in the flea have yet to be elucidated. We hypothesize that the Imd pathway is critical for controlling Gram-negative *R. typhi* burden within the flea. Dissecting the flea immune response to *R. typhi* has been hampered by the lack of a flea genome. Therefore, *R. typhi* infection was modeled in *Drosophila* S2R+ cells. *R. typhi* infected and replicated within S2R+ cells. To identify which innate immune signaling pathway(s) is critical for controlling *R. typhi* burden, S2R+ cells were treated with dsRNA to knockdown either the Imd or Toll pathway. Knockdown of negative regulators *caspar* and *cactus*, of the Imd and Toll pathways, decreased *R. typhi* burden suggesting both the Imd and Toll pathways control *R. typhi* burden. Knockdown of the NF-KB factor *Relish* but not *Dorsal* increased *R. typhi* burden, further confirming the role of the Imd pathway in control of *R. typhi* burden. More extensive screens will provide further evidence of which pathway(s) is controlling *R. typhi* burden. Additionally, a flea dorsal-like sequence has been identified and we are working to identify a flea relish-like sequence in hopes of parlaying the knowledge garnered from the *Drosophila* model into the flea. Identification of immune pathways in the flea and understanding the flea innate immune response to *R. typhi* could potentially lead to new treatment modalities that will decrease transmission and burden in the flea.

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SIRNA NANOPARTICLE-MEDIATED TARGETING OF DOUBLESEX, A REGULATOR OF SEX-SPECIFIC DEVELOPMENT IN *AEDES AEGYPTI*

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Sexually dimorphic behaviors, including blood feeding and other sex-specific behaviors linked to reproduction, contribute to the global spread of mosquito-borne illnesses. Exploration of the developmental genetic basis for sexual dimorphism in mosquitoes has been hampered by a lack of methods to pursue functional developmental genetic studies. Although male and female splice variants of Doublesex (Dsx), a terminal transcription factor in the sex-determination pathway, have been detected in the dengue vector *Aedes aegypti,* the sex-specific expression patterns of these transcripts have not been detailed in developing tissues. Furthermore, although sex-specific *dsx* splice forms are believed to regulate sex-specific development in mosquitoes, this hypothesis has not been functionally tested. We have implemented a powerful methodological toolkit, including molecular markers for developing neural tissue subtypes and siRNA nanoparticle-mediated gene targeting, to characterize *dsx* function during *A. aegypti* development. These studies uncovered sex-specific *dsx* expression patterns in developing tissues, including the pupal brain mushroom body, antennal lobe, and optic lobe. 732 Dsx consensus binding sites were identified in the *A. aegypti* genome, including sites associated with 48 genes dimorphically expressed in the pupal head. *A. aegypti* genes associated with Dsx consensus sites group under a number of significant neural-related gene ontology terms, including neuron fate commitment, neuron differentiation, and neurological system processes, as well as numerous processes related to the sensory system and sensory development, particularly the compound eye and eye development. siRNA-mediated knockdown experiments confirmed that Dsx regulates sex-specific gene expression in the developing nervous system and uncovered adult phenotypes, including reproductive defects, associated with loss of *dsx* function. These studies are revealing the developmental genetic basis of mosquito sexual dimorphism and may one day be exploited in the development of novel control strategies.

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THE SACRIFICED FLAGELLUM OF *TRYPANOSOMA CRUZI* PROVIDES VERY EARLY TARGETS OF PROTECTIVE CD8+ T CELLS

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The CD8+ T cells crucial to the control of infection with *Trypanosoma cruzi*, the agent of human Chagas disease, are predominantly directed at members of large gene families, including trans-sialidase (ts)-like proteins that are diverse and variable within and among *T. cruzi* isolates. We hypothesized that CD8+ T cell responses directed against sub-dominant, invariant proteins may induce more potent cross-strain protection from *T. cruzi* infection and thus sought to identify such targets. Investigation of the early events in intracellular invasion by *T. cruzi* revealed that trypomastigotes sacrifice their flagella via an asymmetric division during amastigogenesis and thus release flagellar proteins into the host cell cytoplasm. Peptides derived from flagellar proteins appear to be among the earliest *T. cruzi* proteins to enter the class I MHC processing and presentation pathway. Overexpression of one of these proteins, the abundant paraflagellar rod protein (PAR) 4, by transgenic *T. cruzi* enhances the potency of the PAR4-specific CD8+ T cell response, resulting in significantly improved control of a challenge infection. This enhanced protection despite the relatively low abundance of PAR4-specific CD8+ T cells was associated with the ability of PAR4-specific CD8+ T cells to detect host cell infection by *T. cruzi* significantly earlier than the immunodominant ts-specific T cells. These results provide insights into previously unappreciated events in intracellular invasion by *T. cruzi* and suggest that the transgenic over-expression of appropriate endogenous proteins may significantly improve the protective capacity of viral vector based or live attenuated vaccines for *T. cruzi* and perhaps other pathogens.

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UNTARGETED METABOLOMICS TO STUDY MULTIDRUG RESISTANCE IN *LEISHMANIA DONOVANI*

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Chemotherapy is the most important tool for the control of visceral leishmaniasis, but its efficacy is jeopardized by the growing resistance and treatment failure against first-line drugs. Antimonials have become obsolete in the Indian subcontinent because of resistance, the efficacy of miltefosine is decreasing, and isolated cases of amphotericin B failure are of concern for both the scientific and health community. To further delay the emergence of resistance, the WHO recommended combinations of anti-leishmanial drugs. Until today, the mode of action and drug resistance mechanisms of the four available drugs (antimonials, miltefosine, amphotericin B and paromomycin) are poorly understood. In this study, we performed untargeted LC-MS metabolomics to identify differentiating metabolites in *Leishmania donovani* promastigotes with induced resistance to single drugs and drug combinations. The most significant metabolic changes were found in the lines resistant (R) towards Sb^{III}, amphotericin B (AmB) and miltefosine, and the combinations Sb^{III} + AmB and Sb^{III} + paromomycin. A clear additive or synergistic effect of the single resistant lines in a combination-resistant line was not found, but significant overlap between differential metabolites in the single R lines and their combination-resistant line was observed. The detected metabolic changes upon drug exposure in wild-type and resistant lines were experimentally validated and showed that resistant parasites have (i) an increased capacity for protection against oxidative stress, and (ii) an altered fluidity of the plasma membrane. Our results elucidate the mechanisms underlying the ability of *Leishmania* to develop resistance to combinations of anti-leishmanial drugs: single and multidrug resistant parasite cell lines show distinct metabolic adaptations, but these all converge on the same defensive mechanisms.

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DISSECTING THE PROMASTIGOTE TO AMASTIGOTE DIFFERENTIATION IN *LEISHMANIA AMAZONENSIS*

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Leishmania is a genus of protozoan parasites transmitted by the phlebotomine sand fly that causes an array of disease ranging from cutaneous to visceral leishmaniasis resulting in significant morbidity and mortality. These parasites have a dimorphic life cycle, existing as a promastigote in the sand fly vector and an intracellular amastigote in the human host. While it is known that temperature and pH changes induce this change, the molecular basis of this differentiation is still unknown. To uncover the molecular mechanisms behind this transition, we used Hsp90 inhibitors, which are also capable of inducing the promastigote to amastigote change. In addition to the expected transition we observed a dose-dependent morphological change in response to the inhibitor, a situation not seen when changing the temperature or pH. We hypothesize that the morphological variability induced by the Hsp90 inhibitor is

associated with intermediate states in the promastigote to amastigote transition not seen under normal circumstances. We have employed different techniques to characterize these forms such as morphological analysis by microscopy and FACS, and gene expression analysis by qRT-PCR. We have also generated reporter plasmids expressing fluorescent proteins under the control of promastigote and amastigote specific promoters to follow the transition between the two forms in response to the typical heat and pH as well as in the presence of the Hsp90 inhibitor. Finally, proteomic analysis of promastigotes, amastigotes and intermediate forms has helped us proposed effector proteins responsible for the parasite transition. Ultimately, these signaling molecules will represent potential drug targets against leishmaniasis, a neglected disease that badly needs new therapeutic options.

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A NOVEL FIELD-APPLICABLE MOLECULAR TEST FOR VISCERAL LEISHMANIASIS

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In the Americas there are several thousand reported annual cases of full blown visceral leishmaniasis (VL) and mortality ranges between 7 and 10%. Furthermore, underreporting of VL is underestimating the actual disease burden. American VL is a zoonotic disease produced by Leishmania infantum and transmitted between dogs and humans by urbanized sand flies. Dogs are the principal reservoir hosts and, therefore, are critical targets for controlling urban transmission. The rK39® serological test is widely used to identify and remove infected dogs from the transmission cycle, but it has low sensitivity (<33%) to detect subclinically infected dogs. The lack of sensitivity of serological tests is considered as the main reason for the inefficacy of control programs. We have developed a sensitive and specific molecular test to detect L. infantum using a Recombinase Polymerase Amplification method coupled with Lateral flow reading (RPA-LF). This innovative isothermal amplification test is as sensitive as real time PCR (gold standard) but it does not require sophisticated equipment, is fast, and the result is read with the naked eye. This makes it ideal for point of care diagnosis and field epidemiology studies. In the lab RAP-LF detected <2 parasites in blood samples. Furthermore, evaluation of dog blood samples from an endemic area showed that RPA-LF detected more subclinically infected dogs than rK39 (51.9% vs. 14.3% positivity, respectively; p= 0.01). The RPA-LF fills the need for an effective diagnostic tool that will play a critical role in control interventions to reduce urban transmission of visceral leishmaniasis to humans.

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IDENTIFYING VACCINE CANDIDATES FOR VISCERAL LEISHMANIASIS

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Leishmaniasis, a neglected tropical disease, prevalent in developing countries with 90% of them in Asia (Bangladesh, India, and Nepal), Sudan, Ethiopia and Brazil. The technical challenges and the complexity in the immunity against the parasites clearly contribute to the absence of vaccines. A major challenge in human vaccine design is to overcome variation in immune response in a genetically heterogeneous population. This is largely determined by genetic heterogeneity in processing and presentation of Ag to T cells, the outcome of which is dependent on binding of T cell epitopes to HLA class I and class II molecules that drive CD8 and CD4 T cell responses, respectively. In silico screening for putative epitopes binding to DRB1 molecules can identify multiple epitopes per single parasite antigen. Our quest here is to determine what actually occurs during the course of a complex infection *in vivo*. We are using in silico prediction tools in an effort to understand more about the processes that direct antigen selection and binding to different DRB1 molecules during natural leishmanial infection. This will be done in concert with analysis of naturally processed leishmanial peptides. A previously conducted GWAS and further sequence based haplotyping on an Indian population has indicated HLA class II as a major genetic risk factor for visceral leishmaniasis(VL) and revealed DRB1*13/14 and DRB1*15/16 as risk and protective alleles, respectively in VL. In a preliminary study, we have obtained data on leishmanial epitopes predicted to bind to DRB1*13/*14 risk vs DRB1*15/*16 protective class II molecules using the in-silico predictive tool NetMHCIIpan v2.1. Data for overlapping 9-mer epitopes has been generated for 43 known *Leishmania* antigens (antigens of diagnostic value, vaccine candidates) and we have found peptides exclusively binding to risk as well as protective group and also some differentially binding peptides. Functional validation of these peptides will be done by measuring immune response against these antigens in individuals carrying different allele group from endemic region in India. This will pave the way for appropriate vaccine candidates which can drive the immune response to protective response in genetically susceptible individuals.

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USE OF CLINICAL PREDICTORS AND MOLECULAR DIAGNOSIS TO IDENTIFY THE SPECIES RESPONSIBLE FOR SNAKEBITE IN RURAL NEPAL

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Snakebite is an important medical emergency in rural Nepal. Correct identification of the biting species is crucial for clinicians to choose appropriate treatment and anticipate complications. This is particularly important for neurotoxic envenomation which, depending on the responsible species, may not respond to antivenoms. Adequate species identification tools are lacking. This study used a combination of morphological and molecular approaches (PCR-aided DNA sequencing from swabs of bite sites) to determine the relative contribution of venomous and non-venomous species to the snakebite burden in southern Nepal. The study also investigated the performance of baseline patient history and clinical characteristics to distinguish between cobra (*Naja* spp.) and krait (*Bungarus* spp.) bites. Out of 749 patients admitted to one of the 3 study centres with a history of snakebite, the biting species could be identified in 194 (25.9%). Out of these, 87 had been bitten by a venomous species, most commonly *Naja naja* (n=42) and *Bungarus caeruleus* (n= 22). When both morphological identification and PCR/sequencing results were available, a 100% agreement was noted. Among patients bitten by venomous snakes, 71 (81.6%) presented with signs of envenomation including neurotoxic signs in 55 (77.4%). Being bitten at night (OR= ∞), while sleeping (OR= 56 [95%CI= 9.9-318.2]), indoors (OR= 9.4 [95% CI= 1.9-46.9]), and presenting with abdominal pain (OR= 23.4 [95% CI=3.8-142.5]) was associated with krait bite, and local signs of envenomation (e.g., edema) with bites by cobras and pit vipers. This study is the first to report the use of forensic genetics methods for snake species identification in a prospective clinical study, and to identify epidemiological and clinical features associated with krait and cobra envenomation in Nepal, thereby providing decisive guidance to improve patient care.

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MULTIPLEX PCR DETECTION OF ENTEROPATHOGENS CAUSING TRAVELERS' DIARRHEA FROM SPIKED STOOL SMEARS ON WHATMAN FTA CARDS USING MULTIPLEX PCR

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Molecular diagnostics offer significant advantages over conventional testing for enteropathogen detection in travelers' diarrhea. There is limited data on the performance of PCR assays using stool samples and smeared stool cards. We sought to determine the limits of detection (LoD) for diarrheal pathogens using a multiplex PCR assay on spiked stool samples, and spiked stool smeared onto Whatman FTA® Elute cards. The multiplex PCR panel used a combination of Luminex xTAG analyte-specific reagents (ASRs) and in-house primers to detect: enterotoxigenic *Escherichia coli* (ETEC), enteroaggregative *E coli* (EAEC), enterohemorrhagic *E coli* (EHEC), *Shigella spp.*, *Salmonella enteritidis, S typhimurium*, *Campylobacter spp.*, norovirus [GI, GII], *Giardia lamblia* and *Cryptosporidium spp.* LoD analysis was carried out by performing serial 1:10 dilutions, with the 4th and 5th dilution performed in triplicate. We evaluated the impact of prolonged storage of stool cards (1 and 3 months) on the LoD. The mean fluorescence intensity value for detection was ≥300. LoDs in spiked stool ranged from 10^2 to 10^4 cfu/g for bacterial pathogens, 10^2 to 10^3 pfu/g for Norovirus GI and GII respectively and 10^3 cysts/g for Giardia. Cryptosporidium could not be reliably detected in the stool or stool card. The LoD for ETEC, EHEC and *S enteritidis* were similar in the stool card and spiked stool, while the LoD for EAEC, *S typhimurium*, Shigella, Campylobacter and norovirus GII were 1 log higher in smeared stool cards compared to stool. Giardia had a lower LoD in the stool card (1 cyst/g). Significant variability (2-4 logs) in the LoDs was observed with prolonged storage of stool cards. LoDs for *S enteritidis* and ETEC increased from 10^1 cfu/g at 1 month to 10^3 cfu/g at 3 months, while EHEC decreased from 10^5 cfu/g to 10^1 cfu/g during the same time-points. *Campylobacter* increased from 10^4 cfu/g at baseline to 10^5 cfu/g at 1 month and could not be detected at 3 months. Further evaluation of the multiplex PCR assay using spiked specimens and diarrheal samples is needed before deployment in field studies.

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A HOSPITAL-BASED SURVEY ON HUMAN BRUCELLOSIS ITS ASSOCIATED FACTORS IN KANO METROPOLIS, KANO STATE-NIGERIA 2011

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The global burden of human brucellosis remains enormous; it causes more than 500,000 infections per year. It is one of the most widespread zoonotic diseases globally. Brucellosis is a multi-systemic, acute to chronic, disease characterized by fever, headache, joint pains, musculo-skeletal pains, sweating, malaise and body wasting. It is a severe debilitating disease that requires prolonged treatment, if untreated can result in permanent disability and loss of productivity. Human brucellosis presents a great variety of clinical manifestations making it difficult to diagnose clinically. In some endemic areas every case of human fever of unknown origin is assumed to be due to brucellosis. Therefore, the diagnosis must be confirmed by laboratory tests. A hospital-based survey on human brucellosis was undertaken to determine its prevalence, associated factors and the extent of missed diagnosis. A descriptive study and laboratory analysis were conducted. A suspected case was defined as any patient $\geq$ 5years with fever (>37.5°C) plus any two of the clinical signs suggestive of human brucellosis. Socio-demographic features, clinical information and assessment of potential risk factors were obtained through questionnaires. Their sera were screened with Rose Bengal plate test (RBPT) and positive cases were subjected to Enzyme Linked Immunosorbent Assay (ELISA) to confirm and detect evolution of the infection. A total of 250 suspected cases were enrolled and 50(20%) were confirmed positive. There were 131 males and 119 females. Mean age was 24years$\pm$16. Sensitivity and specificity were 90% and 85%. Of the 50 cases, 31(62%) were males; clinical signs consistent were recurrent fever (p 0.04), cough/sneeze (p 0.02) and osteomyelitis (p 0.00). Risk factors for human brucellosis were consumption of fresh milk (p 0.02) and local yoghurt kindirmo (p 0.00), keeping goats (p 0.00), assisting animal parturition (p 0.01), processing and eating raw meat (p 0.03 and p 0.00). Thirty six (72%) of the 50 cases were positive by IgG thus chronic infection. Diagnosis of human brucellosis is missed in hospitals in Kano. Risk factors identified were processing and consumption of unprocessed milk and meat; keeping goats and assisting parturition. Authorities should educate the populace on the risks of this disease and its prevention; physicians should raise the index for diagnosis of human brucellosis in patients that present with signs suggestive of it.

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REAL-TIME QUANTITATIVE PCR SURVEILLANCE OF GASTROINTESTINAL PARASITES IN A SYMPTOMATIC RURAL ARGENTINIAN POPULATION: INITIAL RESULTS OF THE LATIN AMERICAN MULTICENTER PARASITE STUDY (LAMPS)

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There are over 2 billion people infected with gastrointestinal (GI) parasites. Diagnosis of GI parasites relies on stool microscopy that has low diagnostic sensitivity and specificity. To better understand the parasitic etiology of abdominal symptoms, we implemented our rapid, high throughput, multi-parallel quantitative real-time PCR (qPCR) for the 8 common GI parasites including the helminths, *Ascaris lumbricoides (Al), Ancylostoma duodenale (Ad), Necator americanus (Na), Strongyloides stercoralis (Ss), Trichuris trichiura (Tt)* and protozoa, *Cryptosporidium parvum (Cp), Entamoeba histolytica (Eh)* and *Giardia lamblia (Gl).* This assay was used to analyze stool samples collected from 99 patients seen in a rural Argentinian clinic. For *Al*, qPCR identified (56.6%) positives whereas McMaster's microscopy technique identified (47.5%) with 91.3% sensitivity and 90.5% negative predictive value (NPV). For hookworm, there was 37.4% detected by qPCR compared to 22.2% by microscopy ($p < 0.05$), with a 95.5% sensitivity and 98.4% NPV. Hookworm ova are indistinguishable by microscopy, but qPCR is species specific. While *Na* was the predominate hookworm detected, *Ad* DNA was detected in higher concentrations (0.61 versus 119.6 fg/µL, $p < 0.0001$). This has important implications, since *Ad* is more aggressive in causing anemia. The difference between qPCR and microscopy was dramatically seen for *Gl* (63.6% versus 8.1%) with 55 additional positives for *Gl* ($p = 0.001$). For *Ss*, qPCR identified (21.2%) positives whereas microscopy identified (3.0%)($p < 0.05$) with 100% sensitivity and negative predictive value. qPCR was also able to detect polyparasitism by a factor of 7 compared to microscopy ($p < 0.05$). We have deployed a quantitative molecular based system that has improved diagnostic accuracy than stool microscopy. This is the first time this assay has been used in Argentina and has shown the prevalence of GI parasite infections in symptomatic patients. The results will help refine treatment options on a public health scale and lead to better health outcomes in endemic settings.

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UTILITY OF PROVISIONAL DIAGNOSES OF DENGUE FROM A SENTINEL-ENHANCED SURVEILLANCE SYSTEM IN PONCE, PUERTO RICO

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Dengue and a number of other acute febrile illnesses (AFI) present with similar signs and symptoms. The unavailability of sensitive and specific rapid diagnostic tests poses a significant challenge to early and accurate diagnosis of dengue because early identification is important for management of patients to reduce mortality. We used data from the first year of a Sentinel Enhanced Dengue Surveillance System (SEDSS) in Ponce, Puerto Rico between May 7, 2012 and May 6, 2013 to evaluate the accuracy of clinicians' diagnoses of patients presenting with an AFI (N = 2,027) in a dengue-endemic area. A classification system was developed, and diagnoses were grouped into syndromes defined as dengue, influenza, viral infection, gastrointestinal, respiratory, genitourinary, and other. Laboratory diagnostic testing was performed for 23 pathogens associated with AFI, including the four dengue viruses (DENV-1-4). Of 1,152 cases in which an infecting pathogen was identified, 579 (50.3%) were DENV-positive, 301 (26.1%) were positive for influenza, and 272 (23.6%) were positive for another pathogen. Among the DENV-positive cases, 243 (42%) received a provisional clinical diagnosis of dengue, while 183 (32%) and 60 (10%) were diagnosed as viral infection and respiratory illness, respectively. At initial clinical presentation, the DENV-positive cases with a provisional diagnosis of dengue had a higher proportion with rash (53% vs. 0.38%; p = 0.001) and diarrhea (44% vs. 32%; p = 0.005), more pronounced leukopenia (3,100 vs. 4,600; p < 0.001), thrombocytopenia (93,203 vs. 155,937; p < 0.001), and elevated aspartate transaminase (175 vs. 112; p = 0.001). Multiple logistic regression modeling and stratification analysis by age group will be performed to determine the clinical and laboratory factors most associated with a clinical and laboratory diagnosis of dengue. This will both inform physicians in their diagnostic approach to dengue and facilitate the use of provisional diagnoses as an early detection tool for increases in dengue in Puerto Rico.

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CARDIAC INVOLVEMENT IN PEDIATRIC DENGUE; A SERIAL ECHOCARDIAGRAPHIC STUDY

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Dengue is the most prevalent vector-borne viral infection of humans. There have been case reports of severe cardiac involvement including myocarditis in dengue, although the true incidence is not well defined. To better characterize cardiac involvement we performed daily echocardiographic studies in children with suspected dengue from 2010 to 2012 at a tertiary care center in Bangkok, Thailand. Plasma levels of cardiac troponin-T were measured daily. We analyzed 180 confirmed dengue cases classified as dengue fever (DF) and dengue hemorrhagic fever (DHF) according to the 1997 WHO case definitions. There were 119 cases of DF, and 12, 27, 21, and 1 cases of DHF grade 1, 2, 3, and 4, respectively. On the day of presentation (approximately 4.5 days of illness), DHF cases had significantly lower stroke volume, left ventricular end-diastolic volume, ejection fraction, and cardiac index, and higher systemic vascular resistance compared to cases with DF (p< 0.001 to < 0.05). Flow and tissue Doppler studies demonstrated a decreased early diastolic component of flow at the mitral valves as well as decreased tissue plane excursion of the mitral valves in DHF cases. These early differences were attributed to findings from DHF cases with ultrasound-documented plasma leakage at this time point. Serial studies showed that differences in hemodynamic and cardiac functional indices between DF and DHF were most pronounced around the time of deferevescence. There were no clinically evident cases of cardiac failure in this study. An abnormal ejection fraction (EF <55%) was observed in 6%, 9%, 18%, and 32% of cases of DF, DHF grade 1, DHF grade 2, and DHF grades 3/4, respectively (p<0.001). However, the abnormal EF was transient and usually coincided with the development of plasma leakage. No cases with abnormally elevated troponin-T levels were identified. We conclude that cardiac involvement is uncommon in pediatric dengue and is not a major contributing factor for dengue shock syndrome. Subtle changes in cardiac function are common but appear to be transient and may reflect plasma leakage and volume status.

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CHAGAS DISEASE TRANSMISSION AND CARDIAC MANIFESTATIONS AMONG TEXAS BLOOD DONORS

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Although well established as an important cause of morbidity and mortality in Latin America, Chagas disease is increasing in recognition as a potential cause of heart disease in the United States. Screening of blood donors in the greater Houston area for Chagas disease (*Trypanosoma cruzi* infection) began in 2007. The transmission mechanisms and potential cardiovascular manifestations of these *T. cruzi* positive individuals have not been previously studied. An one-time assessment of Houston area blood donors that screened positive from 2008-2012 for *T. cruzi* infection (n=30) included 1) blood draw for confirmatory *T. cruzi* diagnostic testing, 2) a questionnaire to evaluate source of infection, cardiac symptoms and health co-morbidities, 3) high-sensitivity troponin T biomarker evaluation, 4) an electrocardiogram, and 5) an echocardiogram if electrocardiogram was abnormal. We found 57% (17/30) of blood donors had two or more positive tests confirming infection. Of those with confirmed infection, 41% (7/17) had an electrocardiographic abnormality consistent with Chagas cardiomyopathy. In addition, 36% (6/17) were suspected to be locally acquired cases. High-sensitivity troponin serum levels were increased in a linear manner with cardiac severity. Cardiologists should consider the changing transmission dynamics associated with Chagas disease in the southern United States and should consider Chagas disease in patients who may have clinically-compatible electrocardiogram or cardiomyopathy, even if the patient has no history of residing in a Chagas-endemic country.

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EXAMINATION OF 2006-2013 MALARIA INCIDENCES IN RELATION TO THE SCALING OF PREVENTATIVE CONTROL INTERVENTIONS IN MULEBA DISTRICT IN NORTHWEST TANZANIA

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Following outbreak of malaria in Muleba district in 2006, intensive malaria control interventions, including Indoor Residual Spraying (IRS), and distribution of LLINs were introduced. IRS was introduced as an outbreak preemptive measure in selected areas of the district in 2007 and 2008, with gradual scale-up of one round of IRS per year covering the entire

district until 2011. About 80,000 LLINs were distributed to under-fives in 2009 and 170,000 nets distributed in early 2011 to the remaining population. However, due to universal attainment of LLINs in 2011, as well as limited resources, IRS was scaled down in 2012-2013, with the last round targeting only 25% (17,000 house structures) of the district. After 2011, no significant efforts have also been made to keep-up LLIN coverage. We compared health facility based malaria incidence per 1000 population of respective village catchment areas from Jan 2006-May 2013, a period in which a total of 352,488 out-patient malaria cases were recorded. District incidence in 2006 (pre-control period) was 118 per 1000, with the rate declining to its lowest level of 37 per 1000 in 2010. This peak decrease of 67% in malaria incidence at 2010 compared to 2006 was most likely due to intensification of effective multiple control interventions over that period. However from 2011 onward, scale down and/or low maintenance of control interventions, coupled with reported stock-outs of ACTs, and low net use (68%-THMIS 2011-12) likely contributed to a rise in malaria incidence; by 2013, increasing to 181 per 1000 (adjusted for 12 month period). The 2013 rate was 35% higher than the pre control era of 2006. Muleba's case suggests that scaling-down malaria control efforts has resulted in loss of initial gains in controlling malaria. This could have serious implications with possible rebound of malaria to pre-intervention levels as well as frequent malaria outbreaks. Once the control of malaria has reached to manageable levels, it is important to advocate for effective monitoring and response so as to sustain the fight against malaria.

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FOCAL SCREENING AND TREATMENT (FSAT) CAMPAIGN IN FOCI OF MALARIA TRANSMISSION: IMPACT ON MALARIA PREVALENCE AND COMPLEXITY

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Mass screening and treatment campaigns have had limited success in curbing malaria transmission, most likely due to the prevalence of subpatent infections missed using the field based diagnostic tools. It has been shown that subpatent malaria infections are more likely to occur in households where patent infections are identified. Therefore it is possible that a more focal approach to treatment campaigns using patent infections as a marker for the presence of a subpatent reservoir may be more effective at reducing the infectious reservoir in humans. To test this strategy, a focal screening and treatment (FSAT) intervention in foci of malaria transmission (prevalence 18,6%, 95% C.I. 10.8-26.5%) was conducted in the western Kenyan highlands as part of a larger cluster randomized trial. All consenting individuals under 15 years old or febrile adults residing within the foci were tested for malaria with a rapid diagnostic test (RDT) and if found positive all individuals residing in the compound received a curative dose of artemisinin combination therapy. Blood spots on filter paper were collected from all household members (N=2083), regardless of RDT result. To assess the impact of FSAT, parasite prevalence and complexity using nested polymerase chain reaction and merozoite surface protein-2 genotyping was determined. The impact of the FSAT approach on parasite prevalence and allelic diversity was assessed with two follow-up cross-sectional surveys at 2-month intervals post intervention. Of the compounds within the intervention foci, 168 of 406 (41.4%) households sampled received treatment. Twenty-seven households declined or had no individuals present during time of sampling. Initial results indicate that, at baseline, PCR prevalence in compounds with a patent infection and therefore targeted for treatment was 34.0% compared to 11.1% in those that had no patent infections ($p<0.0001$) with the FSAT approach successfully identifying 78.3% of parasite carriers. This strategy could provide a useful and operationally attractive alternative to detecting subpatent infections in foci of infection.

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SPATIAL PATTERNS OF MALARIA TRANSMISSION OVER ONE YEAR IN A CLINIC CATCHMENT AREA OF CHONGWE DISTRICT, ZAMBIA

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As malaria transmission declines, it may be more cost-effective and efficient to focus control efforts on high-risk areas. Describing spatial patterns of malaria transmission may identify opportunities to develop and improve current interventions. Individuals testing positive for malaria at a single clinic in Chongwe District, Zambia, were approached for enrollment in the study. These index cases were administered a questionnaire and a blood sample was taken for a rapid diagnostic test (RDT) and microscopy. Upon enrollment their homes were visited, all household contacts were enrolled, administered a questionnaire, and gave a blood sample for RDT and microscopy. GPS coordinates were recorded at each household. If an individual from a previously enrolled household sought care, these were considered re-infection households. Spatial patterns of malaria in this clinics catchment area were analyzed using SaTScan for cluster detection and programs from R statistical software to describe patterns of spatial clustering. From June 2012 to June 2013 a total of 472 index cases and 1,901 household contacts (43% RDT positive) were enrolled. Two statistically significant space-time clusters of index case households were identified controlling for age and gender; one in December 2012 and one in January 2013 (the peak transmission season). No clustering of RDT positive household contacts was identified; factors associated with being an RDT positive household contact included distance to the clinic, socioeconomic status, and bednet ownership ($p<0.05$ for all comparisons). In conclusion, transmission in this area appears to be high with a seasonal peak in incidence during the rainy season. Two space-time clusters of index case households were detected during the peak transmission season in close proximity to the clinic. No clustering of malaria comparing household contacts was detected likely because of high transmission in the entire catchment area.

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CESSATION OF COTRIMOXAZOLE PROPHYLAXIS IN HIV EXPOSED CHILDREN DOES NOT INCREASE THE INCIDENCE OF MALARIA AND OTHER MORBIDITIES

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HIV-exposed infants born to mothers with HIV should receive cotrimoxazole prophylaxis (CPT) until HIV infection can be excluded and the child is no longer exposed through breastfeeding. As daily CPT provides prophylaxis against malaria, it could modulate the development of malaria-specific immunity and increase the incidence of malaria after it is stopped (a rebound effect). To determine if this is the case, we investigated the incidence of malaria and other morbidities during and after CPT in the first two years of life in HIV exposed children in southern Malawi. A cohort of 500 HIV exposed children on CPT for 12 months from the age of 6 weeks to one year and 500 non-HIV exposed children not on CPT were followed until their second birthday. HIV exposed children were recruited at the Prevention of Mother to Child Transmission (PMTCT)

HIV clinic while location and age-matched non-HIV exposed children were recruited from the same population. The incidence of malaria, all-cause morbidity, admissions and mortality was compared during the first and second year of life in the two study groups using multivariate, negative binomial regression analyses. In year-1, the incidence of uncomplicated malaria in HIV exposed children was 65% lower compared to the non-HIV exposed group (IRR = 0.35, 95% CI 0.25, 0.49, p<0.001). In year-2 the incidence of malaria in the HIV exposed group was similar to that in non-HIV exposed group (IRR 0.94, 95% CI: 0.53, 1.68, p=0.839) among the first 315 children that completed the follow-up period. The same pattern was observed for all-cause morbidity and hospital admissions. CPT was associated with marked reductions in the incidence of uncomplicated malaria, all-cause morbidity and hospital admissions during the period in which it was given. The follow-up in year-2 is on-going but preliminary results suggest that the incidence of malaria does not increase after cessation of CPT at around 14 months of age.

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STANDARDIZED MULTIDISCIPLINARY METHOD FOR THE EVALUATION OF THE EFFECTIVENESS OF MALARIA CONTROL INTERVENTIONS

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In a global context of international funding plateauing, control programs are looking for innovative options to better perform with a constant budget. In absence of groundbreaking new control tools, the only option is to make the best use of the control measures that are already available. In order to guide policy making, we propose to carry out a broad and comprehensive evaluation of interventions deployed in a given setting, including an evaluation of their effectiveness and the identification of the key determinants affecting their effectiveness. We wrote and selected 20 Standard Operating Procedures (SOP) through a multi-country multidisciplinary workshop, including experts from Belgium, Benin, Cameroon, Côte d'Ivoire, France, Madagascar and Niger. These SOP cover the following fields: analysis of health systems (1), anthropology (1), biological diagnosis (4), drugs resistance (2), entomology (3), epidemiology (4), health economics (2), and immunology (3). All 20 SOP are combined in a single toolbox that is being implemented in Benin and Madagascar in 2014. Preliminary results will be presented. For each malaria control intervention the following indicators are evaluated: coverage, protective effectiveness against infection, protective effectiveness against morbidity, cost-effectiveness, socio-anthropological determinants of effectiveness, entomological determinants of effectiveness (vector behavior, insecticide resistance) if applicable, and *in vivo* and *in vitro* measure of antimalarial drug resistance. Results also include an analysis of health systems and management of malaria control in general. The toolbox -named PALEVALUT- will be further reviewed before and after implementation in Cameroon, Côte d'Ivoire and Niger in 2015. The whole tool will be soon available with free access on the internet.

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CHANGING HEALTH CONDITIONS AND THE DECLINE OF ALL-CAUSE UNDER-FIVE MORTALITY IN RWANDA 2000-2010: A DECOMPOSITION ANALYSIS

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All-cause child mortality (ACCM) has declined rapidly over the past decade in Rwanda, from 196 deaths per 1,000 live births during 1996-2000 to 76 deaths per 1,000 live births during 2006-2010. Rapid development and improvements in many socioeconomic and health indicators between 2000 and 2010 are likely to have played a role in determining these declines. For example, the percentage of households with improved toilets increased from 9% to 72%, the percentage of households with mosquito nets rose from 8% to 94%, and the percent of children whose mothers used ITNs increased from 5% to 77%. In this analysis, decomposition models were used to describe the decline in ACCM between two Demographic and Health Surveys (DHS) (Rwanda DHS 2000 and Rwanda DHS 2010). The relative importance of changes in the distribution of key socioeconomic and health-related variables (coverage) and changes in the magnitude of association between these variables and ACCM (coefficients) across these surveys was examined. The model explains a decline of 83 deaths per 1,000 live births from 2000 to 2010, or 69% of the reduction observed from the surveys. Results show that the combined effect of all of the changes in intervention coverage explained almost all (99%) of the modeled reduction in ACCM. Holding other factors constant, the observed increase in household bednet ownership could have explained as much as 45% of the modeled decline in ACCM between 2000 and 2010, and 31% of the observed decline in ACCM from survey data. The increasing percentage of children whose mothers used ITN between 2000 and 2010 could have explained an additional 4.2% of the modeled reduction in ACCM, presumably through reductions in neonatal mortality. Improvements in coverage of antenatal care and increasing birth intervals could have explained an additional 13.4% and 3.1% of total modeled ACCM decline, respectively. Changes in the distribution of household wealth and of multiple births between surveys would have led to small but significant increases in ACCM, holding other variables constant. These results clearly show the important role of malaria control interventions in the reduction of child mortality in Rwanda.

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VARIATIONS IN MALARIA EPIDEMIOLOGY IN RELATION TO VECTOR CONTROL COVERAGE IN NINE DISTRICTS OF UGANDA

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Malaria has been a major public health problem in Uganda. The disease is highly endemic in 95% of the country where 90% of the population live. A scale up of malaria control interventions in the past decade coupled with environmental, social and economic factors appears to have contributed to a changing pattern of the epidemiology of the disease in the country. This study was part of a larger study on the distribution of insecticide resistance and resistance management. The aim of this component of the study was to understand the impact of varying coverage of vector control interventions on the observed epidemiological pattern of malaria

in different groups of districts. Three groups of nine districts were selected among all highly endemic districts that were in existence since 2001, using criteria based on coverage with long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS): 1) Districts that had undergone several rounds of IRS (Apac, Gulu, Pader), 2) Districts where LLINs had been distributed but no IRS had taken place (Kayunga, Kiboga, Mbale), and 3) Districts that had not received IRS or LLINs as part of large campaigns at the time of selection (Bugiri, Mayuge, Soroti). Forty-five cluster were sampled in the nine districts in which a cross-sectional survey was conducted during September-October 2012 transmission season. A total of 528 interviews were conducted in the 45 clusters, an average of 12 households per cluster. Blood samples were gathered from all household members and mosquitoes were collected using the pyrethrum spray catch method. *Anopheles gambiae* s.l. and A. funestus s.l. were the main vectors collected. Prevalence of *Plasmodium falciparum* and density of malaria vectors varied between groups of districts with different levels of vector control coverage. The highest prevalence was observed in the unsprayed group with historically low coverage with LLINs. Pyrethroid resistance was widespread in nearly all districts. The role of vector control interventions is discussed in the light of observed variations in malaria epidemiology.

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PERCEPTIONS OF COMMUNITY-LED TOTAL SANITATION ON SANITATION BEHAVIORS IN RURAL ZAMBIA: A QUALITATIVE STUDY

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Despite emerging initiatives in the sanitation sector, inadequate sanitation remains a leading global contributor to morbidity and mortality in children. The government of Zambia, UNICEF, and partners are engaged in the rollout of an ambitious hygiene and sanitation program aimed at reaching 3 million new users of improved sanitation hand washing practices. One of the key approaches used to reach this objective is Community Led Total Sanitation (CLTS), a grassroots, subsidy-free strategy with the goal of changing sanitation practices to create open defecation free (ODF) villages. During CLTS, community members participate in activities that lead them to realize and declare that they are "eating each other's stool". This qualitative study explores community members' and stakeholders' sanitation behaviors, knowledge and perceptions during early CLTS implementation in Zambia. We conducted 67 key informant interviews and 24 focus group discussions with community members and other WaSH stakeholders in 6 districts in Zambia who were selected using purposive sampling based on recommendations from project staff. The study was conducted in July and December of 2013, 12 to 18 months after initiation of CLTS implementation. According to key informants, triggering activities elicit a strong emotional response involving shame, disgust and peer pressure which persuades individuals to build and use latrines. Pride and dignity were also reported as important influential factors, as individuals and communities become empowered. Traditional leaders and community Sanitation Action Groups have strong hierarchical influence that is also persuasive in changing behaviors. Respondents frequently mentioned that children help to influence their families to improve sanitation behaviors. Overall, participants reported an increase in latrine construction and usage after triggering; however, poor (e.g. rocky or sandy) soil conditions and taboos prohibiting certain family members from sharing the same toilet act as barriers in many areas. CLTS results in powerful individual and community emotional responses that serve to encourage construction and use of latrines and adoption of improved hygiene practices. This formative research suggests that CLTS has potential to be an effective approach for improving sanitation beliefs and behaviors in Zambia, which in turn may result in improved child health.

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FACTORS ASSOCIATED WITH PUPIL LATRINE USE IN KENYAN PRIMARY SCHOOLS

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Little empirical data exist on how the type, design, and maintenance of school latrines affect pupil latrine use. The purpose of this study was to characterize how school sanitation conditions are associated with latrine use patterns in 60 primary schools in Nyanza Province, Kenya. We conducted a longitudinal assessment, using structured observations to measure both latrine conditions and latrine use during the 30 minute morning break. We modeled the association between pupil to latrine ratio and latrine use (pupil used a latrine vs. not), using multivariable logistic regression. Lower pupil to latrine ratios were associated with increased latrine use, with the odds of use more than doubling when comparing schools with the lowest pupil to latrine ratios (<15:1) to schools with the highest ratios (>75:1; odds ratio=2.18, 95% CI: 1.69-2.80). We also modeled the association between different latrine characteristics and the number of uses at specific facilities, using multivariable negative binomial regression. Pupils preferred to use newer latrines over older latrines (incidence rate ratio (IRR)=1.13, 95% CI: 1.01-1.26), and also preferred ventilate improved pit latrines (IRR=1.15, 95% CI: 1.00-1.31) and urinals (IRR=1.96, 95% CI: 1.57-2.44) over prefabricated plastic latrines (IRR=0.68, 95% CI: 0.54-0.85) and traditional pit latrines (referent). An increased number of latrines in a block (a group of conjoined latrines) was associated with increased use at that block, although the increase in use was not proportional to the block's added capacity (IRR for two doors compared to one=1.13, 95% CI: 0.96-1.33; IRR for four doors=1.71, 95% CI: 1.39-2.10; IRR for six or more doors=2.26, 95% CI: 1.73-2.93). We found some evidence suggesting latrine dirtiness was a deterrent to pupil latrine use, although the 95% CI included one (IRR=0.91, 95% CI: 0.82-1.02). Our study provides insights into factors affecting latrine use, potentially leading to a better allocation of resources for school sanitation, with the end goal to improve pupil's health and educational outcomes.

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THE IMPACT OF HOUSEHOLD IMPROVED SANITATION ON HUMAN FECAL EXPOSURE IN RURAL INDIA: APPLICATION OF MICROBIAL SOURCE TRACKING TECHNIQUES

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In India, where 626 million mostly rural people practice open defecation and 535,000 children under age five die each year from diarrhea, improved sanitation is clearly necessary to reduce fecal exposure. As part of a large cluster randomized controlled sanitation trial in Odisha, India, to measure the impact of household latrines on diarrhea diseases, human fecal contamination and exposure in intervention and control communities was measured and compared using microbial (fecal) source tracking (MST) methods based on host specific genetic markers of the gut organism Bacteroidales. MST is an emerging approach to discriminate and quantify human and other animal sources of fecal contamination. The goals of this study were (1) to measure levels of total, human and animal fecal contamination in the public domain (improved and unimproved water sources) and the domestic household domain (household stored drinking water, mother and child hand rinses) of fecal-oral disease transmission, and (2) to compare observed levels of total and human

fecal contamination in intervention and control villages for a better understanding of pathways by which latrines reduce exposure in rural India. In intervention (n = 30) and control (n = 30) villages, 20-L samples were collected from improved (public deep and private shallow tube wells for drinking, n = 209) and unimproved (community ponds for bathing and hygiene activities, n = 94) water sources along with samples from 5 to 6 households per village, comprising 300-mL of household stored drinking water (n = 348), and hand rinses of mothers (n =349) and children (n = 346), during the monsoon seasons of 2012 and 2013, after the intervention had ended. After concentration by filtration, total-, human-, and ruminant-associated markers were measured using quantitative PCR assays validated in India. Our results show that despite relatively high detection rates of total Bacteroidales in the public domain (55 to 100%), human-associated markers were rarely detected (1 to 5%). In contrast, detection rates of human-associated markers were significantly higher in the domestic domain (18 to 21%) while total Bacteroidales detection rates were lower (53 to 70%). We present and discuss results of in-depth analyses of observed levels of human fecal exposure, including effects of household latrines on the different pathways tested in the public and domestic domains of disease transmission in study communities.

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IMPACT OF A COMMUNITY-LED TOTAL SANITATION INTERVENTION ON CHILD HEALTH IN RURAL MALI: EVIDENCE FROM A CLUSTER RANDOMIZED CONTROLLED TRIAL

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Globally 2.5 billion people lack access to an improved sanitation facility; only 15% of rural households use improved sanitation in Mali (JMP 2014). Community-led total sanitation (CLTS) uses participatory approaches to mobilize communities to build their own toilets, facilitate sustained behavior change, and eliminate open defecation. Although CLTS has been implemented in over 50 countries, there is a lack of rigorous and objective data on its impacts on sanitation behaviors and child health. This cluster-randomized trial evaluated a CLTS program implemented by the government of Mali with support from UNICEF among 121 villages from the Koulikoro district of Mali. Household survey data (N=4299) collected 1.5 years post intervention delivery revealed that CLTS almost doubled access to private latrines (65% *vs.* 35%), as well as reduced open defecation rates by over 70% among adults and by 50% among children (p<0.001). CLTS households were half as likely to have human feces observed in courtyards (p<0.001). Latrines in CLTS communities were 3 times more likely to have soap and have a cover over the pit, as well as 20% less likely to have flies visible inside (p<0.001). Among children under five, CLTS did not reduce the case definition of diarrhea (relative risk [RR] 0.96, 95% CI 0.80-1.16), although risk of loose stool as measured by an image chart was reduced by 24% for those children not exclusively breastfeeding (RR 0.76, 95% CI 0.59-0.98). When accounting for baseline height, children under five in CLTS villages were taller (+0.16 height-for-age Z-score, 95% CI 0.0-0.32) and less likely to be stunted (RR 0.87, 95% CI 0.75-1.0). Improvements in child weight (+0.09 weight-for-age Z-score, 95% CI -0.04 -0.21) and a reduction in the proportion of children underweight (RR 0.86, 95% CI 0.71-1.04) were observed but were not statistically significant. This study provides evidence that a pure behavioral intervention with no monetary subsidies substantially increased access to sanitation facilities in rural Mali. CLTS may have improved child growth through pathways other than preventing diarrhea, such as lessening the subclinical condition of environmental enteropathy through potential reduced exposure to environmental fecal contamination.

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THE EFFECTIVENESS OF A RURAL SANITATION INTERVENTION ON HEALTH AND ORISSA, INDIA: A CLUSTER-RANDOMIZED, CONTROLLED TRIAL

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We undertook a cluster-randomized, controlled trial to assess the effectiveness of a rural sanitation intervention under the Government of India's Total Sanitation Campaign (TSC) to prevent diarrhoea, child malnutrition and soil transmitted helminth infection. 100 rural villages in Orissa, India were selected for participation in the study. We enrolled households with a child <4 years or a pregnant woman at the time of enrolment. Following a baseline survey, 50 villages were randomized to the intervention arm and underwent latrine promotion and construction in accordance with the TSC; control villages received no intervention. Following the implementation period, we collected and assayed stool samples for soil-transmitted helminths (STH) and provided deworming tablets to assess the rate and intensity of reinfection; we also measured the height of children <2 to assess HAZ. Thereafter, we visited study participants 7 times over 18 months, collecting self-reported diarrhoea prevalence for children <5 (primary outcome) and all members of the household as well as weights for children <5 to assess WAZ. At endline, stools were again assessed for STH eggs and heights measured for children <2. In sub-samples of households, we also assessed faecal contamination of household water supplies, hands of child caretakers and sentinel toys given to children and monitored the density of synanthropic flies that can serve as mechanical vectors of faeces. We assessed latrine coverage and use throughout the study villages with spot checks at mid-line and end-line of the surveillance period. The intervention increased mean village-level latrine coverage from 9% to 63% in intervention villages compared to an increase of 8% to 12% in control villages. 63% of households with any latrine reported using them. Health surveillance data was collected from 1437 households with children under 5 in the intervention arm (1919 <5s, 10014 individuals overall), and 1465 (1916 <5s, 10269) in the control arm. The intervention had no effect on diarrhoeal disease among children <5 (period prevalence ratio 0.97, 95%CI: 0.83-1.12) or all ages (1.02, 95%CI: 0.88-1.18). Neither did it impact HAZ for children under 2, WAZ for children <5, or the prevalence or egg count of STHs. There was no evidence that the intervention impacted contamination of household drinking water, hands or sentinel toys, or impacted density of flies in food preparation areas.

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CURRENT STRATEGIES AND CHALLENGES TO IMPLEMENTING HANDWASHING HARDWARE IN HUMANITARIAN EMERGENCIES

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Diarrhea and respiratory infections are prevalent in humanitarian emergencies but can be prevented by handwashing with soap. The challenges to handwashing promotion in emergencies have not been systematically documented. Our objective was to examine current strategies and barriers to implementation of hardware to facilitate handwashing among emergency-affected populations. We conducted key informant interviews with representatives at the global, regional or country level of agencies supporting handwashing promotion in emergencies. We identified key informants at an emergency environmental health forum and used snowball sampling to identify additional respondents with similar expertise. We coded themes based on key concepts and used content analysis to identify data trends. Our 12 respondents were

staff of United Nations agencies, non-governmental and government organizations. They reported that communal handwashing stations are common in the acute phase of an emergency but maintenance of soap and water are problematic due to lack of ownership. Organizations aim to distribute soap according to SPHERE standards but SPHERE does not recommend quantities needed for handwashing. Consistency and frequency of distribution of water dispensers and soap is highly variable in the post-acute phase due to funding constraints, prioritization by response agencies, and local market availability. There is a tradeoff between using local materials which are lower in cost and readily available and improved materials that are typically costly but more desirable. Sanitizer was not deemed a viable option for community-wide use due to acceptability, cost and sustainability. Evaluations of hardware uptake and acceptability are rare despite perceived utility of such data. Respondents indicated a strong interest in identifying hardware that is most acceptable among what is already available to emergency-affected populations. Assessing the type of soap most attractive to populations and how hardware choices affect handwashing behavior could provide useful guidance to improve handwashing promotion during emergencies.

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HANDWASHING BEHAVIOR CHANGE STRATEGIES IN HUMANITARIAN EMERGENCY SITUATIONS: THE PERSPECTIVES OF EXPERTS

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Handwashing can prevent diarrhea and acute respiratory infections, but is practiced infrequently in long-standing refugee camps. Little information is available on handwashing behavior change strategies targeting displaced persons during emergencies. We conducted key informant interviews between February and April 2013 with professionals supporting water, sanitation and hygiene (WASH) services for emergency-affected populations to understand behavior change strategies and to identify research gaps. We used purposive and snowball sampling to identify experts involved in handwashing promotion. Twelve respondents representing United Nations agencies, non-governmental organizations, and a government agency were interviewed. Respondents reported that technical and infrastructure aspects of WASH are not well coordinated with behavioral approaches; behavior change strategies are less of a priority. Typically, implementing organizations set no specific goals related to improving handwashing practices. Respondents described a dearth of behavior change expertise at the global, regional and local levels. Information on pre-existing knowledge and practices related to hand hygiene is generally not collected prior to implementation of handwashing promotion. Messages focusing on health benefits are given over prolonged timeframes, with little effort to understand motivators to handwashing or assess effectiveness and modify messages. A relatively unskilled workforce is expected to deliver often complex, participatory methods to improve behavior. Effectiveness of behavior change strategies is rarely assessed. Our findings underscore the need to strengthen behavior change expertise at all levels. The current reliance on anecdotal evidence hampers promotion of appropriate handwashing behavior. Lack of understanding of pre-existing behaviors and motivators for handwashing restricts adaptations to the local context and likely undermines behavior change efforts. Establishing specific behavior change objectives and developing contextually specific approaches could improve the effectiveness of handwashing promotion in emergency settings.

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DETERMINANTS OF MORTALITY AMONG HUMAN IMMUNODEFICIENCY VIRUS AND TUBERCULOSIS (HIV/TB) CO-INFECTED PATIENTS IN AMINU KANO TEACHING HOSPITAL, KANO, NIGERIA

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Management of HIV and tuberculosis co-infection remains a major challenge for clinicians and public health authorities. We conducted a prospective cohort study to determine risk factors of death among HIV/TB co-infected patients in Aminu Kano Teaching Hospital, Kano, Nigeria. We recruited 160 consenting, newly diagnosed HIV/TB co-infected patients 18 year or older between June and December 2012. We diagnosed TB by clinical features and any of positive sputum acid fast bacilli (AFB), chest radiographic features of tuberculosis or biopsy proven TB adenitis. We excluded patients with previous exposure to anti-TB or anti-retroviral drugs. We administered structured questionnaires to collect socio-demographic, clinical and laboratory information. Patients started on highly active antiretroviral therapy (HAART) within 8 weeks of starting anti-TB were considered as commencing early treatment; delayed commencement defined as starting HAART after 8 weeks of starting anti-TB. Patients who reported ever missing a dose of either or both of anti-TB and HAART drugs were considered as having sub-optimal adherence. All patients were followed up for 6 months. We conducted bivariate and multivariate analyses to determine independent risk factors of death during the study. A total of 71 (44.4%) patients were females. The median (IQR) age was 33 (28 – 401) years). The mean (range) hospital stay for admitted patients was 20 (5 – 37) days. The mean CD4 counts for patients who commenced early and those who delayed treatment were 144 cells/mm^{3}and 112 cells/mm^{3}respectively. On bivariate analysis, sputum AFB positivity HR (P-value): 3.1 (0.01); Hepatitis C co-infection HR (P-value): 9.8 (0.03) and sub-optimal adherence HR (P-value): 4.3 (0.001) to increase risk of dying among HIV/TB co-infected patients. In contrast, early commencement of HAART HR (P-value): 0.2 (<0.001) was found to decrease risk of dying. On multivariate analysis, risk of dying was reduced by early commencement of HAART HR (P-value): 0.2 (0.005), while hepatitis C co-infection HR (P-value): 12.3 (0.03) and sub-optimal adherence HR (P-value): 2.8 (0.04) remained independent risk factors of death. Early commencement of HAART among HIV/TB co-infected patients improves survival. Clinicians should adhere to universally accepted guidelines on timing of commencement of HAART. Sub-optimal adherence should be addressed by strengthening adherence units in HIV programs.

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RECOVERY OF CD4+ T CELL COUNTS IN HIV-ASSOCIATED TUBERCULOSIS ACCORDING TO AGE, NUTRITIONAL STATUS AND ANTIRETROVIRAL THERAPY

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We quantified associations between age and the monthly change in absolute CD4+ T cell count among HIV-infected tuberculosis (TB) patients during six months of TB therapy with or without concurrent antiretroviral therapy (ART). We determined whether this association is modified by sex and baseline nutritional status. The parent study for this secondary analysis was a randomized clinical trial of concurrent versus delayed ART during TB treatment. The study population included 208 non-pregnant, HIV-seropositive TB patients who had CD4+ T cell counts >350 cells/μL and were naïve to anti-retroviral therapy. Age at enrollment was

defined in years within categories as ≤24, 25-29, 30-34, 35-39 and ≥40. Nutritional status was classified as normal (BMI > 18.5 kg/m2) or low (BMI ≤18.5 kg/m2). Multivariate random effects linear regression models were used to estimate mean differences in absolute CD4-cell count in relation to concurrent ART status and baseline age. Concurrent ART during TB therapy was associated with a monthly rise of 15.7 CD4+ T cells/μL (P<0.0001). There was no overall difference in CD4+ T cell response by age during TB therapy (P=0.6550). However, among patients who received ART with TB treatment, greater gains in CD4+ T cell counts were seen among younger patients (age*time*ART, p-value=0.0443), whereas the same effect was not seen among patients who delayed ART. This inverse association between age at ART initiation and CD4+ T cell increase during concurrent ART and TB therapy was strongest in females (p-value: age*time=0.0059) and in patients with BMI ≥18.5kg/m2 at enrollment (p-value: age*time=0.0061). Our findings suggest that older HIV-seropositive patients on ART might experience a slower rate of immune restoration, especially if female or BMI ≥18.5kg/m2 at initiation. Older HIV-positive patients may benefit from closer monitoring of immune status and nutritional support during ART.

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FOOD INSECURITY, DIET DIVERSITY AND BMI OF HIV INFECTED INDIVIDUALS ON ANTIRETROVIRAL THERAPY IN RURAL HAITI

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Food insecurity and malnutrition are both important risk factors for poor outcomes in the treatment of people living with HIV. Food supplementation is increasingly offered in conjunction with HIV programs. Current programs focus on those with acute or chronic malnutrition. The optimal target population is unknown. We evaluated 490 HIV-infected individuals receiving antiretroviral therapy (ART) in rural Haiti. Baseline data including body mass index (BMI), household food security (using the Household Hunger Scale), socioeconomic status (SES), medication adherence and dietary diversity were collected via structured interview. We analyzed factors associated with low BMI and severe food insecurity using logistic regression. Moderate to severe food insecurity was present in 89% of individuals. Amongst severely food insecure subjects, 49% had a normal or above normal BMI. After adjusting for age, sex, BMI and presence of a garden in the home, severe food insecurity was associated with relative poverty [odds ratio (OR) 2.37, 95% confidence interval (CI) 1.58 - 3.54, p<0.001], illiteracy (OR 1.79, 95% CI 1.19 - 2.71, p< 0.01), and having no source of income generation (OR 1.63, 95% CI 1.04 - 2.56, p< 0.05). Individuals with severe food insecurity had a less diverse diet, with less frequent consumption of proteins. Food insecurity was highly prevalent in patients with HIV infection receiving ART. Normal or high BMI did not rule out severe food insecurity. Current guidelines regarding the use food support to supplement HIV treatment that are based on BMI will miss a significant proportion of patients who may benefit from this intervention.

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HIV AND CHAGAS DISEASE: AN EVALUATION OF THE CLINICAL PRESENTATION AND THE USE OF REAL-TIME QUANTITATIVE PCR TO MEASURE LEVELS OF *TRYPANOSOMA CRUZI* PARASITEMIA IN HIV PATIENTS

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With the persistence of Chagas disease in Latin America and with increased migration from rural to urban settings- where rates of HIV/AIDS are increasing- there has been an increase in the number of persons co-infected with HIV and Chagas disease in recent years. This study evaluated the clinical presentation and laboratory results of patients co-infected with HIV and Chagas in Cochabamba, Bolivia, with a focus on the levels of *Trypanosoma cruzi* parasitemia measured by real-time quantitative PCR (qRT-PCR) of blood clot. Clinical evaluation, electrocardiogram, chest radiograph, CD4 count, viral load, serology for *T. Cruzi*, direct microscopy of blood, and real-time PCR of blood clot was performed on each patient. Some patients also have a sample for qRT-PCR from whole blood with EDTA. 38 of the 133 HIV patients evaluated were co-infected with Chagas disease (28.6%). Four of the 38 (10.3%) were positive by direct microscopy, thus meeting the criteria for reactivation. Two of the patients with reactivation had very high levels of parasitemia (>1000 parasites/ml) by qRT-PCR of clot and by whole blood, however two patients with reactivation had no parasitemia detected by qRT-PCR of clot and only low levels by whole blood (<40 parasites/ml). Our results demonstrated that high levels of parasitemia are associated with high HIV viral loads and low CD4 counts; however, quantifiable levels of parasitemia did not show a strong correlation with symptoms of Chagas disease or reactivation. Although the levels of *T. cruzi* parasitemia detected by qRT-PCR do not show direct correlation with reactivation of Chagas disease or with clinical symptomatology in all patients, the level of parasitemia in HIV patients may be an indication of those at risk for progression of disease. Further studies are needed to determine the significance of parasitemia detected by PCR, and whether asymptomatic patients with detectable *T. cruzi* parasitemia should be treated with antiprotozoal agents.

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RISK OF ANTIRETROVIRAL TREATMENT FAILURE AMONG CLINICALLY STABLE ADULTS IN BLANTYRE, MALAWI

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Millions of people living with HIV infection are receiving antiretroviral therapy (ART) in sub-Saharan Africa without access to routine viral load monitoring due to its expense and associated logistical challenges. If those likely to have detectable HIV viral load could be identified by readily identifiable risk factors, such patients could be prioritized for targeted viral load monitoring. Targeted monitoring in patients who are clinically well will allow for interventions before they develop severe illness or advanced ART resistance and will likely be a more affordable and feasible strategy than universal testing. As part of an on-going clinical trial at Ndirande

Health Centre in urban Blantyre, we conducted viral load and CD4 count assessments for 945 healthy adult outpatients who had been on ART for a minimum of six months. Ninety (10%) had HIV viral load >1000 copies/mL and 64 (7%) >5000 copies/mL. Among patients with a detectable viral load of greater than 40 copies/mL, the geometric mean was 3157 copies/mL (95% CI 2083 - 4786 copies/mL). The median CD4 cell count was 454 cells/mm3 (95% CI 433 - 475), 147 (16%) had a CD4 cell count <250 cells/mm3. We will use logistic regression to investigate factors associated with elevated viral load. The covariates in the modelling will include age, sex, BMI, CD4 cell count, reason for ART initiation, current ART regimen, duration of ART and self-reported adherence. We will also explore the association between infections including TB and malaria and viral load. Our goal is to identify patient characteristics that are associated with increased risk of virological failure on ART that can be identified prior to overt ART clinical failure. Targeted viral load testing among high risk individuals may be a cost-effective strategy to improve patient survival and limit the spread of ART resistance in resource-limited settings.

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TRYPANOSOMA CRUZI PARASITEMIA IN IMMUNOSUPRESSED PATIENTS WITH HIV INFECTION OR ORGAN TRANSPLANT RECIPIENTS

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Chagas disease is now an active and emergent disease in urban centers of endemic and non endemic areas and affects around 7 million inhabitants in Latin America and a contingent of infected immigrants in other continents. Considering the high morbimortality of chronic Chagas disease reactivation associated with immunosupression, we analyzed the levels of parasitemia in immunosupressed patients under solid and stem cells transplantation (N=12) and in patients with HIV/*Trypanosoma cruzi* co-infection (N= 43 patients). Blood samples from organ transplants recipients were analyzed between the 4-6 months post transplant. A control group of 91 patients with Chagas disease was also included. Inclusion criteria for Chagas disease and HIV infection were the presence of anti-*Trypanosoma cruzi* antibodies by ELISA or immunofluorescence and presence of antibodies for HIV antigens by ELISA confirmed by imunoblot, respectively. For diagnosis of Chagas disease reactivation, the parasite was identified by direct microscopy on peripheral blood or other biological fluid. Quantitative PCR was performed with genomic sequencies as previously described (Freitas *et al,* 2011). Higher parasitemia was observed in the co-infected group in comparison to the control group with Chagas disease, and is related to the presence of reactivation of Chagas disease in seven HIV infected patients in this group. Increased parasitemia was detected in two of the seven kidney transplant patients: one patient with reactivation of Chagas disease, and one patient without reactivation but increased number of parasite DNA copies patient (higher than 200x the pre transplant levels). Increased parasitemia is also observed in co-infected HIV/*T. cruzi* patients without reactivation in association with high parasitemia showed in xenodiagnosis test (higher than 20% of nymphs positive in each exam). Monitoring parasitemia by quantitative PCR should be considered as an useful tool for the management of chronic Chagas diseases in patients under immunosupression.

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COPING WITH CHRONIC DISEASE AND DISABILITY IN ISOLATED COMMUNITIES OF THE PERUVIAN AMAZON: A CASE SERIES

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Healthcare, physiotherapy and social services are limited in the Peruvian Amazon and visits from boat-clinics infrequent. Qualitative research in Peruvian Amazonian communities concerning the impact of chronic disease and disability on households is scarce. We aimed to explore real-life situations faced by families in which a family member had chronic disease or disability. Whilst conducting a descriptive pilot project of patients accessing healthcare from a mobile boat-clinic serving 13 Peruvian Amazon communities, we performed four qualitative, extended case studies with consenting families. Interviews were performed in patient households and explored disease and disability burden, healthcare access, social support, and use of traditional and modern medicine. All interviews were reviewed with a translator and transcribed in Spanish and English. Case 1: Mrs B, a 52 year old school teacher, experiences chronic, inflammatory small joint pains for which she takes ibuprofen and a self-prepared natural home remedy. Case 2: Mr C, a 49 year old subsistence farmer, has hypertension managed quarterly by a visiting medical boat and lives with his bedbound 85 year old mother without social support. Case 3: Mrs P, a 56 year old subsistence farmer, consulted the mobile medical boat-clinic due to problems with her 6 year old daughter's speech and mobility due to which she has been denied local schooling. During the consultation her daughter was diagnosed for the first time with cerebral palsy. Case 4: Miss R, a 29 year old female, was diagnosed with epilepsy when she was 13 years old and after initial local treatment with traditional medicine has been intermittently treated by a distant clinic (3-4 hours by boat) and visiting medical boats. Miss R was also more recently diagnosed with pseudoseizures. Her seizure activity remains uncontrolled. In conclusion, these cases exemplify the difficulties of coping with chronic disease and disability in the Peruvian Amazon with constrained healthcare infrastructure and minimal formal social support. The cases reveal ongoing use of traditional medicine in addition to modern medicine potentially relating to local health beliefs and reduced access to modern healthcare. Whilst mobile medical boats may be an adjunct to existing local healthcare services, treating chronic diseases such as hypertension or epilepsy, is limited by infrequent and sporadic consultations.

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DEVELOPMENT AND EVALUATION OF MULTIMEDIA INFORMED CONSENT TOOL FOR A LOW LITERACY AFRICAN RESEARCH POPULATION

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International guidelines recommend the use of appropriate informed consent procedures in low literacy research settings because written information is not known to guarantee comprehension of study information. This study developed and evaluated a multimedia informed consent tool for people with low literacy in an area where a malaria treatment trial was being planned in The Gambia. Methods: We developed the informed consent document of the malaria treatment trial into a multimedia tool integrating video, animations and audio narrations in three major Gambian languages. Acceptability and ease of use of the multimedia tool were assessed using quantitative and qualitative

methods. In two separate visits, the participants' comprehension of the study information was measured by using a validated digitised audio questionnaire. The majority of participants (70%) reported that the multimedia tool was clear and easy to understand. Participants had high scores on the domains of adverse events/risk, voluntary participation, study procedures while lowest scores were recorded on the question items on randomisation. The differences in mean scores for participants' 'recall' and 'understanding' between first and second visits were statistically significant ($F(1, 41) = 25.38$, $p<0.00001$ and $F(1, 41) = 31.61$, $p<0.00001$ respectively. In conclusion, our locally developed multimedia tool was acceptable and easy to administer among low literacy participants in The Gambia. It also proved to be effective in delivering and sustaining comprehension of study information across a diverse group of participants. Additional research is needed to compare the tool to the traditional consent interview, both in The Gambia and in other sub-Saharan settings.

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PANDEMIC INFLUENZA PREPAREDNESS IN CAMBODIA: AN ECONOMIC EPIDEMIOLOGICAL DECISION ANALYSIS

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Pandemic burden is predicted to be high in low income countries, relative wealthy well-resourced countries. Yet evaluations of cost effective pandemic preparedness investments focus almost exclusively on high income countries. We undertake an economic evaluation of two pandemic preparedness investment options in Cambodia. We developed a dynamic pandemic simulation model including age structure, health system capacity and cost of illness. The model was used to perform cost utility analysis (CUA) on three pandemic preparedness investment options i) antiviral stockpiling and ii) additional mechanical ventilators and iii) a 1:1 mixed investment strategy. The analysis was repeated at three investment levels US$250k, US$4 million and US$20 million. Due to the inherent uncertainty in pandemic modelling we use sampling distributions in place of single point estimates for most parameter inputs. We also present a cost-consequence analysis (CCA) to place model results within the context of non-quantified costs and consequences. At investment levels of US$250k and US$4 million stockpiling is most cost effective, investment level of US$20 million a mixed investment is preferable, reflective diminishing marginal returns from increased stockpile size. However there is substantially more uncertainty in incremental cost effectiveness ratio (ICER) estimates for antiviral stockpiling. Also, the CCA highlights that investing in ventilators would have considerable utility between pandemics. It is likely that both antiviral stockpiling and investment in mechanical ventilators are cost effective pandemic preparedness options. The caveats to this are the considerable uncertainty inherent in these estimates and that depending on the payer, they may or may not be affordable or the most urgent public health investment for Cambodia. Note: In light of the recent Cochrane publication on antiviral effectiveness (10th April 2014) we will be updating our analysis within the coming weeks.

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RELATIONSHIP OF ANGIOGENIC AND INFLAMMATORY BIOMARKERS AT MID-PREGNANCY TO SMALL-FOR-GESTATIONAL AGE OUTCOMES IN A PROSPECTIVE COHORT OF TANZANIAN WOMEN

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Intrauterine growth restriction (IUGR) is a major public health problem that affects an estimated 27% of pregnancies in low and middle-income countries. Inadequate fetal growth is associated with increased risk of neonatal morbidity and mortality as well as developmental delay and cardiometabolic disorders in adulthood. We examined the relationship between a panel of angiogenic and inflammatory biomarkers measured in mid-pregnancy and small-for-gestational age (SGA) outcomes in Tanzanian women. Plasma samples were collected from a prospective cohort of 432 primigravid women at enrolment (12-27 weeks gestation). Levels of 18 biomarkers (Ang-1, Ang-2, Ang-L3, VEGF, sFLT-1, sTNFR2, PlGF, MIP-1β, MCP-1, Leptin, IL-1β, IL-18 BP, sICAM-1, FAC-D, sEndoglin, CRP, CHI3L1, C5a) were analyzed by ELISA. Infants falling below the 10th percentile of birth weight for gestational age relative to the applied growth standards were considered SGA. Multivariate binomial regression models were used to determine the relative risk of SGA associated with levels of each biomarker. Receiver operating curves obtained from multivariate logistic regression models were used to assess the discriminatory capability of selected biomarkers. A total of 60 participants (13.9%) gave birth to SGA infants. Compared to those in the first quartile, the risk of SGA was reduced among those in the fourth quartiles of VEGF-A (adjusted risk ratio (RR) 0.38, 95% Confidence Interval (CI), 0.19-0.74), PGF (adjusted RR 0.28, 95% CI, 0.12-0.61), sFlt-1 (adjusted RR 0.48, 95% CI, 0.23-1.01), MCP-1 (adjusted RR 0.48, 95% CI, 0.25-0.92), and Leptin (adjusted RR 0.46, 95% CI, 0.22-0.96). Our findings provide evidence of altered angiogenic and inflammatory mediators, at mid-pregnancy, in women who went on to deliver small for gestational age infants. Studies are currently under way to validate these findings in both internal and external cohorts.

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MULTINATIONAL DISEASE SURVEILLANCE PROGRAMS FOR CROSS-BORDER EPIDEMIOLOGIC INFORMATION EXCHANGE

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Cross-border disease surveillance and response efforts in regions sharing borders depend on effective international collaboration. The Border Infectious Disease Surveillance (BIDS) program facilitates epidemiologic information exchange between the United States and Mexico. To understand the global context for BIDS, we conducted a survey of multinational disease surveillance programs (MNDSPs) operating worldwide. Our survey assessed program organization, goals, operations, decision-making processes, and evaluation. From January 2013 to March 2014, we identified MNDSP representatives through internet search and querying domestic and international colleagues. We contacted 12 MNDSPs and obtained responses from ten programs spanning all continents except Antarctica. In general, responding programs aim to enhance epidemiologic surveillance capabilities, strengthen cooperation for infectious disease monitoring and prevention, and increase consistency among countries. Reportable conditions are jointly selected by participating countries based on common importance. Most programs have specific algorithms for disease surveillance and laboratory testing. Eight (80%) of the ten programs have a central database that obtains information through

manual data entry or electronic linkage. E-mail is the primary mode of communication. Eighty percent of the programs have multinational emergency notification contact lists. All ten programs meet at least annually in person or via video or teleconference. Fewer than half (40%) of the programs share specimens or laboratory testing reagents among countries. Local and national public health laboratories are the primary infrastructure for diagnostic testing. Seven of the ten programs have dedicated funding allocated to MNDSP operation. Few programs have implemented a routine quality assurance program. Despite variation in health priorities, geography, and socioeconomic context, our survey identified key operational commonalities among MNDSPs and provides an important perspective on global disease surveillance efforts.

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KNOWLEDGE, ATTITUDES AND BEHAVIOR TOWARD PARASITE INFECTIONS AMONG HIGH SCHOOL STUDENTS IN SOLOMON ISLANDS

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Pacific region is an epidemic area of different parasite infections. The countries have provided examinations and medical supports to their citizens. However, few statistic data and information on attitudes and behavior toward parasite infections are available. The survey was trying to investigate the knowledge, attitudes and behavior toward parasite infections among high school students in Solomon Islands. The survey was conducted in August, 2012. Total of 679 participants, aged from 12 to 18 years old, were randomly selected from St. Nicholas and St. Joseph high schools. Approximately 95% of participants indicated that they had clean water for food preparation or cooking, however nearly 72% showed they didn't have drinking water boiled. 67% and 13% of the students said the main source of their drinking water was from rain and bottled water, respectively. 90% of the students had toilets at home, and 82% indicated they had the habit of hand washing. However, up to 53% of participants didn't wear shoes, while 40% with excessively long fingernails. Nearly 28% of them had been diagnosed for parasite infections. 40% claimed they had never learned about parasite prevention. A screening program executed by Taiwan Health Center in Solomon Islands showed the prevalence of parasite infection among local students was up to 34%. The understanding of parasite prevention among high school students in Solomon Islands is still deficient, and it is not taught as part of the curriculums in schools. Appropriate medical and educational resources need to be prompted to make significant changes in parasite prevention.

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A LOW-COST ICT TOOL KIT FOR IMPROVED DENGUE SURVEILLANCE, LABORATORY MANAGEMENT AND CLINICAL DECISION SUPPORT IN NICARAGUA

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Dengue is a mosquito-borne viral disease of major medical and public health importance worldwide. We have developed and tested three low-cost informatics tools in Nicaragua to help improve laboratory, surveillance and clinical management practices to reduce dengue-related morbidity and mortality. As part of the Dengue FIRST initiative (Fighting Infections through Research, Science and Technology), these tools were developed collaboratively between the NGO the Sustainable Sciences Institute and the Ministry of Health of Nicaragua. Input from stakeholders at the national, state and local levels was incorporated at multiple phases. "DENGUE-ALERT" is an innovative automated early warning system for outbreak detection and response that provides a customizable "dashboard" with information from traditional epidemiological disease reports and entomological, climatic and crowd-sourced data. It targets a wide range of end-users including public health and community-based actors and is designed to enable earlier detection and reporting of virus circulation or outbreak indicators to increase the efficiency of response and resource use. "DENGUE-SPECIALIST" is a web-based mobile application designed to improve the efficiency and accuracy of clinical data access and analysis in hospitalized dengue cases. End-users are clinicians and nurses in public sector hospitals. Simulations of SPECIALIST were tested with data from multiple years of a clinical study in a Nicaraguan public hospital. "DENGUE-LAB" is a web-based platform that supports a national-level information management system for integrating laboratory results and reporting to streamline information flow around the numerous tests used for dengue diagnosis. LAB is designed to improve quality control measures, simplify and automate some of the complexities of dengue diagnosis, and impact both the quality and the reliability of diagnostic results. Both quantitative and qualitative indicators were used in a mixed method evaluation of the pilot of these tools to assess their ability to support earlier and more accurate disease response and outbreak prevention. Following this design and testing phase, the goal is to extend the use of these tools to Mexico and to other Central American countries that are interested and able to adapt and implement "customized" versions of ALERT, SPECIALIST and LAB as appropriate in their country contexts.

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DENGUE TORPEDO: A NOVEL APP TO MOTIVATE COMMUNITY-BASED DENGUE VECTOR CONTROL

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Dengue is a mosquito-borne viral disease that continues to expand geographically in part due to failed efforts in vector control. Residents of communities affected by dengue are potentially the best agents to control vector breeding sites, since the *Aedes* mosquitoes that transmit dengue virus breed in clean water in and around people's homes. We present the design and piloting of Dengue Torpedo (DT), an interactive cellphone and web platform that combines mobile technology and game concepts to motivate community residents to report and eliminate mosquito breeding containers. DT (a) crowdsources the identification and mapping of breeding sites (problem of information); (b) motivates residents to act (problem of execution); (c) develops a collaborative model of software production that involves local user-residents in researching, testing, and designing the application; (d) promotes civic engagement and active citizenship; (e) involves residents in public health education; and (f) engages youth in the creative development of communication technologies. Using a community-based collaborative model of software design and development, we created alpha and beta versions of DT in Rio de Janeiro, Brazil. The mobile and web interface is interactive and allows residents to create their own profiles and exchange information about dengue in their neighborhoods. DT also has an educational component that relates to other relevant issues in the community as well as specific information regarding dengue. Players earn badges and points that can be exchanged for community or personal prizes donated by local sponsors and small businesses. DT has 5 participatory features in dengue vector control that make it pioneering: it is interactive, connects mobile and web technologies, uses gameplay to motivate residents, institutes a community-based collaborative model of app development, and helps local institutions sponsor related educational activities. In parallel, we are developing DT for the Mexican health sector, incorporating contextually appropriate information and designing the interface with input from community members and researchers in Morelos, Mexico. DT can improve

public participation in combating dengue, generate new correlations and visualizations of scientific data of potentially great scale and low cost, reduce mosquito infestation, and eventually decrease rates of dengue infection and disease.

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OPENHDS: USE OF TABLET COMPUTERS IN HEALTH AND DEMOGRAPHIC SURVEILLANCE

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Health and Demographic surveillance systems (HDSS) can provide essential information in areas where routine vital registration is absent or incomplete. also play an essential role in health intervention studies in such areas. Setting up and running an HDSS poses an operational challenge, and a reliable and efficient platform for data collection and management is a key prerequisite. OpenHDS is an HDSS data system that provides data entry, quality control, and reporting to support demo-graphic and health surveillance. It consists of two components: web and mobile. OpenHDS mobile is integrated with the Open Data Kit (ODK) a software platform for data collection using mobile devices running the Android operating system, and allows direct data entry using tablet computers. This offers a number of advantages over traditional paper-based systems: it reduces the workload of the data management team, no IDs need to be typed in (removing one of the biggest causes of errors on data collection in HDSS systems); it allows for near real-time quality control; and it can provide guidance for the project logistics. The web interface allow viewing of the data collected and correction of errors/perform eventual amendments. Here we present an overview of the openHDS/ODK software platform, and report on the experience of using this platform to set up a HDSS in support of a trial of odour baited traps as a malaria intervention study on Rusinga Island, Kenya, (Solarmal) Project. We also present the experience of migrating the data systems of established HDSS sites from an older system (HRS) to openHDS (Ifakara and Rufiji, Tanzania). We show how OpenHDS addresses specific operational problems and the use of the complete OpenHDS/ODK system for data collections offers a number of advantages over paper-based systems both with respect to data quality and cost savings.

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AVAILABILITY OF SIGNAL FUNCTIONS AND QUALITY OF EMERGENCY OBSTETRICS CARE FOR POPULATION IN RURAL BANGLADESH

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The maternal mortality ratio in Bangladesh is high at 220 deaths per 100,000 live births. Emergency obstetrical care (EmOC) interventions in health facilities are effective at reducing maternal mortality. Facility deliveries in Bangladesh have grown from 16% in 2000-2006 to 29% in 2007-2012. This study assessed the quality of obstetrical services in health facilities serving rural areas of Bangladesh in order to identify opportunities to improve EmOC. We randomly sampled 50 rural villages in 46 of 64 districts of Bangladesh and interviewed the administrator of 8-9 hospitals nearest to each sampled village. We categorized the quality of EmOC at each hospital according to its capacity to provide 6 basic EmOC signal functions (administrations of antibiotics, oxytocin, and anticonvulsant; assisted vaginal delivery; manual removal of placenta and removal of retained products after delivery), staffing, and availability of phone and ambulance for referring patients. EmOC quality categories included high (6 signal functions; ≥3 staff on call [24-hour coverage]; an ambulance and a phone), moderate (≥4 signal functions; ≥2 staff [or no 24-hour coverage]; a phone), low (≥2 signal functions; ≥1 staff; a phone), and sub-standard (no minimum criteria). Administrators of 432 hospitals were interviewed. Administration of antibiotics was available in 99% of the hospitals, whereas anticonvulsant administration was only available in 65%. The quality of EmOC was high in 31%, moderate in 55%, low in 4%, and sub-standard in 9% of the hospitals; 32% did not have 24-hour coverage of skilled birth attendance. Approximately one-third of health facilities providing obstetric care in rural Bangladesh lack anticonvulsants needed to manage eclampsia, an important cause of maternal mortality. Similarly, the lack of 24-hour availability of skilled birth attendants increases the risk of peripartum complications. Given the high rate of maternal mortality, there is a pressing need to improve provision of EmOC in health facilities in rural Bangladesh.

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OCCURRENCE OF SEVERE DENGUE FEVER IN AN ENDEMIC CITY OF BRAZIL: AN ECOLOGICAL STUDY

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Background: Brazil accounts for more than 70% of dengue cases notified on the American continent. In this context, Rio de Janeiro became one of the most endemic cities in the country during the last decades, presenting a long history of co-circulation of all four dengue serotypes with a recent trend for the clinical and epidemiological profile of the disease to change. Methods: This ecological study aimed to analyze the relationship between incidence of severe dengue during the 2008 epidemic in Rio de Janeiro City and socioeconomic, previous circulation of other dengue serotype and health service availability indicators. The data was incorporated into a negative binomial regression model. Results: Districts with more cases of dengue in the 2001 epidemic and where higher percentages of the residents who declare their skin color/race as black showed higher incidence rates of severe dengue in the 2008 epidemic. Meanwhile, districts with lower incidences of severe dengue in 2008 were those with more Family Health Strategy (FHS) clinics. Conclusion: The findings suggest persisting health inequities possibly due to greater socioeconomic vulnerability among black population. Additionally, the protective effect of FHS clinics may be due to facilitated access to other levels of health care or even by reducing vulnerability to transmission afforded by local practices in health promotion. These aspects reinforce the importance of better understanding of social determinants in order to identify key-points for developing and implementing interventions for dengue control.

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CLIMATE-DRIVEN DENGUE EPIDEMIC EARLY WARNINGS FOR BRAZIL

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In this study, we address the potential to predict dengue fever epidemics across Brazil, during the peak dengue season (austral summer period). We produce probabilistic dengue forecasts for the 553 microregions of Brazil, from 2000 to 2014, using a novel spatio-temporal modelling framework. The dengue forecasts are driven by multi-model ensemble seasonal climate forecasts and the observed epidemiological situation in Brazil at the forecast issue date. The multi-model ensemble comprises several quasi-operational forecast systems, among them two systems

from the European EUROSIP initiative and six systems from the North American Multi-Model Ensemble (NMME) project. This precursory information allows dengue warnings to be released three months ahead. We evaluate the past performance of the dengue early warning system by verifying probabilistic predictions against out-of-sample observed data. Timely dengue early warnings provide the opportunity for the ministry of health and local authorities to implement appropriate, city-specific mitigation and control actions. This model framework could be applied to predict outbreaks of other climate-sensitive diseases in other parts of the world. This is especially pertinent as climate change is likely to make diseases, such as dengue and malaria, more widespread. The successful implementation of seasonal climate forecasts in disease early warning systems depends on close collaboration between public health specialists, climate scientists and mathematical modellers. The overall objective of this study is to bring awareness to scientists, policy makers and international health surveillance teams of the data and tools required in order to make timely predictions. We hope that this early warning framework will help to improve prevention strategies for vector-borne diseases and establish a landmark towards the use of climate data to benefit society.

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COMMUNITY HEALTH WORKER TIME USE: METHOD EVALUATION AND TIME USE FINDINGS IN MALAWI

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Understanding community health worker (CHW) workload is critical for maintaining program quality in the face of competing tasks. Time motion studies are considered the gold standard in time use research, but are prohibitively time and resource intensive. Four alternative measurement methods (3 interview methods and time diary) were piloted to assess time use among CHWs as a subcomponent of a cost effectiveness evaluation of the Rapid Scale-up of MNCH in Malawi in 2013. Based on pilot results, 2 interview methods (week-long vs. year-long reference period) were deployed to measure time use among 248 CHWs (126 implementing community cases management (CCM)) in 6 districts. Reported total hours and hours spent on CCM activities in an average week were higher and more variable using the year-long vs. week-long interview method. Correlation between methods in estimated work hours/week (0.12) and CCM hours/week (0.27) was very low. Based on implausible variability in time use estimated by the year-long method, final results were reported using the week-long method only. On average a CHW reported working 42.29 (95% CI: 38.88, 45.70) hours/week and those who implemented CCM spent 18.08 (95% CI: 16.24, 19.92) hours/week on CCM. CCM (Adjusted coeff: 10.32; 95% CI: 5.09, 15.56) and each additional intervention implemented (Adjusted coeff: 1.86; 95% CI: 0.81, 2.90) increased total number of hours worked per week. CHWs who implemented CCM spent significantly less time on nutrition (Coeff: -2.24; 95% CI: -3.12, -1.37), HIV (Coeff: -3.49; 95% CI: -6.56, -0.42), and community based maternal and newborn health interventions (Coeff: -1.31; 95% CI: -2.33, -0.30), compared with CHWs implementing those services but not CCM. Findings highlight the importance of an accurate and efficient means of assessing CHW time use to determine feasibility and impact of introducing additional tasks. Time use interviews offer promise for collecting time use data when a large number of CHWs are targeted, although further refinement and validation against a gold standard time assessment is needed.

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BREASTFEEDING PRACTICES AND CHILDHOOD DIARRHEA MANAGEMENT AMONG WOMEN IN RURAL COMMUNITIES IN MOZAMBIQUE

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Early childhood nutritional practices, including exclusive breastfeeding for the first 6 months of life and adequate hydration of children with diarrhea, are critical for minimizing morbidity and mortality from infectious causes in the developing world. Gorongosa National Park in Mozambique is surrounded by rural communities with significant geographic and economic barriers to health resources. These communities are located in two groups: those located in the mountain region and those in the plains region, with the mountain communities being especially isolated. In 2011, a comprehensive household survey was conducted to study health behaviors in these regions as part of an ongoing Ecohealth Program. 1625 women of reproductive age were surveyed by trained interviewers in the local language: 1074 women from 4 mountain communities and 551 women from 2 plains communities. 192 women with children age 6 months or younger answered questions about breastfeeding, and 1035 women with children age 5 years or younger answered questions about previous nutrition and diarrhea education, and diarrhea management. Results showed that women in the plains communities were more likely to have received education about early childhood nutrition vs. women in the mountain communities (85% vs. 28%, p<.0001), but in both populations, there was no association between previous nutrition education and exclusive breastfeeding (plains: RR .85, CI .31-2.28; mountain: RR 1.01, CI .81-1.26). In the mountain communities, 46% of women withhold water completely during a child's episode of diarrhea; however, those who received previous education were significantly more likely to hydrate their child though the episode (RR 3.43, CI 2.19-5.38). No such association was found in the plains communities (RR 1.10, CI .92-1.32), where only 7% of women will withhold hydration. These results can be used to tailor a planned intervention involving a new nutrition educational program. The data suggest that previous programs did not effectively teach best breastfeeding practices, and that there is an opportunity to increase the number of women exclusively breastfeeding, especially in the plains communities where women are more likely to use breast milk alternatives. The data also suggest that prior education about rehydration in diarrhea has been successful in the mountain communities, and any future nutrition programs should model educational materials accordingly.

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ACCEPTABILITY AND EFFECTIVE USE OF A RAPID HOME DIAGNOSTIC TEST FOR MULTIPLE INFECTIOUS DISEASES: FORMATIVE RESEARCH IN PERU AND CAMBODIA

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In 2012, the U.S. Defense Threat Reduction Agency Joint Science and Technology Office initiated a program to develop novel point-of-need

diagnostics for surveillance of emerging infectious diseases including dengue, malaria, plague, and melioidosis. Prior to distribution of devices to community members in Iquitos, Peru, and Phnom Penh, Cambodia, research was conducted to assess acceptability of use, determine conditions in which use would occur, examine incentives to use the device on someone with fever >5 years of age in their home, and assess device use competency. In February 2014, 9 focus group discussions (FGD) with community members and 5 FGD with health professionals were conducted in Iquitos, and 9 FGD with community members and 9 in-depth interviews with health professionals in Phnom Penh. In both places, participants agreed to use the device themselves (involving finger prick) or could identify someone to do so. The main incentive identified in Iquitos was the ability for tentative results to be used for care facilitation (as devices would not provide usable results for individuals), specifically reduced wait times to be seen or obtain a diagnosis. In Phnom Penh, the main incentives were monetary compensation (~US$2.50/test) or results from a simultaneous rapid test; also, free medicine for the sick was acceptable in lieu of usable results. To assess device use competency in Iquitos, participants were asked to read instructions and apply the device to research team members. Most steps were done correctly; the most difficult was proper recording of test results. In Phnom Penh, participants were asked to describe each step after reviewing the instructions; however, they struggled with comprehension. Health professionals' main concerns were their community's ability to accurately use the test, complicated instructions, and safety. Motivation and ability to use home diagnostic devices depended on local attitudes that varied between the two disease endemic sites, illustrating the value of formative research before deployment of novel technologies.

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THE PERCEPTIONS OF PHYSIOTHERAPISTS AND PATIENTS ABOUT INTEGRATION OF HEALTH PROMOTION IN PHYSIOTHERAPY PRACTICE

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This study was undertaken to determine the status of integration of health promotion in physiotherapy practice from both physiotherapists and patients' perspectives. A qualitative research method was followed. A rich purposive sample of physiotherapists patients from different health care settings provided information for data collection during semi-structure interviews which lasted from 45 to 60 minutes. Data were collected over a period of six months. All interviews were audio taped followed by verbatim transcription. The Nvivo version 10 program was used. The following sequence was followed in data analysis: Familiarisation with data, code book construction, thematic analysis, synthesis and identification of similarities, identification of overarching themes and formulation of concept. A total of 35 physiotherapists, 69% females and 31% males, as well as 21 patients, 55% females and 45% males, participated in the interviews. All physiotherapists had a work experience of more than 5 years whilst patients' age ranged between 24 years to 60 years. Physiotherapists' workload comprised of in- and out-patients whilst patients attended physiotherapy sessions at hospital and Primary Health Care (PHC) settings. Concepts formulated for both groups were knowledge, attitude and practice. Patients do have an understanding of what health promotion means and expect health promotion service during physiotherapy treatment as well as aspire to be self-efficient in looking after their health. The physiotherapists are unable to differentiate between health education and health promotion though they have a positive attitude towards health promotion in practice. They rarely integrate health promotion in their daily practice. In conclusion, the need to integrate health promotion in practice exists. Hospital and PHC based physiotherapy differ. There are gaps between the patients' needs and physiotherapy practice requiring policy and guidelines to drive health promotion in physiotherapy. Focus should be on continuous professional development to improve knowledge. Education and training curriculum should be reviewed.

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TREATMENT OF POSTPARTUM HEMORRHAGE WITH MISOPROSTOL BY GUATEMALAN TRADITIONAL MIDWIVES

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Postpartum hemorrhage (PPH) due to uterine atony is a leading cause of maternal mortality in Guatemala, especially in rural areas where homebirth is common. In a pilot project, 36 Guatemalan traditional midwives received training on the recognition and treatment of PPH using 800 µg buccal misoprostol. Training was designed to be linguistically and culturally appropriate. Pre- and post-evaluation showed participants' knowledge of PPH signs, prevention and treatment using misoprostol significantly increased following training. Ongoing data collection, to be completed in June 2014, demonstrates that midwives are capable of recognizing PPH and using misoprostol for its treatment. We plan to expand our work to reach more midwives, further addressing the shortage of data on misoprostol for PPH treatment in homebirth, particularly in Latin America.

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MAPPING THE ENVIRONMENTAL AND SOCIOECONOMIC COVERAGE OF THE 2012 INDEPTH INTERNATIONAL HEALTH AND DEMOGRAPHIC SURVEILLANCE SYSTEM NETWORK

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The International Network for the Demographic Evaluation of Populations and their Health (INDEPTH) was initiated in1998 and has produced reliable longitudinal data about the lives of people in low- and middle-income countries (LMICs) and the impact on those lives of development policies and programs through a global network of health and demographic surveillance system (HDSS) sites. Since stable and reliable health and demographic data are scarce across many LMICs, here we examine the environmental and socioeconomic similarities between existing HDSS field sites and the rest of the LMICs in Africa and Asia, so as to provide evidence in terms of levels of confidence in extrapolating the findings from HDSS field sites to other regions. A 'signature', consisting of 15 environmental and socioeconomic variables, was constructed for each HDSS field site. The field sites were then hierarchically grouped by the similarity of their signatures to quantify the variability in terms of environmental and socioeconomic conditions between sites, and these similarities were mapped. The current INDEPTH HDSS field site network covering Africa and Asia spans a wide range of climatic and environmental conditions. The similarity maps produced provide valuable information in determining the confidence with which relationships derived from present HDSS field sites can be extended to other areas, and to highlight areas where the location of new HDSS field sites would improve significantly the environmental and socioeconomic coverage of the network. The results also indicate suites of field sites that form cohesive groups and from which data can be logically summarized.

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EVALUATION OF POINT-OF-CARE AND POINT-OF-NEED MULTIPLEX DIAGNOSTIC DEVICES FOR EASE OF USE AND FOR TRANSMITTING RESULTS THROUGH A SECURE ENCRYPTED REMOTE NETWORK

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The United States Department of Defense's (DoD) Defense Threat Reduction Agency recently initiated a program designed to drive development of novel point-of-need (PON) diagnostic devices within a biosurveillance network for capturing emerging infectious diseases in austere locations. DoD biomedical laboratories are evaluating two devices in South America (Peru), Australia, Southeast Asia (Thailand, Cambodia), and Africa (Sierra Leone) to mimic real-world settings similar to those encountered by deployed military personnel. These multiplex devices are designed to detect pathogens that cause dengue fever, malaria, plague, and melioidosis: a clinic/medic-based point-of-care (POC) molecular diagnostic device for use in health care clinics and hospitals with health-care providers as the end-user, and a patient-administered "buddy-test" PON device to be used in community households with the lay person as the end-user. For POC testing, 11 Investigational Use Only (IUO)-labeled systems and 1000-2000 test pouches will be distributed to each site. Nearly 1400 POC subjects will be enrolled in Peru with an expected dengue disease rate of 20-25%, and 2500 subjects will be enrolled in SE Asia with an expected melioidosis disease rate of 10%. For PON testing, 7000 to 9000 Research-Use Only (RUO)-labeled devices will be distributed across all locations to detect dengue (Peru), malaria, plague, and melioidosis (all other locations). De-identified and blinded results from these PON and POC tests will be compared to gold standard testing for study of device sensitivity and specificity (aiming for 85% for Role 1; 75% for Role 0), then wirelessly communicated through a secure encrypted remote network (SERN) using an FIO reader (success considered at 90% accuracy). Successful demonstration of delivery of de-identified and encoded test results through the SERN will enable forward operators direct access to surveillance and reach-back analysis remotely in locations devoid of established medical treatment facilities, and will provide information about infectious disease threats.

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U.S. DEPARTMENT OF DEFENSE GLOBAL FEBRILE AND VECTOR-BORNE ILLNESS SURVEILLANCE PROGRAM

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Originally established by Presidential Directive in 1997, the Department of Defense (DoD) Global Emerging Infections Surveillance and Responsive System (GEIS) was expanded in 2006 and subsequently incorporated as a Division of the Armed Forces Health Surveillance Center (AFHSC) in 2008. AFHSC-GEIS divides its surveillance approach into five infectious disease categories: respiratory, gastrointestinal, febrile and vector-borne, antimicrobial resistant, and sexually transmitted. The goal of the GEIS febrile and vector-borne illness (FVBI) surveillance program is to integrate febrile illness, arthropod-vector, and pathogen discovery surveillance systems that contribute to Force Health Protection and global public health through: 1) characterizing the geographic distribution, transmission, and risk of FVBI pathogens and related illnesses; 2) outbreak detection and response; 3) generation of actionable surveillance data supporting informed patient care and FVBI disease risk assessments; 4) promoting FVBI-related research, training, and capacity-building initiatives. During fiscal year 2014, the FVBI program funded 36 competitive proposals supporting surveillance efforts in 32 countries. Recent program accomplishments include extensive global surveillance for drug-resistant malaria with initiation of a multi-site clinical trial evaluating *Plasmodium falciparum* artemisinin resistance in Thailand, Kenya, and Peru. Flea, chigger, and animal reservoir collections data (>90,000 data points) were also incorporated into the GEIS-funded VectorMap program, a publicly available repository for global arthropod vector collection data (www.vectormap.org). AFHSC-GEIS coordinates a worldwide surveillance network comprised of military and civilian laboratory partners who conduct FVBI surveillance in US military as well as foreign military and civilian populations. GEIS-funded initiatives yield enhanced recognition of the risks and threats from FVBI supporting the public health needs of military and associated populations in a growing number of partner nations.

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PROGRAMMATIC ASSESSMENT TOOL FOR RISKS OF MEASLES OUTBREAKS IN THE PHILIPPINES

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Measles is a highly contagious viral disease and remains an important cause of death and disability among children globally. In 2012, the World Health Organization (WHO) Regional Committee for the Western Pacific Region (WPR) reaffirmed its commitment to eliminate measles and urged member states to interrupt endemic measles virus transmission as rapidly as possible. Despite the Philippines' continued commitment to this goal and a nationwide supplemental immunization activity conducted with measles and rubella vaccine targeting children aged 9 months to <8 years in 2011, a total of 1499 cases of measles were reported in 2012. Identifying areas at risk of outbreaks can provide an effective strategy in targeting immunization efforts and preventing future outbreaks. Based on a polio risk assessment tool developed in WPRO and applied in the Philippines, an assessment tool was developed to assess risk of measles outbreaks. Overall risk of an outbreak was assessed as a function of indicator scores that fall into four main categories of risk components (with corresponding weights): population immunity (50%), surveillance quality (24%), program delivery performance (6%), and threat probability assessment (20%). Cut-off criteria for each indicator score were created to assign an overall risk category score (RCS). The RCS was categorized into very high, high, medium, or low risk using available data at the second subnational administrative level. Preliminary results of the risk assessment compared with reported measles cases in 2013 showed clusters of laboratory-confirmed measles cases in areas identified as high and very high risk. Risk assessments can be used for advocacy to communicate risk to policymakers, mobilize resources, and strategically guide immunization response accordingly to the level of risk. Ongoing evaluation of indicators is needed to effectively plan risk mitigation activities and to evaluate performance towards elimination of measles. Further studies are needed to pilot test the risk assessment tool in other countries and regions.

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RISK FACTORS AND PREDICTORS OF PRETERM BIRTH IN DAR ES SALAAM, TANZANIA

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Preterm birth is associated with early life mortality and morbidity. In Tanzania, 1900000 babies are born each year of which 11.45% are preterm (less than 37 weeks gestation). Complications of preterm birth account for 40% of neonatal mortality and survivors are at high risk for long-term neurodevelopmental and behavioral sequelae. We aim to identify the incidence, risk factors, and predictors for preterm birth in Dar es Salaam, Tanzania. We prospectively followed 8428 mother-newborn pairs enrolled from 2001-2004. Maternal and socioeconomic characteristics, obstetric history, and medical illnesses were collected during pregnancy. Birth outcomes included small for gestational age (SGA, weight below the 10th percentile for gestational age) and preterm birth (less than 37 weeks gestation). We conducted bivariate and multivariate analyses using log-binomial regression models to examine the effect of predictor variables on outcomes. Of the 8428 pregnant women, 8003 (95.0%) gave birth to live newborns and were eligible for analysis. The incidence of preterm birth was 16.7%. Risk of preterm birth was associated with older maternal age greater than 34 years of age (RR=1.14 [unit=1 year]; 95%CI 1.07-1.20), wealth in the lowest quintile (RR=1.24; 95% CI 1.11-1.39), less than one year of education (RR=1.67; 95%CI 1.44-1.93), and lower mid-upper arm circumference (RR=1.06 [unit=1cm]; 95%CI 1.04-1.07). In Dar es Salaam, preterm birth is associated with maternal age, wealth, and education, which may be surrogates to unmeasurable factors affecting fetal development. Mid-upper arm circumference, a measure of maternal nutrition, is one modifiable factor associated with preterm birth.

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PREDICTORS OF EARLY MODERATE TO SEVERE STUNTING IN BOLIVIAN INFANTS (0-6 MONTHS)

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Childhood malnutrition, particularly stunting (low length-for-age), can have long-term adverse effects like impaired cognitive function, increased risk of chronic disease, decreased economic potential, and increased risk of maternal mortality. Bolivia, a lower-middle-income country, has one of the highest prevalence of infant malnutrition in the Americas with an estimated 9.5% of six month old infants being moderately to severely stunted. The purpose of this study was to identify predictors of moderate-to-severe stunting (length-for-age Z score <-2) among urban and peri-urban Bolivian children in early infancy (0-6 months of age). Convenience sampling at outpatient well-child visits was used to recruit 185 mother-infant pairs in El Alto, Bolivia, from June to October 2011. Researchers collected anthropometric data from mothers (height, weight) and infants (length, weight, head circumference) at two visits (4 to 6 weeks apart) as well as baseline socio-demographic, clinical, and perinatal characteristics. Multivariable logistic regression was used to identify predictors of being stunted at both visits. The prevalence of being stunted at both visits was 15.7%. Multivariable logistic regression showed that breastfeeding (OR:0.29 95%CI[0.1-0.81]), preterm birth (OR: 10.25 95% CI[3.26-32.23]), small-for-gestational age (OR:6.67 95% CI[2.21-20.18]), and inter-birth spacing of less than 24 months (OR:7.21 95% CI[2.08-24.94]) were significantly associated with stunting in this study population. Although this study was limited in its small sample size (leading to large standard deviations), its results were consistent with prior literature identifying preterm birth and small-for-gestational age (SGA) as predictors of childhood stunting. These results emphasize the need for targeted interventions to foster optimal in-utero growth and prevent small-for-gestational age and preterm births, which would lead to lower rates of stunting in Bolivia.

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THE PUBLIC HEALTH IMPACT AND COST-EFFECTIVENESS OF DIAGNOSTIC AND PROGNOSTIC TOOLS FOR CASE MANAGEMENT OF NON-MALARIAL FEBRILE ILLNESS IN CHILDREN UNDER FIVE

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As malaria transmission declines globally, there is increasing recognition of the importance of correct identification and treatment of non-malarial febrile illness (NMFI). Studies have shown that in many regions, a decrease in antimalarial therapy has been accompanied by a simultaneous increase in antibiotic use. In the absence of appropriate diagnostic and prognostic tools, currently recommended guidelines for integrated management of childhood illness (IMCI) can potentially lead to overuse of antimalarials and antibiotics as well as under-referral of patients with early signs of severe illness requiring hospital treatment. We developed a Markov chain model to assess a range of diagnostic and prognostic tools for NMFI implemented at different levels of the healthcare system in terms of their impact on mortality and cost. The analysis was undertaken in a range of developing country healthcare infrastructure scenarios, including a comparison of factors such as high vs. low health facility access and public vs. private vs. community health systems. The model is paramaterised using data on healthcare access, care-seeking behaviour, and epidemiological and clinical characteristics of febrile illness across a range of countries. Sensitivity analysis is conducted using data on the sensitivity and specificity of diagnostic and prognostic tools for identifying NMFI etiology and symptoms of early severe illness. The methods outlined here can be used to optimise case management strategies across a portfolio of potential diagnostic and prognostic tools being considered for development and implementation.

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STRENGHTENING MALARIA AND ANTENATAL CARE CONTROL PROGRAMS WITH A SYSTEM COMBINING AVAILABLE TECHNOLOGIES

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RDTs are considered a viable alternative to deliver accurate results at POC. However, there are still obstacles to the widespread implementation of this strategy, such as lack of proper quality assurance of RDT-based programmes at POC and reporting constraints, especially in remote areas of low-income countries where the strategy is mostly needed. In collaboration between IHI and NIMR, an implementation research study was conducted in the Geita District to test the feasibility and usability of a system designed to overcome the issues described above. In brief, the system called Fionet is designed to assist peripheral HCWs in the processing and interpretation of RDTs, and collect and transmit clinical data to a cloud information service using local cell phone networks. Information captured is useful for evaluation of quality of RDT by district authorities. HCWs were trained in the use of mobile devices which contained electronic survey forms easily completed through a touch-

screen interface and at the same time guide RDT processing and perform automated interpretation of the results. All information collected and a high-resolution image of the RDT was transmitted to a central database located in a cloud information service. Data were safely stored and organized according to predetermined reports and was accessed via a website . Two patient populations were included in the current study: 1- general population (children and adults, male and female) in whom a malaria test was required; and 2- pregnant women attending ANC clinics in whom screening for both malaria and syphilis was performed to prevent mother to child transmission and adverse outcome of pregnancies. HCWs at all government facilities participating in the pilot were able to operate the system and collected over 5,000 patients in the course of 8 weeks. Health program managers were able to login to the portal and review cases uploaded, aggregated and organized in predefined reports, enabling to make recommendations about program management including monitoring of RDT processing in the field. Scale up of a system such as Fionet to at least a full-district level is warranted after the encouraging results of the present study, to fully demonstrate health system strengthening opportunities in delivery of care, monitoring and evaluation and improvement in system efficiencies.

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TRAINING AND CAPACITY BUILDING INITIATIVES IN SUPPORT OF GLOBAL HEALTH SECURITY, 2012-2014

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The Global Health Security Agenda, launched on 13 February 2014, called upon US government agencies to work together towards a vision of "a world safe and secure from global health threats posed by infectious diseases" by engaging with partner nations to strengthen their capacity for preventing avoidable epidemics, detecting infectious disease threats early, and responding rapidly and effectively. The Global Emerging Infections Surveillance and Response System Division of the Armed Forces Health Surveillance Center (AFHSC-GEIS) supports programs that strengthen global networks for real-time biosurveillance capabilities, train an effective biosurveillance workforce, and strengthen laboratory systems. In fiscal years 2012-14, AFHSC-GEIS supported a global health capacity building portfolio that included 67 projects in 34 countries. Specifically, AFHSC-GEIS partners executed health system improvements in six main areas: 1) electronic disease and biosurveillance systems (29 projects in 15 countries); 2) workforce development in WHO-approved epidemiology and outbreak response methods (23 projects in 20 countries); 3) tropical medicine training for host-country civilian and military personnel (11 projects in 10 countries); 4) training in, and capacity for, entomological surveillance and control methods (10 projects in 7 countries); 5) accredited laboratory practices and quality assurance efforts (34 projects in 21 countries); and, 6) health care facility and laboratory diagnostic capacity development (18 projects in 16 countries). The results of qualitative analysis of quarterly and annual reports from AFHSC-GEIS partners indicate that, while challenging, there has certainly been a progressive evolution of health security capacities within the network.

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DETERMINANTS OF DENGUE MORTALITY BEYOND BIOLOGICAL FACTORS: A SCOPING REVIEW

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Dengue is a viral disease which clinical spectrum varies from unapparent to severe forms and fatal outcomes. Though dengue death is avoidable in 99%, every year around 20000 deaths are estimated to occur in more than 100 countries. Beyond biological factors, social determinants of health (SDH) are related to fatal outcomes. A scoping review and content analysis using QDA Miner was conducted to document the role of SDH in dengue mortality. Inclusion criteria were any document with information of dengue fatal cases in humans, written in English, Spanish, Portuguese or French, peer reviewed or grey literature. Using a set of key words related to dengue mortality, PubMed /LILACS /COCHRANE/Scielo/Science Direct/WHOLIS and Google Scholar, were the electronic databases used. From a total of 971 documents retrieved, 78 articles met the criteria and were reviewed. The documents were published in the Americas region (50%), Asia (38%), Europe (9%) and Africa (3%). The main article's source of information was hospital records (56%) followed by a mix of surveillance data and hospital records (33%). Ninety-three percent included any information about the SDH. Information about individual dimension was found in 88.5% of reviewed articles, where age, education and type of infection/immunological status were considered determinants for dengue deaths. Sixteen articles (20.5%) did mention about health systems and described determinants were access, opportunity, quality of attention and health staff knowledge. Three articles (3.8%) reported socio-economic and political context were poverty and social behavior were the determinants described. Gender and opportunity of attention were considered as dengue determinants dependent on social/personal health seeking behavior. Ethnicity was considered as biological determinant that also depends on cultural and socioeconomic context. These findings reveal the need for more studies about the role of SDH in dengue mortality, in order to design and implement interventions beyond biological factors in areas such education and the health systems (more data will be available at the presentation)

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LABORATORY QUALITY MANAGEMENT SYSTEM: EXPERIENCE WITH IMPLEMENTATION IN LATIN AMERICA AND THE CARIBBEAN

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Laboratories are essential for public health activities to include disease surveillance and control and in the treatment of diseases. The Quality Management System (QMS) in clinical laboratories emphasizes efficient use of resources, processes and personnel to reduce errors and ensure reliable and safe results. The aim of the joint CDC DHAPP NAMRU-6 collaborative effort sponsored by DHP and PEPFAR is to strengthen QMS in clinical laboratories throughout Latin America and the Caribbean (LAC) to improve and measure compliance with quality laboratory services and prepare regional laboratories towards accreditation. Since 2012, 18 clinical laboratories in Belize, El Salvador, Guatemala, Honduras, Nicaragua, Dominican Republic, Colombia and Peru were enrolled in an improvement program. It began with a 5-week training workshop in Lima, Peru to include 2-weeks of training in Strengthening Laboratory

Management Towards Accreditation (SLMTA), followed by distance mentoring and technical assistant visits. We conducted baseline laboratory assessments using the Stepwise Laboratory Improvement Process towards Accreditation Checklist (SLIPTA WHO/AFRO), developed by WHO/AFRO for evaluating and monitoring the improvement progress of laboratories aiming to achieve the ISO 151889 standards. We report 12 laboratories which have now completed 12 months of the program with before and after evaluations. Among the 12 laboratories assessed, average baseline score was 118(78-144), representing 44% compliance with the SLIPTA WHO/AFRO checklist. The average score after 12 months was 14% higher at 151(120-180)representing 58% compliance. Among the 12 quality management systems evaluated, the three areas with greatest improvement included, customer management and customer service (28%), purchasing and inventory process control (16%), and Evaluation of internal and external quality control (15%). Information management (9%) and facilities and safety (4%) showed the least improvement. In conclusion, implementation of the QMS improved multiple areas of laboratory service and processes in diverse settings across LAC. Results of the initial post-testing evaluation will guide future training and resources for further improvement and help lead to the permanent adoption of effective laboratory practices.

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CHARACTERIZATION OF *ANOPHELES GAMBIAE* HEME OXYGENASE

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Heme is a double-edged sword, being vital for oxygen transport yet highly toxic at high concentrations. Heme oxygenase (HO) plays a key role in detoxification through heme catabolism. Present in many organisms it is particularly well characterised in humans. Surprisingly, however, HO is poorly characterised in hematophagous insect vectors such as mosquitoes, yet such organisms deal with enormous influxes of dietary heme in their bloodmeals. Thus one might expect the enzyme to play a key role in survival. Here we are investigating the role of HO in mosquitoes and tsetse flies, which transmit malaria and sleeping sickness respectively. HOs in both organisms were identified via genome database searches, amplified, cloned, and expressed in E. coli. Spectroscopic assays have been done to confirm the catalytic attributes of vector HO *in vitro*, as well as Western blotting, qRT-PCR and immunolocalisation studies to investigate tissue-specific and temporal variation in HO expression. HPLC analysis is being conducted to identify the products of the HO reaction pathway. Finally, inhibition of HO activity has been carried out *in vivo* to determine the physiological role of HO. Here, a competitive HO inhibitor (zinc protoporphyrin) was fed to *Anopheles gambiae* populations. This resulted in a significant reduction in oviposition, suggesting a key role for HO in egg production. It is hoped that the results of these studies will identify new targets for the development of novel vector control agents.

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THE CIRCADIAN CLOCK AND LIGHT/DARK CYCLE INFLUENCE RNA EXPRESSION IN THE *AEDES AEGYPTI* MOSQUITO

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The biological rhythms of mosquitoes regulate and/or modify many behaviors and physiological processes underlying disease transmission, survivability, and fitness. The circadian clock regulation of RNA expression influences physiological and behavioral output on a daily basis. The *Aedes aegypti* mosquito is the major vector of Yellow fever and dengue virus, and exhibits 24 hr rhythms in biting, flight, and oviposition. To better understand the patterns of specific gene expression controlled by the daily light/dark (LD) cycle or the circadian clock in *Ae. aegypti*, we undertook a microarray analysis (>90% transcriptome) of adult female mosquito heads collected every 4 hr over 2 days maintained under LD or constant dark (DD) conditions. Data were subjected to cosine wave analysis (JTK_CYCLE) to determine for ~24 hr rhythmicity. We generated the *Aedes aegypti* Circadian Database, available at http://www.nd.edu/~bioclock/, in which these gene expression profiles and accompanying stastical analysis are searchable. This database provides the capability to examine the potential rhythmicity of the expression profile for each gene, as influenced by the LD cycle or the endogenous circadian clock. We identified 4 classes of genes present within the transcriptome: rhythmic only under LD cycle conditions, rhythmic only under DD conditions, rhythmic under both conditions, and non-rhythmic. The sets of genes active in vision, metabolism, and olfaction were investigated further, and those associated with vision were in good agreement with an earlier study on *An. gambiae* mosquitoes (Rund et al., 2011 PNAS 108:e421-30). Similarly, *Ae. aegypti* genes involved in metabolism and detoxification were under circadian control or non-rhythmic, as predicted by studies of *An. gambiae*. Our *Ae. aegypti* studies demonstrate similarities and differences in regulation of transcripts in different mosquito species, and suggest temporal modulation of processes such as vision and metabolism. These gene expression patterns likely identify both shared traits and the basis of species-specific behaviors.

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DISSECTING THE FINE-SCALE MATING INTERACTIONS IN THE DENGUE VECTOR MOSQUITO *AEDES AEGYPTI*

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Understanding mosquito mating biology is an important step towards identifying novel targets for mosquito control. We have begun dissecting the fine-scale mating biology in male *Aedes aegypti*. Seminal fluid proteins are produced in the reproductive tract tissues of male insects, primarily in the accessory glands, and are transferred _ along with sperm _ to females during mating. Seminal fluid proteins induce numerous physiological and behavioral changes in mated females, each of which might be targeted for novel control strategies. Our work on top candidates for genetic manipulation and the biology of male accessory gland function will be presented. Finally, and to further comprehend mosquito mating, we analyzed transcriptional changes within the lower female reproductive tract in response to male seminal fluid protein receipt. Our results suggest a wealth of new targets for control of these important disease vectors.

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IMPACT OF INCREASED INSULIN SIGNALING IN THE FAT BODY OF *ANOPHELES STEPHENSI* AND *AEDES AEGYPTI* MOSQUITOES ON LIFESPAN AND REPRODUCTION

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Lifespan is a key factor in determining the transmission efficiency of mosquito borne diseases. Finding a novel mechanism affecting mosquito lifespan could be a valuable tool to control mosquito-borne disease transmission. The insulin/insulin-growth factor signaling (IIS) pathway may provide a novel endogenous solution to vector control. In mosquitoes, the IIS plays an important role in the regulation of many physiological processes, including longevity and reproduction. Here we aimed to increase insulin signaling in the fat body of *An. stephensi* and *Ae. aegypti* mosquitoes by creating a transgenic line expressing an active form of Akt, a key component of the IIS, specifically in the fat body tissue. We observed the effects on longevity and reproduction in a heterozygous line. However, contrary to the expected results, we observed an increase in the life span of heterozygous females positive for the transgene. We also observed no significant difference in the reproductive potential of heterozygous positive versus heterozygous negative females, an effect that was opposite of the

anticipated result. Ongoing work on this transgenic mosquito may yield unique insights into how the insulin signaling cascade regulates lifespan in mosquitoes and other eukaryotes.

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CROSSTALK OF IMD AND TOR SIGNALING PATHWAYS IN THE IMPORTANT DENGUE VECTOR *AEDES AEGYPTI*

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Mosquitoes transmit several devastating infectious disease such as malaria, dengue fever, yellow fever, Japanese encephalitis and filariasis. Due to the lack of effective vaccine and the increasing drug and insecticide resistance, alternative approaches for these mosquito-borne diseases are urgently required. It has been demonstrated that the Toll and the Immune Deficiency (IMD) signaling pathways play crucial roles in the production of antimicrobial peptides in the Drosophila. Recently, the JNK pathway was shown to be activated by transforming growth factor-β-activated kinase 1 (TAK1), the component of IMD pathway, and to play an important role in tissue modeling in Drosophila. On the other hand, Matrix metalloproteinase 1 (MMP1) was demonstrated to be essential for the embryonic development in the Drosophila. Our previous study showed that *Aedes aegypti* TAK1 (AaTAK1) is responsible for the production of Cecropin A. We also showed the novel role of *Aedes aegypti* Matrix metalloproteinase 1 (AaMMP1) in the regulation of vitellogenesis. Our results revealed that silence of AaTAK1 by RNA interference approach resulted in the inhibition of AaMMP1 in the translational level. In this study, we showed that the transcriptional pattern of AaTAK1 is highly expressed from 12 to 72 hours after a blood meal and particularly in the ovary and midgut. By RNAi-mediated silencing of AaTAK1, we showed that the egg production was reduced in the absence of AaTAK1. Interestingly, the expression of Vitellogenin (Vg) was inhibited in the absence of AaTAK1 or with the application of JNK inhibitor (SP600125) in the *in vitro* fat body culture system. In addition, by RNAi mediated silencing of AaJNK, the egg production was also reduced. Furthermore, we showed that silencing of AaTAK1 or AaJNK inhibit the phosphorylation of S6K, a key component of TOR pathway, and also inhibit the expression of Vitellogenin (Vg) and AaMMP1 in the fat body. Taken together, our data suggest a novel function of AaTAK1 and AaJNK in the regulation of vitellogenesis and AaMMP1 through TOR signaling pathway.

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GENETIC BASIS OF PYRETHROID RESISTANCE IN A POPULATION OF *ANOPHELES ARABIENSIS*, THE PRIMARY MALARIA VECTOR IN LOWER MOSHI, NORTHEASTERN TANZANIA

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Pyrethroid resistance has been slower to emerge in *Anopheles arabiensis* than in *An. gambiae* s.s and *An. funestus* and, consequently, studies are only just beginning to unravel the genes involved. Permethrin resistance in *An. arabiensis* in Lower Moshi, Tanzania has been linked to elevated levels of both P450 monoox ygenases and β-esterases. We have conducted a gene expression study to identify specific genes linked with metabolic resistance in the Lower Moshi *An. arabiensis* population. Microarray experiments employing an *An. gambiae* whole genome expression chip were performed on *An. arabiensis*, using interwoven loop designs. Permethrin-exposed survivors were compared to three separate unexposed mosquitoes from the same or a nearby population. A subsection n of detoxification genes were chosen for subsequent quantitative real-time PCR (qRT-PCR). Microarray analysis revealed significant over expression of 87 probes and under expression of 85 probes (in pairwise comparisons between permethrin survivors and unexposed sympatric and allopatric samples from Dar es Salaam (controls). For qRT-PCR we targeted over expressed ABC transporter genes (ABC '2060'), a glutathione-S-transferase, P450s and esterases. Design of efficient, specific primers was successful for ABC '2060'and two P450s (CYP6P3, CYP6M2). The primers for CYP4G16 used were previously used in a microarray study of *An. arabiensis* from Zanzibar islands. Over expression of CYP4G16 and ABC '2060'was detected though with contrasting patterns in pairwise comparisons between survivors and controls. CYP4G16 was only up regulated in survivors, whereas ABC '2060'was similar in survivors and controls but over expressed in Lower Moshi samples compared to the Dar es Salaam samples. Increased transcription of CYP4G16 and ABC '2060'are linked directly and indirectly respectively, with permethrin resistance in Lower Moshi *An. arabiensis*. Our study provides replication of CYP4G16 as a candidate gene for pyrethroids resistance in *An. arabiensis* though its role may not be in detoxification and this requires further investigation.

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WNT SIGNALING IS ESSENTIAL FOR THE REGULATION OF TOR SIGNALING-MEDIATED EGG PRODUCTION IN THE MOSQUITO *AEDES AEGYPTI*

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Mosquito-borne diseases are the most devastating agents for human being, due to its high diversity of transmissible pathogens like protozoan and viruses. Despite the efforts from government agencies that have contributed the eradication of the mosquito-borne diseases for several decades, the goal has not been achieved yet. Therefore, many research institutes turn their attentions toward the mosquito life cycle and immune system to halt the disease transmission. Previous studies have already been demonstrated that Target of Rapamycin (TOR) pathway plays an important role in mosquito vitellogenesis, whereas WNT pathway participates in the embryonic development and cell polarity in Drosophila. However, the interactions between these pathways are poorly understood. In this study, we propose a hypothesis that factors of TOR and WNT signaling pathway play synergistically in the mosquito vitelloginesis. We attempt to characterize Wnt signaling components in the mosquito, *Aedes aegypti*. Our results showed that silencing of *Aedes aegypti* Frizzled2 (AaFz2), a transmembrane receptor of Wnt signaling pathway, resulted in the decrease of fecundity in *Ae. aegypti*. We showed that AaFz2 is highly expressed in the mosquito fat body at 6 hours post blood meal in turns of transcriptional and translational level, suggesting the amino acid-stimulated feature of AaFz2. Interestingly, the phosphorylation of S6K and the expression of Vg were inhibited in the absence of AaFz2. These findings determine a direct link between Wnt and TOR signaling in the regulation of mosquito reproduction.

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FUNCTIONAL ANALYSIS OF THIOESTER-CONTAINING PROTEIN COMPLEX IN DENGUE VIRUS INFECTION

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Dengue fever is one of the most devastating arthropod-borne diseases. The WHO reported some 2.5 billion people are now at risk from dengue and estimates that there may be 50 million cases of dengue infection worldwide every year. Up to now, no effective dengue vaccine or drug has been developed. Therefore, intensive study for potential host factors in the mosquito vector and construction of transgenic mosquito together with gene drive technique to replace the vector populations became an alternative strategy to combat dengue virus. Here, we made use of

the well-established reverse genetic approach by silencing potential host factors in the mosquitoes. Our results showed that two immune responsive genes, Thioester-containing protein 1 (TEP1) and Leucine-Rich Immune Gene 1 (LRIM1), were highly expressed in response to dengue virus infection in the mosquito midgut. Silenced of TEP1 or LRIM1 resulted in the over-expression of transcript of dengue virus 2 in the mosquito midgets. Immunofluorescent assay revealed that silenced of TEP1 or LRIM1 resulted in the over-expression of DENV E-protein in the mosquito midgets. We also demonstrated that the translation level of TEP1 is highly expressed in the mosquito midgut after an infectious blood meal. Taken together, our results suggest TEP1 or LRIM1 are important candidates for the establishment of transgenic mosquito against dengue virus. We are currently in the construction of gain-of-function TEP1 transgenic mosquito line with the blood meal inducible carboxypeptidase promoter. Our results will provide new insights into the understanding of dengue-vector interaction and new strategy for dengue eradication program. Data revealed by this proposal will be crucial for future studies on vector competence and vector control in the field.

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MOSQUITOCIDAL PROPERTIES OF IGG TARGETING THE GLUTAMATE-GATED CHLORIDE CHANNEL IN THREE MOSQUITO DISEASE VECTORS (DIPTERA: CULICIDAE)

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Mosquito-borne diseases account for an estimated 1,434,000 deaths annually and 60,056,000 disability adjusted life years. Historically, the most successful strategies to control disease transmission have been by targeting mosquito vectors through the use of chemical insecticides. The glutamate-gated chloride channel (GluCl) is a conserved and highly sensitive target of the drug ivermectin. As an alternative to using chemical insecticides to kill mosquitoes, we tested the effects of purified immunoglobulin G (IgG) targeting the extracellular domain of GluCl from *Anopheles gambiae* (AgGluCl) on the survivorship of three mosquito disease vectors that vary in their sensitivity to ivermectin: *Anopheles gambiae* s.s. > *Culex tarsalis* > *Aedes aegypti*. AgGluCl IgG mixed in a single blood meal significantly reduced the survivorship of *An. gambiae* (LC_{50} =2.82mg/mL [2.68-2.96]), as did serially blood feeds containing 1/10th of the LC_{50} concentration. However, AgGluCl IgG did not affect the survivorship of *A. aegypti* or *C. tarsalis*. Transcriptional analyses showed AgGluCl mRNA was present in the *An. gambiae* adult female head and thorax, but not the abdomen and immunohistochemical staining showed AgGluCl expression only in the antenna, Johnston's Organ, supraesophageal ganglion and thoracic ganglia. Interestingly, injection of AgGluCl IgG into the hemocoel equally reduced the survivorship of *An. gambiae*, *A. aegypti* and *C. tarsalis*, suggesting that AgGluCl IgG sensitivity of blood fed *An. gambiae* is due to permissive antibody diffusion across the midgut. To analyze AgGluCl IgG's mode of action, we fed *An. gambiae* blood meals containing both AgGluCl IgG and ivermectin (a GluCl agonist). AgGluCl IgG attenuated the mosquitocidal effects of ivermectin, suggesting that AgGluCl IgG acts as a GluCl antagonist. These data characterize mosquito GluCl as an important insecticide and immunological target, further the science of developing mosquitocidal vaccines, and lend insight into the unique properties of the *Anopheles* midgut relative to other mosquitoes.

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POST-INSEMINATION TRANSCRIPTIONAL RESPONSE IN THE LOWER REPRODUCTIVE TRACT OF *AEDES AEGYPTI* FEMALES

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Insemination induces substantial changes in female *Aedes aegypti* mosquitoes. Physiologically, inseminated females accelerate blood digestion and oogenesis. Behaviorally, they increase oviposition, alter host-seeking behavior, and become refractory to subsequent insemination. While it is known that these changes are induced by male seminal fluid proteins, what happens in the female to produce these changes is largely unknown on the molecular level. In order to better understand these changes, their associated pathways, and the specific genes involved, we used RNA-seq to conduct a differential expression transcriptome analysis of the lower reproductive tract (LRT; i.e. the bursa copulatrix, spermathecae, common oviduct, and lateral oviducts) of female *Ae. aegypti*. Comparisons were made between virgin females and those at 0, 6, and 24 hours post insemination. Our results provide a framework for further investigation of the postcopulatory physiology and behavior of this important disease vector.

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INVESTIGATION ON ENTOMOPATHOGENIC FUNGAL FAUNA OF MOSQUITOES IN BURKINA FASO: TRANSGENIC BIOCONTROL PERSPECTIVES

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Unlike bacteria and viruses, fungi infect mosquitoes through direct contact with the cuticle and so lend themselves to strategies currently used for delivery of chemical insecticides, and in addition may provide environmentally preferred alternatives. Much attention has focused on the ascomycetes Metarhizium spp. and Beauveria spp. However it should be noted that not much is known about fungal pathogens of adult malaria mosquitoes in the field. In that respect this study aims to investigate entomopathogenic fungi which naturally infect mosquitoes in Africa and their potential use as genetically engineered biocontrol agents. The field collection of mosquitoes was carried out in Burkina Faso. Mosquitoes were collected alive and maintained in insectary conditions with access to sugar water till death. Every morning, dead mosquitoes were collected and isolated individually in a petri dish for fungal growth. Fungal isolation and culture was conducted in a sterile environment. Two different growth media were used. Potato Dextrose Agar, a common medium for isolating fungi, and Potato dextrose agar + CTAB + Chloramphenicol, selective medium. The analysis is ongoing to unravel an exhaustive list of entomopathogenic fungi. The natural virulence of these fungi toward malaria vectors will be subsequently tested. Final results will be available in coming months, before October 2014.

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DETECTION OF POINT-MUTATIONS IN THE *KDR* GENE OF THE DENGUE VECTOR *AEDES ALBOPICTUS*

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The Asian tiger mosquito, *Aedes albopictus*, is the most invasive mosquito in the world and a main vector of dengue, chikungunya and other viruses. Though *Ae. albopictus* originated from east Asia, in recent 30 years, it has migrated to all of the continents except Antarctica. Treatment of public areas with high population density of *Ae. albopictus* with adulticides, mainly pyrethroids, is a recommended protective measure in response to Dengue epidemics and routine treatments with insecticides and is performed alongside source reduction through environmental modification. Intensive pyrethroid application poses selection pressure on mosquito populations for increased resistance. Physiological resistance to pyrethroids has been documented already in several *Ae. albopictus* populations, mainly from the native home range in Asia, abrading the sustainability of control programs based on these insecticides. Field collections of *Ae. albopictus* were conducted in six sites throughout the Guangdong province in South China and subjected to standard WHO tube assays with 0.045% deltamethrin based on discrimination dose determined by the *Ae. albopictus* reference susceptible strain. Between

375 and 658 females were tested per site. Mortality ranged between 28.3% and 84.9%, indicating wide-spread phenotypic resistance in south China. Apart from standard WHO tube assay, rapid and reliable detection of resistance is recognized as an important element of resistance management. Historically, the most used markers for pyrethroid resistance are mutations in the *para*-gated sodium channel gene (*kdr* gene) the pyrehtroid target site. Here we report the current polymorphism of the *kdr* gene in 10 world-wide populations across the world. A total of seven non-synonymous mutations were identified. Although the frequency of non-synonymous mutations was generally low, one population from southern China showed 41.9% frequency, suggesting that *kdr* point mutations are widely spread among *Ae. albopictus* population in various part in the world.

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THE *ANOPHELES GAMBIAE* MOSQUITO MIDGUT TRANSCRIPTOME BY RNA-SEQ

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The mosquito midgut is the first organ to interact with malaria parasites. This tissue can mount effective antiplasmodial responses that limit parasite survival and disease transmission. RNA-Seq by Illumina sequencing of the midgut transcriptome was used to identify new genes and transcripts, contributing to refinement of *Anopheles gambiae* genome annotation. We sequenced ~223 million reads from *An. gambiae* midgut cDNA libraries generated from susceptible (G3) and refractory (L35) mosquito strains after *Plasmodium berghei* or *P. falciparum* infections. In total, 22,889 unique midgut transcript models were generated from both *An. gambiae* strain sequences combined, and 76% are potentially novel. Of these novel transcripts, 49.5% aligned with annotated genes and appear to be isoforms or pre-mRNAs of reference transcripts, while 50.5% mapped to regions between annotated genes and represent novel intergenic transcripts (NITs). Predicted transcripts were validated for midgut expression using qRT-PCR and microarray analysis, and novel isoforms were confirmed by sequencing. Coding potential analysis revealed that 43% of total midgut transcripts appear to be long non-coding RNA (lncRNA), and functional annotation of NITs showed that 68% had no homology to current databases from other species. Reads were also analyzed using *de novo* assembly and predicted transcripts compared with genome mapping-based models. Finally, variant analysis of G3 and L35 midgut transcripts detected 160,742 variants with respect to the *An. gambiae* PEST genome, and 74% were new variants. This in-depth sequencing and assembly of the *An. gambiae* midgut transcriptome doubled the number of known transcripts and tripled the number of variants known in this mosquito species. It also revealed existence of a large number of lncRNA and opens new possibilities for investigating the biological function of many newly discovered transcripts.

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PYRETHROID SUSCEPTIBILITY OF MALARIA VECTORS IN FOUR DISTRICTS IN WESTERN KENYA

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Implementation of insecticide resistance monitoring programs is necessary to ensure continued efficacy of insecticide-based interventions long lasting insecticidal nets (LLINs) and indoor residual spraying (IRS). This study was designed to investigate the extent and distribution of pyrethroid resistance in 4 districts of western Kenya (Nyando, Rachuonyo, Bondo and Teso). All four districts have received long lasting insecticide treated nets (LLINs) while Nyando and Rachuonyo districts have had indoor residual spray (IRS) campaigns for 3-5 years using a mix of pyrethroids. This study is part of a project (Implications of Insecticide Resistance) that aims to determine the impact of insecticide resistance on the efficacy of malaria control interventions. Three day old adult mosquitoes from either larval samples collected in the field, or F1s raised from blood-fed females collected from houses, were used for WHO tube bioassays with mortality recorded 24 hours post exposure. Resistance level was assigned based on the WHO 2013 guidelines. Once exposed, samples were identified to species using PCR. Results *Anopheles arabiensis* comprised at least 94% of all *An. gambiae* s.l. in Bondo, Rachuonyo and Nyando. Teso was a marked contrast case with 77% of all samples being *An. gambiae* s.s. Mortality to insecticides varied widely between clusters even in one district with mortality to deltamethrin ranging from 45-100%, while to permethrin the range was 30-100%. Mortality to deltamethrin in Teso district ranged from 44-95.4% in An arabiensis and 28-85.4% in *An. gambiae* s.s. To permethrin, mortality ranged between 5.9-95% and 34.6-100% in *An. arabiensis* and *An. gambiae* s.s. respectively although a Wilcoxon signed-rank test failed to show consistently higher or lower resistance in any one vector compared to the other ($Z = 0.1$, $P = 0.9203$). Cluster specific mortality of *An. arabiensis* between permethin and deltamethrin were not correlated ($Z = 2.9505$, $P = 0.2483$) . In conclusion, our results show high levels of pyrethroid resistance in western Kenya with intense spatial heterogeneity. The observation that insecticide resistance can vary within small geographical areas may allow evaluation of the impact of resistance on the efficacy of malaria control interventions within similar eco-epidemiological zones.

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LIFE SHORTENING EFFECT OF OLYSET® DUO, A LONG-LASTING INSECTICIDAL NET INCORPORATING A MIXTURE OF PYRETHROID AND PYRIPROXYFEN, AGAINST PYRETHROID-RESISTANT MOSQUITO

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The increase in pyrethroid resistance in mosquitoes across Sub Saharan Africa has become a serious threat for malaria vector control, and threatens to reverse the recent gains achieved by the widespread use of bed nets. New bed net products are clearly needed to maintain these gains. It has previously been shown that contact of adult mosquitoes with pyriproxyfen treated materials can dramatically reduce egg laying and shorten the life expectancy of exposed females. Pyriproxyfen treated bed nets could therefore be used as a resistance management tool against to help prevent the selection of pyrethroid resistance. These findings led to the development of Olyset® Duo which is a long-lasting insecticidal mosquito net incorporating a mixture of 2% w/w permethrin and 1% w/w pyriproxyfen on all sides of the net. In this study we evaluate the life shortening effect of this net against susceptible and pyrethroid-resistant malaria vectors in the laboratory. Olyset Duo and PPF LN, a pyriproxyfen alone net, were washed 3 times according to WHO guidelines and exposed to a susceptible (Kisumu) and a kdr resistant strain (VK7) of *Anopheles gambiae* in the WHO tunnel test. Contact with the PPF LN significantly reduced the survival rate of blood-fed females of both strains, while contact with Olyset Duo killed the susceptible strain and reduced the longevity of surviving blood-fed females of the resistant strain, when compared with the effect of exposure to untreated netting. These results indicate that Olyset Duo could reduce vectorial capacity of malaria vectors through the life shortening effect of pyriproxyfen and thus disrupt the malaria transmission cycle (since a reduction in longevity of as little as 30% can reduce vectorial capacity by approximately 300 times). The cumulative impacts of mortality of mosquitoes from exposure to permethrin, and the suppression of progeny combined with the observed life shortening effects on females that survive permethrin exposure are anticipated to have significant impacts on malaria transmission under field conditions.

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ANOPHELES GAMBIAE SENSU LATO POPULATION SUSCEPTIBILITY TO THE COMMONLY PUBLIC USED INSECTICIDES FOR MALARIA CONTROL IN MALI

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Current vector control strategies are mainly based on the use of chemical insecticides for insecticide-treated nets (ITNs) and indoor residual spraying (IRS) of houses. Mosquito resistance to at least one of the insecticides used for malaria control has been observed in many countries including Mali. Consequently, monitoring insecticide resistance is a key element of the implementation of insecticide-based vector control interventions. The aim of this study was to update data on vectors resistance to insecticides in National Malaria Control Program's sentinel sites in Mali. World Health Organization standard bioassay method was used to assess resistance in 3-5 days old F0 or F1 adult female *An. gambiae* s.l.. Insecticides were Lambda-cyhalothrin 0.05%, DDT 4%, Permethrin 0.75%, Deltamethrin 0.05%, Bendiocarb 0.1% and Fenitrothion 1.0%. Results: Suspected to confirmed phenotypic resistance of *Anopheles gambiae* s.l. population were observed for all tested pyrethroids and DDT in all sites except in Yanfolila where it was susceptible to lambda-cyhalothrin (98.0%) [IC 95%, 98_99.8] and to DDT (100%). *An. gambiae* s.l. was susceptible to the bendicarb in Gao, Bougouni, Djenné, Yanfolila and Tombouctou, while suspected resistance was observed in Kati, Niono, Bandiagara and Kita. Except in Niono, a rice cultivation area (92% [IC 95 % 88.3_94.8] mortality), *An. gambiae* s.l. population was fully susceptible to the Fénitrothion in all sentinel sites. These results showed resistance of *Anopheles gambiae* s.l. population to pyrethroids in the majority of the sentinel sites. Therefore, we suggest to the NMCP to alternate the pyrethroid with the Fenitrothion as management strategy to the current resistance.

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EVALUATING THE EVIDENCE FOR EFFECTIVENESS OF VECTOR CONTROL OF DENGUE OUTBREAKS BY SYSTEMATIC REVIEW AND META-ANALYSIS

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Dengue is the most widespread mosquito-borne arboviral disease worldwide with an estimated 400 million cases occurring annually and almost half of the world's population at risk. At present, preventing and responding to dengue outbreaks both rely on vector control to suppress vector populations or reduce dengue virus transmission, most commonly by eliminating or larviciding mosquito breeding sites and space-spraying with insecticide, respectively. Other approaches are also available and used widely, but with none considered to be sufficiently proven or reliable enough for recommendation, perceptions or beliefs regarding the effectiveness of each approach vary widely among decision-makers in the public health community, often without foundation. Addressing this within the aims of the European Union supported IDAMS research consortium (International Research Consortium on Dengue Risk Assessment, Management and Surveillance; www.idams.eu), we undertook a systematic review and meta-analysis of the evidence available from published studies, to determine if specific vector control tools could impact on vector abundance and/or dengue incidence. A total of 945 studies were found during systematic searches of peer-reviewed databases and grey literature; 35 of these satisfied all inclusion and exclusion criteria and were subject to the Quality Assessment Tool for Quantitative Studies (QATQS). The PRISMA guidelines were followed to ensure rigorous application of review principles. Control approaches included insecticide fogging/space-spraying, insecticide-treated materials, vector trap devices, house screening, mosquito coils and other repellents, clean-up or environmental management and biological control agents. Initial analyses indicate that house screening and community-based programmes can potentially have a beneficial impact, while evidence suggests that repellents and traps do not. Complete results of all final analyses will be presented and the implications of the findings for dengue control in the face of today's realities will be fully considered.

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INVESTIGATING THE MECHANISMS OF DDT AND DIELDRIN RESISTANCE IN FIELD POPULATION OF *ANOPHELES FUNESTUS* IN SENEGAL

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Insecticide resistance in *Anopheles funestus*, one of the main malaria vectors, is threatening malaria control in Africa. Elucidation of the mechanisms of resistance is crucial to the design of suitable resistance management strategies. Therefore, we have investigated the mechanisms responsible for DDT and dieldrin resistance in *An. funestus* population

from Senegal. Insecticide susceptibility assays were carried out using 2-5 day old F1 adults generated from indoor-collected, blood-fed female of *An. funestus* from Gankette, in Northern Senegal. WHO bioassays indicated that *An. funestus* is resistant to lambda-cyhalothrin 0.05% (74.64% mortality / n = 222), DDT 4% (83.36% mortality / n = 158) and deltamethrin 0.05% (88.53% mortality / n = 114). Suspected resistance was observed to permethrin 0.75% (91.19% mortality / n = 139), bendiocarb 0.1% (94.13% mortality / n = 157) and dieldrin 4% (96.41% mortality / n = 306). However this population is fully susceptible to malathion 5 % (100% mortality / n = 50) and fenitrothion 1% (100% mortality / n = 55). Genome-wide transcription analysis using microarray and quantitative RT-PCR revealed that the cytochrome P450 CYP6M7 was the detoxification gene most commonly over-expressed in DDT resistant mosquitoes and field unexposed to insecticide compared to a laboratory susceptible strain. In addition, several others genes with diverse functions including glutathione S-transferases were also overexpressed. Using the pyro-sequencing method, The A296S Rdl(R) target site mutation was detected in all dieldrin resistant mosquitoes but at a low frequency (14%) in the field sample. Our study has revealed a strong association between the dieldrin resistance phenotype and the presence of the Rdl mutation. TaqMan genotyping revealed that the L119F mutation in the GSTe2 gene conferring DDT resistance in Benin is completely absent in Senegal. This indicates a shift of DDT resistance mechanisms in West Africa *An. funestus*. These results could help to guide the implementation of suitable control interventions against this vector in Senegal.

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EXTENSIVE ALTERNATIVE SPLICING IN THE VOLTAGE GATED SODIUM CHANNEL OF *ANOPHELES STEPHENSI*

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Anopheles stephensi, a sub-tropical species distributed throughout the Middle-East and South Asia is one of the major malaria vectors in India mainly in urban areas. Resistance to DDT in this vector has been reported in India since 1965, and development of incipient resistance against pyrethroids has recently also been reported. One of the mechanisms of resistance in insects is reduced sensitivity of the insects to these two insecticides due to alteration in the target site_the voltage gated sodium channel (VGSC). A single amino acid substitution in the VGSC (*kdr* mutation) is known to significantly alter the susceptibility of vector to insecticides by altering channel kinetics. The role of alternative splicing which reorganizes a primary transcript to generate multiple transcripts, in insecticide resistance is not yet known in insects although such events are known to significantly alter the channel kinetics. To understand the extent of alternative splicing in *An. stephensi*, and the possible role in generating altered sensitivity to DDT and pyrethroids, the whole coding region of VGSC gene of DDT- and pyrethroid-resistant mosquitoes was amplified in two overlapping fragments, cloned and sequenced. Analysis of sequences revealed extensive alternative splicing events. These include seven different exon-skipping events, four alternative acceptor sites, two mutually exclusive exons and one intron-retention. In addition to these alternative splicing events, modification at the 5′ un-translated region has also been recorded where quadruplet repeats of 30 nucleotides are seen. Preliminary data in our study showed two splicing events to be pronounced in resistant clones. The role of such splicing events is being examined to determine correlation with DDT and pyrethroid resistance._

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INSECTICIDE RESISTANCE IN *ANOPHELES FUNESTUS* IN SOUTHEASTERN AFRICA AND ITS IMPACT ON MALARIA CONTROL PROGRAM FAILURE

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Malaria parasites are transmitted to humans by anopheline mosquitoes. The parasite undergoes an obligatory sexual stage within the mosquito midgut that takes up to 14 days to complete. This presents a window of opportunity for us to control the mosquito populations before they have sufficient time to become infective. Unfortunately, both mosquitoes and parasites have been around a lot longer than humans and so far have managed to find ways of getting around all the insecticides and drugs that we throw at them. In Africa today there are approximately 140 recognised species of *Anopheles* mosquitoes. Only 4 of these are really good vectors of malaria parasites and of these 4, *Anopheles funestus* is the most important vector in the south-eastern African region. It is highly adapted to humans and human habitations and should, therefore, be easy to control using current technology. Unfortunately, it has developed high levels of resistance to the pyrethroid insecticides that are used for both treating bed nets and spraying on the inside walls of houses. Recent entomological surveys in Zimbabwe, Zambia, Malawi and Mozambique show an almost uniform profile of resistance in *An. funestus* with unpublished data from Zimbabwe indicating a definite impact on programme failure. There is an urgent need for resistance management strategies to be implemented in these countries.

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UNDERPINNING SUSTAINABLE VECTOR CONTROL THROUGH INFORMED INSECTICIDE RESISTANCE MANAGEMENT

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There has been a rapid scale-up of vector control for malaria in the last ten years. Both of the primary strategies, long-lasting insecticidal nets and indoor residual spraying, rely on the use of a limited number of insecticides. Insecticide resistance has rapidly increased in prevalence and has come to the forefront as an issue that needs to be addressed in order to maintain the sustainability of control efforts in the drive to elimination. Zambia's programme reported high levels of resistance to the insecticides it used in 2010. As a result, it increased its investment in resistance monitoring to support informed resistance management decisions. A national survey on insecticide resistance in Zambian malaria vectors, covering 26 districts in all 10 provinces, was performed using WHO bioassays to detect resistant phenotypes. Molecular techniques were used to detect target-site mutations and microarray to detect metabolic resistance mechanisms. *Anopheles gambiae* s.s. was resistant to pyrethroids, DDT and carbamates, with potential organophosphate resistance in one population. The resistant phenotypes were conferred by both target-site and metabolic mechanisms. *Anopheles funestus* s.s. was largely resistant to pyrethroids and carbamates, with potential resistance to DDT in two locations. The resistant phenotype was conferred by elevated levels of cytochrome p450s. Currently, the Zambia National Malaria Control Centre is using these results to inform their vector control strategy. The methods employed here can serve as a template to all malaria-endemic countries striving to create a sustainable insecticide resistance management plan.

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INCREASED EXPRESSION OF METABOLIC RESISTANCE CANDIDATE MUTATIONS IN THE MALARIA VECTOR *ANOPHELES GAMBIAE* SENSU STRICTO IN DAR ES SALAAM, TANZANIA

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

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EFFICACY OF LLIN MIXTURES OF CHLORFENAPYR AND ALPHACYPERMETHRIN AGAINST PYRETHROID RESISTANT *ANOPHELES GAMBIAE*: AN EXPERIMENTAL HUT TRIAL IN BENIN

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Insecticide Treated Nets (ITNs) and Indoor Residual Spraying (IRS) have and will continue to save millions of lives in Africa. However, malaria vectors are developing incredible resistance to existing insecticides and current gains may be lost if new generation tools are not made available soon. The mixture of dual unrelated active ingredients on mosquito net maybe a solution as it presents an opportunity for improved control and management of pyrethroid resistance through the simultaneous exposure to both compounds. With industry and IVCC, we have come to develop a new generation of bednet incorporating alphacypermethrin and chlorfenapyr that may counter growing insecticide resistance in west Africa. Samples of these new nets treated with chlorfenapyr or chlorfenapyr-alphacypermethrin mixtures with binders were washed gradually, 10 and 15 times and evaluated each time in experimental huts against pyrethroid resistant *Anopheles gambiae* in Central Benin. Interceptor 1, an alphacypermethrin-impregnated polyester LLIN (WHO approved) washed to same extent was used as positive control. The nets were deliberately holed with six holes to examine the effect of wear and tear on protectiveness. Mortality rate of *A. gambiae* with Interceptor 1 was constantly less than 20% after 10 or 15 washes, and was presumably due to the high level of pyrethroid resistance in this species. Nets treated with the insecticide mixtures and washed 15 times induced 3 times higher mortality of *A. gambiae* (58-60%) than the Interceptor 1. The differences in mortality of *An. gambiae* between mixtures with low and high dosages chlorfenapyr were not significant, nor was the difference between low and high dosed binders. After washing 15 times, the LN treated with the mixtures inhibited *A gambiae* biting (32–44%) by a greater margin than the Interceptor 1 (20%). The study demonstrates the availability of a resistance-combating dual active ingredient mosquito nets that resist at least 15 washes of the WHO standard practices and restore the effectiveness of ITNs in areas compromised by the spread of pyrethroid resistance.

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A NOVEL LONG-LASTING INSECTICIDAL NET BASED ON A MIXTURE OF CHLORFENAPYR AND ALPHACYPERMETHRIN CONTROLS MULTI-RESISTANT *ANOPHELES* MOSQUITOES UNDER FIELD CONDITIONS AFTER MULTIPLE WASHING: EXPERIMENTAL HUT RESULTS FROM EAST AFRICA AND IMPLICATIONS FOR WHOPES TESTING GUIDELINES

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The emergence and spread of pyrethroid-resistant *Anopheles* across Africa is a grave threat to malaria control. There is an urgent need for non-pyrethroid, wash-resistant LLINs to be developed. Chlorfenapyr is a pyrrole insecticide with a distinct mode of action which disrupts cellular respiration. Interceptor 2 is a 'coating' LLIN containing a mixture of chlorfenapyr (CFP) and alphacypermethrin-α. This study assessed wash-resistance in experimental hut field trials and explored the properties of CFP in laboratory tests based on current WHOPES guidelines and behavioural tests. Hut trials in Tanzania with the mixture LLIN killed 63% and 66% of *An. arabiensis* when unwashed and 10 times washed respectively, compared with 46% for the pyrethoid LLIN (Interceptor 1-α only) washed 10 times. HPLC showed that after 10 washes Interceptor 2 had 75% CFP and 95% α still remaining. This is the first report of a non-pyrethroid insecticide on a LLIN performing successfully after multiple washes. By contrast the standard WHO bioassays failed to predict the performance of chlorfenapyr and therefore need urgent revision otherwise suitable insecticides may be overlooked. The three minute bioassay of CFP nets produced <15% mortality while LLIN require >80% mortality to pass the LLIN test. In laboratory testing there was a strong positive correlation between temperature and mortality above and below standard test temperature. Bioassay tests conducted during the night produced consistently higher levels of mortality than the same tests in the day time. It appears that conversion of CFP to the active metabolite and its disruption of respiratory pathways is enhanced at night when the host seeking mosquito is more metabolically active during the active phase of its circadian rhythm. Testing according to WHOPES guidelines are not suitable for certain types of non-neurotoxic insecticide which, though highly effective in field trials, would be overlooked at the screening stage of evaluation through conventional bioassay.

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A NEW PROMISING LONG LASTING INSECTICIDE NET IN THE CONTROL OF INSECTICIDE RESISTANT VECTORS: OLYSET DUO®: A PYRIPROXYFEN AND PERMETHRIN MIXTURE NET

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Insecticide resistance is threatening the reduction in malaria burden achieved by the massive scale up of vector control measures. One of the most effective vector control tool is the long lasting insecticidal nets (LNs). LNs rely on pyrethroid insecticides to provide a repellent barrier between humans and mosquitoes and kill mosquitoes before they can transmit malaria. Pyrethroid resistance is now occurring in most countries and can undermine the effectiveness of the LN. The development of new tools combining chemical with different mode of action is a necessity to control resistant vector and sustain the gain achieved in controlling malaria. Olyset Duo is a new LN combining a permethrin and pyriproxyfen an insect growth regulator. Pyriproxyfen can sterilize adult mosquitoes and incorporated on a net reduce the vector density. The efficacy and wash resistance of Olyset Duo is currently evaluated in Lower Moshi, Tanzania. Standard WHO cone, cylinder and tunnel test has been performed in the laboratory to evaluate mortality, blood feeding inhibition, reduction in fecundity and fertility of adult female anopheles exposed to Olyset Duo. Experimental hut trial has been also carried out to evaluate the impact of the mixture net on the wild free flying An.arabiensis compare to standard LNs. *Anopheles* exposed to unwashed Olyset Duo in cone, cylinder and tunnel show higher mortality and lower blood feeding rates than to the standard Olyset Net (permethrin alone). The surviving *Anopheles* exposed to Olyset Duo were all sterilized. This should have impact on the population size of the next generation. Initial results of this novel LN's mixture show great potential for the control of resistant malaria vectors in Sub Sahara Africa. The full outcomes of the study will be presented.

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FIELD TESTING OF A PYRETHROID QUANTIFICATION KIT (PQK) IN TANZANIA - AN EASY-TO-USE TOOL FOR MONITORING THE QUALITY OF INDOOR RESIDUAL SPRAY CAMPAIGNS

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Insecticide treated nets (ITN) and indoor residual spraying (IRS) are two of the primary methods of malaria prevention in Africa. In order for these methods to be effective it is essential that adequate concentrations of insecticide are present on nets and wall surfaces to kill mosquitoes. There is no easy assay to quantify insecticide levels without expensive laboratory equipment and procedures. To address this, LSHTM has developed a simple field-applicable kit for monitoring pyrethroid residues on insecticide-treated nets- the Pyrethroid Quanitification Kit (PQK)- which can be adapted to other types of treated surfaces. During the initial trial the PQK kit was calibrated against a variety of sprayed surfaces and with different concentrations of lambdacyhalothrin before being taken into the field. Mosquito cone bioassay was conducted to show whether the surface concentrations of insecticide detected by the PQK were sufficient to kill a susceptible strain of mosquitoes. Houses in six villages were visited 3 months after IRS had been conducted in Muleba, Tanzania. The samples were analysed in the field using a handheld spectrophotometer. In each house, five areas of the wall were examined to give an indication of insecticide distribution and within-wall variation. Results showed that the actual spraying results differed from expectation. Preliminary results showed that only 28% of houses had all rooms sprayed, leaving 72% of houses partially sprayed, and insecticide concentration varied dramatically across sprayed walls. The PQK is an easy to use quality assurance tool for monitoring of pyrethroid application rates and improving the quality of IRS campaigns.

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INSECTICIDE RESISTANCE AND COPY NUMBER VARIATION IN THE *ANOPHELES GAMBIAE* ACETYLCHOLINESTERASE (*ACE-1*) GENE

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The *Anopheles Ace-1* gene encodes the neurotransmitter acetylcholinesterase, the target of organophosphate and carbamate insecticides, which are of growing importance for vector control. A single base mutation (G119S), which causes a major conformational change in the protein, gives resistance to both insecticide classes in mosquitoes. However, the nature, evolution and phenotypic consequences of the more recently identified copy number variation (CNV) of *Ace-1* are far less well understood in *An. gambiae*. We show that CNV is present throughout West Africa and appears to be ubiquitously associated with resistant (119S), but is much less commonly associated with wild-type susceptible (119G) alleles. In *Culex pipiens Ace-1* CNV is well known and involves duplication which pairs a 119G and a 119S allele on the same chromosome to create a permanent heterozygote. However, in *An. gambiae* many *Ace-1* allele copies can be found in 119S homozygotes and sequence analysis suggests that resistant alleles may have originated, or at least spread, following gene duplication. Duplicated alleles are expressed though not necessarily in direct proportion to copy number, and *Ace-1* overexpression is linked to high-level carbamate resistance. Diagnostic assays and neutral markers show that *Ace-1* 119S duplicated resistant alleles are strongly selected and increasing in frequency. Given strong predictive links with carbamate and organophosphate resistance, molecular monitoring should be a key component of programmes using these insecticide classes for control of *An. gambiae*.

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A RETROSPECTIVE EVALUATION OF THE DURABILITY OF LONG-LASTING INSECTICIDAL NETS FROM TWO NATIONAL CAMPAIGNS IN TANZANIA

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Long-Lasting Insecticidal Nets (LLINs) are the mainstay of malaria control, particularly in sub-Saharan Africa. However, despite many National Malaria Control Programs (NMCPs) adopting partial or universal LLIN coverage, there is still only limited knowledge of the effective life of nets after user conditions - commonly known as LLIN durability. Our ABCDR study retrospectively investigates four aspects determining LLIN durability - attrition, bioefficacy, chemical content and physical degradation - of national campaign LLINs in eight districts in Tanzania covering a range of epidemiological and ecological settings. Nets were collected from 3,420 households, and a questionnaire was conducted to determine net ownership and net use. The number, size and location of holes and bioefficacy against anopheline mosquitoes were measured in a sub-sample

of identified campaign nets. A total of 6,832 nets were collected, though not all of them were LLINs. Preliminary results suggest that net ownership, coverage and use are highly variable between districts (household with at least 1 net per sleeping space ranged from 29 - 66%) and by equity (those in lower socio-economic quintiles used nets less frequently). 27% of nets were reported not to have been used the night before the survey, mainly because there were no mosquitoes, the primary user did not sleep at home that night and net age. The results of the bioefficacy and physical degradation analysis are still outstanding, but will be presented and correlated with net use characteristics. Increasing the data on LLIN durability after user conditions to understand the median lifespan of nets for programmatic and cost-effectiveness decisions is becoming a priority for the World Health Organization and NMCPs. In addition to informing the Tanzanian NMCP about the nets distributed during national campaigns between 2009 and 2011, we are also extending the ABCDR study prospectively to compare the durability of three LLIN products over at least three years.

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DIRECT EVIDENCE OF CHEMICAL CONTAMINATION OF *ANOPHELES GAMBIAE* S.L. BREEDING SITES UNDERLYING THE SELECTION OF PYRETHROID RESISTANCE IN COTTON GROWING AREAS REVEALED BY GPC: POTENTIAL IMPACT ON THE EFFICACY OF VECTOR CONTROL TOOLS IN BURKINA FASO

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Since the detection of the first case of *Anopheles gambiae* resistance to pyrethroid recorded in Ivory Coast in 1993, several studies had reported the role of agriculture in the selection and the spread of pyrethroid resistance in natural populations of *An. gambiae* s.l. Unfortunately no direct evidence was reported enhancing the presence of such chemicals in anopheline breeding sites. It was what we addressed in the current study performed in Dano, a cotton growing area located in the South West of Burkina Faso by monitoring the insecticide content both in water and sediments sampled from randomly selected breeding sites using GC analysis from August to October 2013. The resistance status of local populations of *An. gambiae* s.l. was estimated using standard WHO tube assays. Early in August some herbicides as Diouron were detected from the soil residue in concentrations ranged from 22.63 to 105.5 mg/Kg of soil without any insecticide in the water. In October two pyethroids namely lambacyalothrin and deltamethrin were found in the breeding water at concentrations ranging from 0.0147µg/l to 1.49 µg/l together with other chemicals occurring in very low concentration from the soil residue (benzoypropenyl, dioxacarb, chloroneb). A reduced mortality rate was observed both with deltamethrin 0.05% and bendiocarb 0.1% reaching 52.04% and 66.67% respectively. High *kdr* allele frequencies reaching 0.95 and 0.4 respectively for 1014F and 1014S alleles and 0.12 for the ace-1 allele accompanied this strength resistance phenotype. Data on the efficacy of long lasting insecticide treated bednets (LLINs) in use in the region obtained by WHO cone test, showed mortality rates ranged from 10% to 83% depending to the type of LLIN. The significance and the impact of such resistance on the efficacy of malaria vector control strategy in short and long terms were discussed.

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ANALYSIS OF THE DNA SEQUENCE OF ACE.1 GENE IN *ANOPHELES GAMBIAE* S.S: DUPLICATION AND RECOMBINATION HISTORY

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Gene duplication is a source of molecular innovation throughout evolution. However, even with massive amounts of genome sequence data, correlating gene duplication with other events in natural history can be difficult. This is especially true in its most interesting cases, where rapid and multiple duplications are likely to reflect adaptation to rapidly changing environments and life styles. This may be so for the *Anopheles* ace-1 gene which encodes for the neurotransmitter acetylcholinesterase, the target of organophosphate and carbamate insecticides. *Anopheles gambiae*-resistant is known to possess only one mutation on the acetylcholinesterase (AChE) gene (ace) that is involved in insecticide (carbamates and organophosphate) target site insensitivity. To better understand a preferred model for the natural history of ace.1 duplication in the main malaria vector, we proceeded to the cloning and sequence analysis of DNA fragment including the G119S mutation from four West Africa countries. Here we show that phylogenetic trees produced from the nucleotide sequences of 34 individual ace.1 gene consisted of 4 main clusters, with ace.1 copies of different specimens grouping together in three out of the four clusters as expected if there was multiple duplication events. Furthermore, Phylogenetic analysis displayed an individual mosquito bearing more than two different resistant haplotypes with unexpectedly high copy number (at least six) of susceptible haplotype in the same individual. Our data indicate that this high copy number in both susceptible and resistant haplotype have evolved through gene duplication following by recombination event.

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EVALUATING THE EFFICACY OF ORGANOPHOSPHATE-COMBINED PAINT AGAINST PRYRETHROID-RESISTANT *ANOPHELES GAMBIAE* S.L. IN VALLÉE DU KOU, BURKINA FASO

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Malaria control is currently treated by the rapid spread of insecticide and drug resistance among mosquito and parasite respectively, and operational difficulties on the field. It is necessary to find alternative and innovative tools for more effective malaria control. In the current study we evaluated the efficacy of organophosphate-combined paint containing insect growth regulator in indoor application on the walls in Vallée du Kou where *Anopheles gambiae* is resistant to pyrethroids. The main entomological parameters such as mortality rates and paint insecticidal residual efficacy were compared in a village scale between treated and untreated huts. The residual insecticidal efficacy of the paint tested both with the susceptible *An. gambiae* "Kisumu" and wild local populations of *An. gambiae* s.l. showed high mortality rates ranged 98 to 100% even six months after the treatment. The indoors catches of wild *An. gambiae* performed during four consecutive days per month from July to December 2013 revealed that all mosquitoes entered in the paint-treated houses were died reaching 100% mortality rates throughout the six months collection. The frequency of kdr-L1014F (98%) mutation which was higher in this area did not differ between treatments. That of the Ace-1R remained low less than 5% and did not differ whatever the treatment. These results are very promising in terms of new perspectives to control resistant malaria vectors. Contrary to the classic indoor sprays, this tool is very simple and does not require

any special equipment or qualified personnel. But a large-scale assay is required to address its impacts on malaria transmission and the perception and acceptability of targeted human communities.

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INSECTICIDE RESISTANCE PROFILES IN *AEDES AEGYPTI* STRAINS FROM THE CARIBBEAN REGION OF COSTA RICA

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Dengue is the most important vector-borne disease in Costa Rica. Although control of the vector, *Aedes aegypti*, includes repeated use of pyrethrins and temephos, insecticide resistance is not monitored continuously. In this study, resistance profiles to deltamethrin, cypermethrin, and temephos were evaluated in strains of *Ae. aegypti* from the cities of Guacimo and Limon in the Caribbean Region. Bioassays were carried out by exposing groups of 20 larvae for 24 hours to each of the insecticides at a series of concentrations that would generate from 2% to 100% mortality. Tests were performed by quintuplicate using larvae from the second to fourth generations, and a 50% lethal concentration (LC50) was estimated. A 50% resistance ratio (RR50) was calculated using the LC_{50} of the Rockefeller strain as a susceptible control. When resistance occurred, the enzymatic mechanism was evaluated by exposing the larvae to the synergists piperonyl butoxide (PB) and S, S, S, tributylphosphorotrithioate (DEF) and repeating the assays. No resistance to temephos or deltamethrin was detected in *Ae. aegypti* strains from Guacimo and Limon. Emerging resistance to cypermethrin was detected in both Guacimo (LC50 = 0.00845 mg/L, range = 0.00664 to 0.01038 mg/L; RR50 = 6.07) and Limon (LC50 = 0.01016 mg/L, range = 0.00876 to 0.01177 mg/L; RR50 = 7.30). The analysis of cypermethrin with PB resulted in synergism ratios of 19.2 and 8.7 for Guacimo and Limon strains, respectively. Synergism ratios for DEF were 0.9 for the Guacimo strain and 1.8 for the Limon strain. Results show that *Ae. aegypti* in both areas studied are in the process of developing resistance to cypermethrin, which is associated at least in part with the activity of cytochrome P450 monooxygenase. Therefore, local authorities should begin replacement of cypermethrin to ensure effectiveness of vector control and limit the development of resistance.

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USE OF NEXT GENERATION SEQUENCING (NGS) TO IDENTIFY NOVEL SNPS ASSOCIATED WITH PYRETHROID RESISTANCE IN *AEDES AEGYPTI*

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The identification of single nucleotide polymorphisms (SNPs) associated with insecticide resistance has being performed mainly through genomic DNA and cDNA sequencing. Several SNPs conferring insecticide resistance have being identified in target site genes and in less extent at some detoxification genes. Recent Next Generation Sequencing (NGS) technology allows screening the whole genome to identify SNPs associated with insecticide resistance. In this study we identified SNPs associated with pyrethroid survival in an *Aedes aegypti* field population collected in Viva Caucel, Yucatan, Mexico, which already has high frequency for pyrethroid associated mutations in *para*. Four genomic DNA libraries were built following the recommended Illumina HiSeq protocols. Two replicate libraries contained DNA from mosquitoes that had survived one hour exposure to 25 µg permethrin (active ingredient per bottle). The remaining two libraries contained DNA from mosquitoes that died from the same exposure. Sequences were obtained from an Illumina HiSeq2000/2500 sequencer. We constructed a reference library including the total transcript sequences released by Vector Base. We aligned the paired read data to our locally build reference library using the GSNAP software. SNPs with coverage <15 or >1000 were excluded. SNPs association with resistance was assessed using LOD scores. SNPs in 38 genes were associated with permethrin resistance; none of these belonged to the expected target site or putative detoxification genes. Instead, we identified genes involved with GTP and ATP binding, calcium ion binding, zinc ion binding, glutamate ion channel transport and enzymes with specific activity. These candidate genes could become markers that will allow tracking pyrethroid resistance in *Ae. aegypti*.

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INSECTICIDE SUSCEPTIBILITY, CHARACTERIZATION OF BREEDING SITES AND COMMUNITY PERCEPTIONS ON MALARIA VECTOR CONTROL INTERVENTIONS ON KNUST CAMPUS

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Control of malaria vectors with insecticides remains an essential component to fight and eliminate malaria. There have been reports of insecticide resistance to all four classes of insecticides. Spread of resistance is said to be due to the excessive use of insecticides in Public health and in Agriculture. Insecticide-treated nets (ITN) and Indoor residual spraying (IRS) have proven to be the two most powerful and most broadly applied vector control interventions over the years. Despite the reports of resistance to pyrethroids, a lot of ITNs are still being mass distributed for free in most communities in Ghana.This study seeks to find out the level of resistance in *Anopheles* mosquitoes, to insecticides on the University campus which has an urban ambience, to determine other chemical vector control methods used by inhabitants and their perception on ITN use. Due to the extensive agricultural practices on the campus, the breeding sites of the *Anopheles* mosquitoes were characterized using physico-chemical parameters to find out the impact of spraying on the larvae. For the susceptibility tests, 2-5 days old non blood fed female *Anopheles* mosquitoes were tested against 0.05% deltamethrin, 4% DDT, 0.1% fenitrothion and 1% bendiocarb using standard WHO tube assay. A well-structured questionnaire was administered to residents to determine their knowledge, attitudes and practices to malaria. A total of 3,766 mosquitoes were identified as *An. gambiae* s.l (98.9%) and *An. funestus* (1.2%). Resistance was recorded to all classes of insecticides with mortality rates of 15-54% deltamethrin, 10-50% bendiocarb, 7.5-38.75% DDT and 5-42.5% fenitrothion. Overall knockdown rates was 60-21% for deltamethrin, 36.25-11.25% for fenitrothion, 26.25-12.5% DDT and 55-10% for bendiocarb across all breeding sites. Susceptibility status of mosquitoes indicate most are resistance. There was no association between susceptibility status and physical parameters of breeding sites. Inhabitants use ITN's, aerosol sprays, mosquito coils and repellents, impregnated curtains and screens on windows amongst other traditional methods; knowledge on use of ITNs was adequate. Knock down resistance (kdr) genes must be assessed. Knowledge on ITN usage was not converted into practice. This study shows the need for continuous monitoring of resistance to mosquitoes in this area to enable better control methods to be formulated and practiced.

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MULTI-COUNTRY PROFILE OF INSECTICIDE RESISTANCE ON MALARIA VECTORS IN THE PRESIDENT'S MALARIA INITIATIVE (PMI/USAID) SUPPORTED COUNTRIES UNDER THE AFRICA INDOOR RESIDUAL SPRAYING PROJECT

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The Africa Indoor Residual Spraying project implemented indoor residual spraying (IRS) in twelve African countries, namely Angola, Benin, Burkina Faso, Ethiopia,Ghana, Liberia, Madagascar, Mali, Mozambique, Nigeria,

Rwanda and Zimbabwe withsupport from the President's Malaria Initiative in 2012/2013. The project regularly collects data on susceptibility of the malaria vectors to the four classes of insecticides approved by WHO for IRS with the aim to guide selection of appropriate insecticides for IRS programs. WHO tube tests were used for susceptibility tests, and most of the tests were carried out against *Anopheles gambiae* s.l. (144 and 188 tests in 2012, and 2013 respectively); and a total of 14 tests were also carried out against the *An. funestus* in Mozambique and Zimbabwe. Data were entered into the database and collated to inform the decision making processes. *An. gambiae* s.l. was found to be fully susceptible (98-100% mortality after 1 hour exposure and 24 hrs. holding period) to pirimiphos-methyl in all the sites where the tests were conducted. However, potential resistance to fenithrothion was observed in sites in Benin, Ethiopia, Ghana, Liberia, Madagascar and Mozambique. Resistance to malathion has been observed in Ethiopia. Variable levels of susceptibility were reported for the carbamate bendiocarb against *An. gambiae* s.l. Resistance to pyrethroid insecticides is widespread for *An. gambiae* s.l. in most countries.However, in Angola, Mozambique, Madagascar and Zimbabwe, *An. gambiae* s.l. is susceptible to deltamethrin for most sites. A high level of resistance to DDT (0-26% mortality after 1 hour exposure and 24 hrs. holding period) has been reported in most countries, except Zimbabwe, Mozambique, and in some areas of Madagascar. *An. funestus* was resistant to pyrethroids in Zimbabwe and in some sites of Mozambique. It was,however, susceptible to organochlorine and organophosphate insecticides in the southern parts of Africa. The results are discussed in the context of malaria vector control interventions, geographical locations and epidemiology of malaria in the countries.

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A SEASONAL COMPARISON OF HIGH AND LOW EPIDEMIOLOGICAL CLUSTERS OF DENGUE TRANSMISSION IN SURINAME

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The primary vector of the dengue virus, *Aedes aegypti*, is well established in the increasingly urbanized capital of Paramaribo, Suriname. Due to the complex interaction of the environment and the mosquito, dengue transmission risk differ within the geographic area of concern. This study characterizes dengue-related entomological and environmental factors affecting disease transmission. Previously, high and low epidemiological rate clusters of dengue were identified in Paramaribo. Within the clusters, houses were randomly selected for inspection before and after the short rainy season. An environmental survey was conducted and entomological indices were calculated for each cluster. Presence of larva and pupae were recorded and samples of pupae were collected during the surveys. All inspected locations were geospatially coded. In total, 536 houses were surveyed in Paramaribo: 242 during the pre-rainy season and 294 post-rainy season. The findings show significant difference between pupae-positive houses and pupae-positive containers (house & container index) pre- (37.60% & 16.53%) and post- (24.83% & 7.73%) rainy season, p=0.001 and p=0.033. The Breteau indices pre- and post- rainy season were 0.68 v. 0.27. These pupae indices were higher in the low cluster pre-rainy season but higher in the high cluster post-rainy season. The dispersion index remained unchanged between the seasons (3.99 and 3.70 for pre- and post-rainy season, respectively) but decreased from 5.10 to 3.81 in the high cluster and increased from 2.95 to 3.16 in the low epidemiological cluster between the rainy seasons. The variation in the dispersion index indicates that factors within clusters and seasonality affect the proportions in which pupae are present among different categories of containers. This is the first attempt to demonstrate how entomological and environmental data influence the rate of dengue transmission in Suriname.

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DENGUE VECTOR COMPETENCE STUDIES IN *AEDES* MOSQUITOES

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Dengue is globally the most important arboviral infection of humans. The main vector worldwide is *Aedes aegypti*. The Asian tiger mosquito, *Ae. albopictus*, is also a competent vector of dengue viruses and its presence in continental USA and southern Europe raises the concern that dengue will spread further into new regions. This study aimed to directly compare the susceptibility of *Ae aegypti* and *Ae albopictus* to dengue by conducting blood feeding experiments on viremic dengue patients. This study directly compared the susceptibility of these two mosquito types by conducting blood feeding experiments on 118 viremic dengue patients on 232 independent exposure events. The likelihood of saliva infection (and thus potential infectiousness) in *Ae. albopictus* was approximately half that in *Ae. aegypti* (OR=0.49; CI: 0.34-0.70). When stratifying by serotype, the odds of saliva infection were significantly lower for *Ae. albopictus* with DENV-2, DENV-3 and DENV-4 , but not with DENV-1. We have demonstrated that disseminated DENV infections and thus transmission are less likely to occur in *Ae. albopictus* than *Ae. aegypti*. These results have implications for understanding the spread, distribution and control of dengue.

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DOES DENGUE VIRUS ENHANCE ITS OWN TRANSMISSION FROM HUMANS TO MOSQUITOES?

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Among other cues, host-seeking mosquitoes are known to be responsive to carbon dioxide and body temperature. Due to a higher body temperature, dengue virus-infected patients, like other febrile patients, may be more attractive to *Aedes aegypti* mosquitoes compared to healthy individuals. That is, the clinical effects of dengue may aid its transmission to further susceptible mosquitoes. An altered distribution of mosquito bites between infectious and non-infectious hosts may impact predictions of transmission dynamics during epidemic periods with a high force of infection. We test the hypothesis, H_0 = both febrile and afebrile hosts are similarly attractive to naïve *Ae. aegypti* mosquitoes, by exposing febrile dengue patients and healthy matched volunteers to uninfected *Ae. aegypti* mosquitoes. We released five *Ae. aegypti* into the center of a large cage (~4m x $2m^2$), with the participants at each end. The time it takes for each participant to be first chosen, and landed upon by a mosquito, is measured, with mosquito landing used as a surrogate for biting. Anticipated completion of enrollment will occur in December 2014. Results from this study will inform us whether dengue virus has the capacity to enhance its own transmission (whether it be directly or indirectly) by increasing the attractiveness of infected human hosts to naïve mosquitoes.

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DO SEASONAL TEMPERATURES MATTER IN THE LARVAL HABITAT? EFFECTS ON ADULT SIZE, LONGEVITY, AND R' IN THE MOSQUITO *AEDES TRISERIATUS*, VECTOR OF LA CROSSE VIRUS

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Mathematical models of mosquito transmitted diseases suggest that female longevity is an important factor for determining disease risk. As ectothermic organisms, temperature regulates mosquito growth and development. It is well understood that bellow a critical maximum, hotter temperatures decrease development time and adult size. Adult size is correlated with fecundity, but it is less well understood how size is related to longevity and how temperatures experienced in the larval habitat influence adult longevity. Additionally, most experimental studies that have tested effects of temperature use constant temperatures and ignore daily or seasonal temperature fluctuations that mosquitoes encounter under natural conditions. I simulated fluctuating daily temperature and photoperiods consistent with those experienced in St. Louis, MO in June and August to test the hypotheses that 1) *Aedes triseriatus* mosquito larvae developing under different seasonal conditions will differ in size and adult longevity 2) that fluctuating temperatures yield adults that differ in size and adult longevity from those produced in stable conditions. There was no effect of size on female longevity. Despite early differences in survival probability and different ages at senescence, there was no statistically significant difference between August and June treatments. The August treatment was significantly different from the constant temperature control, which experienced reduced longevity. Simulated August, June, and constant treatments also differed in larval survivorship, median day to eclosion, size, and r' (a cohort performance index). The June and constant treatments only differed in female size. These results suggest seasonal temperature fluctuations in the larval habitat do not affect adult longevity in *Ae. triseriatus*, but do affect other measures of performance typically reported in studies that investigate down- stream effects of the larval habitat.

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TRANSGENIC *ANOPHELES STEPHENSI* FITNESS AND SUSCEPTIBILITY TO VARIOUS INFECTIONS

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Various mosquitoes from the genus *Anopheles* spread the causative agents of human malaria. Traditional malaria control efforts have been unable to eliminate the disease, mainly because of insecticide and drug resistance arising among both the mosquitoes and the *Plasmodium* spp. parasites that cause the disease. Therefore, new tools to combat the disease are needed, and one potential new technology to limit malaria transmission is the use of transgenic mosquitoes refractory to malaria infection. Multiple laboratories have created multiple mosquito lines that do not transmit the malaria parasite, but have not yet reached the stage of releasing such mosquitoes, partly due to concerns about their fitness relative to wild-type conspecifics and their ability to resist a broad range of malaria parasites and other human pathogens. We studied various aspects of mosquito fitness in five separate transgenic lines representing different transgenes, insertion points and promoters in order to determine the fitness costs that may arise due to transgenesis. Of the five lines tested, four have shown no fitness cost and the fifth has some fitness reduction due to position effects. We also challenged our mosquitoes with different *P. falciparum* strains. Our results indicate that the mosquitoes are highly resistant to infection by multiple *P. falciparum* strains and do not suffer a large fitness cost as a result of transgenesis, thereby illustrating their potential utility as part of a larger malaria control program and warranting further testing in large cage or field trials. Ongoing studies are focused on testing transgenic mosquito resistance to the *P. vivax* parasite, O'nyong'nyong virus and the filarial worm *Brugia malayi*.

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AEDES AEGYPTI POPULATION STRUCTURE IS DRIVEN BY BOAT TRAFFIC IN THE PERUVIAN AMAZON

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In the Americas, as in much of the rest of the world, the dengue vector *Aedes aegypti* is predominantly found in urban areas. Its presence in rural areas is more limited, and the factors favoring its potential geographic expansion to rural and smaller urbanized settings are poorly understood. In the Peruvian Amazon, this vector has been expanding its range into rural communities over the last 5-10 years. Understanding *Ae. aegypti* dispersal patterns is important for anticipating the future range expansion of dengue and other viruses transmitted by this mosquito. To examine if human transportation networks play a significant role in the population expansion of this mosquito, we analyzed *Ae. aegypti* population structure using a panel of 10 microsatellite markers. Adult and immature *Ae. aegypti* (>20 individuals per site) were collected from the city of Iquitos (pop: 380,000) and several neighboring communities that are connected by river transport, including Nauta (pop: 14,000), Indiana-Mazan (pop: 7,000), Barrio Florida (pop: 750), Tamshiaco (pop: 4,500), and Aucayo (pop: 800). We detected significant departures from Hardy-Weinberg Equilibrium in all study sites due to lower than expected number of heterozygotes. FST statistics show significant differentiation for the majority of study site pairs. Population structure among *Ae. aegypti* in different towns is not correlated with the geographic distance between towns, suggesting that human transportation networks may be responsible. Furthermore, *Ae. aegypti* gene flow among sub-populations is greatest between locations with heavy boat traffic such as Iquitos-Nauta (which also has heavy road traffic) and Iquitos-Indiana-Mazan, and lowest between locations with little or no boat (or road) traffic such as Barrio Florida-Iquitos interior. Bayesian clustering analysis using Structure program suggested definite admixture, with 5-6 genetic clusters. Our results provide strong evidence for the hypothesis, especially via boats, is responsible for *Ae. aegypti* spread in the Peruvian Amazon.

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ADVANCEMENTS AND CHALLENGES IN USING NEAR INFRARED SPECTROSCOPY (NIRS) TO DETERMINE THE AGE OF FEMALE *AEDES AEGYPTI* MOSQUITOES WITH VARYING LARVAL AND ADULT DIETS

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Interventions targeting adult mosquitoes are often used to combat the transmission of vector-borne diseases, including dengue. In the absence of a vaccine, we rely on control measures targeting the primary dengue vector, *Aedes aegypti*, to prevent transmission. Due to the 7 day extrinsic incubation period of the virus in the mosquito, older mosquitoes (>7 days)are of most concern in the dengue transmission cycle. Identifying the age of female mosquitoes is therefore a crucial step in determining if vector control interventions are altering the age structure of the mosquito population, thereby reducing transmission potential. We have developed

a model using near infrared spectroscopy (NIRS) to determine the age of adult female *Ae. aegypti.* This technique quantitatively measures organic compounds, and has previously been used successfully to age-grade other mosquito species. To determine if the larval and/or adult diet of female *Ae. aegypti* affects the ability of NIRS models to predict mosquito age, 2 groups of mosquito larvae were raised in identical settings and fed either fish food or infant cereal. Emerged adult females were separated and fed either blood or sugar, resulting in four experimental groups. These adult females were then killed 1, 4, 7, 10, 13 or 16 days post-emergence. The head and thorax of each mosquito were scanned using a LabSpec 5000. The spectral scans from each group were analyzed independently and collectively, to determine if the diet of the mosquito affected the spectrum. Multiple models were developed using GRAMS PLSPlus/IQ, with different spectral ranges. The best model included all experimental groups, and was able to positively predict the age group (< or ≥7 days) of 88.8% of mosquitoes. Models using a single experimental group to predict the others were less robust. These results indicate both larval and adult diet affect the predictive ability of models developed using the spectral scans of female *Ae. aegypti.* This potentially complicates the application of NIRS age grading to field populations, as larval and adult nutrition can vary greatly in the field.

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SPECIES DIVERSITY, SEASONAL, AND SPATIAL DISTRIBUTION OF MOSQUITOES (DIPTERA: CULICIDAE) CAPTURED IN *AOTUS* MONKEY-BAITED TRAPS IN A FORESTED SITE NEAR IQUITOS, PERU

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This study was conducted to determine the relative abundance, diversity, seasonal, and vertical distributions of potential mosquito vectors in the Amazon Basin, Peru. A total of 66,097 mosquitoes (50 mosquito species from 12 genera) were collected from May 2001 through March 2002 at a forested site near Iquitos, Peru. Mosquitoes were collected using *Aotus nancymae* Hershkovitz monkey-baited CDC light traps set for 12-h day and night periods at varying heights (*e.g.* ground and canopy) in the forest. Of the 12 genera, three accounted for 75% of all mosquitoes collected: *Culex* (33%), *Aedes* (23%) and *Psorophora* (18%). The most prevalent species collected were *Aedes serratus* (Theobald), *Culex pedroi* Sirivanakarn & Belkin, *Psorophora albigenu* (Peryassu), and a combination of *Mansonia indubitans* Dyar & Shannon and *Mansonia titillans* (Walker), which accounted for 56% of all mosquitoes captured. In general, mosquitoes were collected more often at night and on the ground. Exceptions include *Coquillettidia venezuelensis* (Theobald), which were collected in relatively even numbers at both day and night and most *Mansonia* and some species of *Anopheles*, which were collected more often in the canopy. Total mosquito populations had two peaks, June-July (*Ma. indubitans/titillans, Cq. venezuelensis*) and December-January (*Ps. albigenu*, *Cx. pedroi, Ae. serratus*). Observations of the eight most collected mosquitoes indicated that behavioral shifts were not observed between collection months. These data provide a better understanding of the species diversity, population density and seasonal distribution of potential mosquito vectors within the Amazon Basin region and allow for the development of appropriate vector and disease prevention strategies.

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HUMAN IGG ANTIBODY RESPONSE AGAINST RECOMBINANT *AEDES AEGYPTI* SALIVARY PROTEINS MODIFIED DURING DENV2 INFECTION

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Arthropod saliva has been shown to modulate the success of infection success for the microorganisms they transmit. Our group has found that DENV infection of *Ae. aegypti* salivary gland induces changes in the expectorated salivary content. We have produced by recombinant technology three of the proteins we found to be reduced in saliva. The immunogenicity against these proteins was evaluated in subjects from a DENV endemic area in Colombia by ELISA. We found a significant increase in the IgG antibody levels after exposure to mosquito bites. We also found a differential antibody response among healthy, febrile DENV (-) and DENV (+) subjects. We hypothesize that the decrease of these proteins in saliva may be advantageous to the virus. Differential IgG response between DENV (+) and negative subjects suggests 1) dengue positive subjects are significantly at higher exposure/risk to mosquito bites 2) the immune response against these proteins may play an important role in disease progression.

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ALTERNATIVE MOSQUITO SAMPLING TO MONITOR LYMPHATIC FILARIASIS TRANSMISSION IN PAPUA NEW GUINEA

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Entomological markers of lymphatic filariasis (LF) transmission provide key information regarding progress toward LF elimination and subsequent monitoring. However, "gold standard" approaches of human landing catches (HLC) can be inefficient and expensive as transmission approaches zero. In addition, HLC can be complicated by and/or violate cultural and ethical standards. Recently barrier screens have been used with success in the Pacific region to estimate human blood index and quantify malaria transmission in anopheline mosquitoes. Since Wuchereria bancrofti (Wb) is exclusively transmitted by anopheline vectors in Papua New Guinea, this study compares HLC, light traps, and barrier screens with regard to mosquito species captured, anopheline infectivity (as detected by microscopy for stage 3 Wb larvae), and presence of Wb DNA. 2mx40m barrier screens were sampled every 15 minutes by trained collectors in communities with simultaneous HLC collections (plus light traps in one sampling week). Collections occurred between 6pm and 6am in four communities for one week in November 2013, March 2014, and July 2014. Mosquitoes were separated according to hour captured, capture method, and the side of the screen collected (village side vs. bush side). Anopheline capture density and composition will be compared for each mosquito sampling method. Xenomonitoring for LF elimination will be quantified by both microscopy of anopheline mosquitos and PCR for Wb DNA in all bloodfed mosquitoes. Human blood index and presence of Wb DNA will be compared across the study communities. Negative binomial models will be fit to compare agreement between mosquito capture

methods for mosquito species densities and presence of L3. The results are relevant to the selection of the most efficient mosquito collection techniques for xenomonitoring LF during elimination programs in PNG.

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IGG ANTIBODY SUBCLASSES AGAINST VECTOR SALIVARY PROTEINS AS A MEASURE TO RISK OF *AEDES AEGYPTI* BITE EXPOSURE AFTER IMPLEMENTATION OF ATTRACTIVE LETHAL OVITRAPS

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Aedes salivary proteins induce specific immune responses in the vertebrate host that can be used a tool to measure vector host-contact. Specifically, an increase in IgG antibody subclasses (IgG1 predominantly associated with the anti-microbial response and IgG4 representing chronic exposure to an antigen) has been associated with chronic exposure to mosquito bites and the risk for arbovirus infection. We evaluated the levels of antibodies against *Aedes aegypti* salivary gland extract in subjects from dengue endemic areas in Iquitos-Peru, before and after the implementation of Attractive Lethal OviTraps (ALOTs). Serum samples were collected from the household occupants before (Time 0) and after one year of the treatment (Time 12) in the ALOT treated and the untreated control areas. PRNT70 tests for DENV were performed on all sera for determination of viral exposure. Our results showed that IgG4 antibody concentrations were significantly higher than IgG1 concentrations for both groups at both times. When comparing the antibody response between times, we found that the concentration of both IgG1 and IgG4 decreased in subjects from the treated area over 12 months. No significant differences were observed in the control area during that time. The relationship between the exposure to DENV and IgG4/IgG1 will be discussed. Our results suggest that IgG4 levels are indicative of cumulative exposure to mosquito bites and it represents a useful tool for monitoring vector control interventions.

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INFECTION EVALUATION ON FIELD-CAPTURED MOSQUITOES FROM THREE COLOMBIAN CITIES WITH DIFFERENT ENDEMICITY PATTERNS OF DENGUE

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Dengue fever is endemic in Colombia, where the four virus serotypes circulate in highly variable epidemiological scenarios. This heterogeneity is illustrated in the following three cities: Riohacha, with the highest values of Breteau index (BI) and lowest number of dengue cases; Villavicencio, which has the highest number of dengue cases and the second highest BI values; and Bello, which has the lowest BI values, but exceeds Riohacha in number of dengue cases. To explain these differences, adult mosquitoes were captured in four neighborhoods in each city over 3 to 5 sampling periods across 2012 and 2013. Virus presence and serotype were detected through examination of pooled mosquitos from each sampled house. We found that mosquitoes from Bello have the highest infection rate, followed by Villavicencio and finally Riohacha. In samples from Riohacha and Villavicencio, we detected the serotypes DENV-1, 3 and 4, while in Bello we detected serotypes DENV-2, 3 and 4, and the highest proportion of pooled mosquitos with more than one serotype. 13.3% of pools presented infection with more than one serotype, including two pools of just one single mosquito, which is the first reported case of a mosquito co-infected with two dengue serotypes in Colombia. DENV-4 serotype is the most prevalent in pools from the three cities (75.5%), followed by DENV-3 (28.8%), DENV-1 (6.6%) and DENV-2 (2.2%). Our results differ from those of dengue serotype presence in humans from the whole country reported by the Colombian National Institute of Health, where DENV-1 is the most prevalent and DENV-4 the least. This suggests that human populations are more refractory to DENV-4 infection, the most abundant serotype, while being more susceptible to DENV-1 and DENV-2 infection. These patterns highlight the importance of including evaluation of mosquito infection when developing dengue control strategies.

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VALIDATION OF A PREDICTIVE MODEL: ENVIRONMENTAL INFLUENCES ON THE PREVALENCE OF WEST NILE VIRUS IN CULEX MOSQUITOES, LONG ISLAND, NEW YORK

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To better understand the dynamics of West Nile Virus (WNV) transmission, we revisit a model describing the spatial-temporal distribution of positive Culex mosquito pools collected in Suffolk County, Long Island, New York. The original statistical model (m09) used meteorological and hydrological conditions to simulate WNV infection in Culex mosquitoes during 2001-2009. Here, we use Culex mosquito WNV infection data collected during 2010-2012 to validate m09 predictions for these years and to explore whether inclusion of additional environmental variables improves model performance. The m09 predictions for 2010-2012 are well correlated with observed WNV activity during the same time period (0.65). In evaluating the full 2001-2012 record (m12), we explored smoothing the meteorological and hydrological data temporally over 3-month seasons, increasing the spatial resolution of the analysis to the locations of the mosquito traps rather than the aggregated scale of the meteorological and hydrological data, and incorporating other socioeconomic and environmental variables (median household income, population density, a metric of the built environment, and household occupancy status); however, the results remained consistent, with wetter winter conditions, wetter and warmer spring conditions, and drier summer conditions favoring increased prevalence of WNV among Culex mosquitoes. In general the socioeconomic variables did not further constrain the models, suggesting that the distribution of WNV activity in Culex is most intimately linked to climate. Temporal cross validation of the m12 model confirmed consistency of the associations between environmental variables and WNV activity. The different spatial and temporal considerations of these analyses indicate robust associations between meteorological and hydrological conditions and WNV activity. Validation of this model indicates that as meteorological observations and hydrology model estimates of land surface wetness are collected, areas at heightened risk for WNV activity can be predicted.

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OCHLEROTATUS GENICULATUS, A NATIVE EUROPEAN MOSQUITO WITH A HIGH POTENTIAL FOR TRANSMISSION OF CHIKUNGUNYA VIRUS

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Ochlerotatus (Finlaya) geniculatus (Olivier), a tree-hole mosquito that bites humans aggressively, is present throughout Europe, North Africa and Asia Minor. In Albania it shares a common habitat with *Aedes (Stegomyia) albopictus* and our ovitrap collections have confirmed its abundance to at least 1350m. In the laboratory, both species were highly susceptible

to chikungunya virus (quantitative titration of saliva, head and body) but reached a higher titre in the head and thorax of *Oc. geniculatus*. Titer of infectious virus in the saliva was also higher and more sustained. Interestingly, in Ae. albopictus, virus was present in the saliva within three days, whereas in *Oc. geniculatus* it was not detectable until day seven, after which the profile of increase in titre was akin to that of dengue virus in *Ae. aegypti*. Given the widespread presence of Oc. geniculatus in peri-urban areas, its propensity to feed on humans and the rapid rise in the frequency of imported cases of chikungunya, we suggest that this species should be considered as a potential vector in the Palearctic region.

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OPTIMIZATION OF FEEDING ASSAYS TO EVALUATE MALARIA TRANSMISSION-BLOCKING VACCINES IN MALI

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Transmission-blocking vaccines (TBV) are critical tools for malaria elimination. However, the assays used to measure TBV functional activity, including direct membrane feeding assays (DMFA) and direct skin feeds (DSF) involve multiple biological systems with inherent variability. The objective of this research study is to establish optimal conditions for our assays used in clinical trials in Mali. Since January 2014, field experiments have been carried out to address different aspects of the feeding assays (DSF/DMFA). Experiments have been conducted to confirm optimal feeding parameters for DMFA including membrane selection (parafilm versus baudruche), mosquito age, and mosquito starvation duration. The impact of anatomical location (arm, calf, ankle) on subsequent mosquito infectivity by DSF is being assessed. Finally, we want to assess the effect the time of day the DSF is performed has on mosquito infectivity by DSF and DMFA. The evaluated endpoints for the exploration of these variables includes: mosquito feeding rates, mosquito survival rates, and mosquito infectivity rates. Optimal observed parameters will be applied to improve the feeding assays conducted for transmission-blocking activity (TBA) measures. Data from pilot experimental hut (EH) assays, procedure by which wild, blood-fed mosquitoes are collected from the room where the participant slept alone overnight, will be presented. First, molecular tools will be deployed to pair fed mosquitoes with the individual volunteer participating in the EH assay. If fed mosquitoes captured are consistently matched to the EH volunteer, the EH assay will be expanded to evaluate TBV impact on naturally occurring transmission.

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DESIGN AND ASSESSMENT OF A MOBILE DATABASE MANAGEMENT SYSTEM FOR ARTHROPOD-BORNE DISEASE SURVEILLANCE IN BELIZE

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Successful implementation of information and communication technologies for development (ICT4D) hinge on dynamic collaboration among all key stakeholders - particularly intended users. In dengue endemic areas, where vector control practices have shown diminishing returns, the gap between surveillance practices and ministerial response represents a unique opportunity for mobile health technologies to fill a need. The incorporation of real-time data collection and archiving, open-source QGIS mapping capabilities, and regression tools into a single platform aims to support ministry officials in streamlined action for both preventive and reactionary measures. This study presents a three-tiered, workshop-based approach to the adoption of a novel mobile application and database management system by the Northern Region of the Ministry of Health (MOH) in Belize. In general, the first phase consists of training MOH field technicians on theory and technical use of the mobile application; the second phase simulates field conditions in an outdoor setting and extends to risk map generation; while Phase III monitors real surveillance conditions in village settings. Shortcomings of the mobile system identified by MOH officials and field technicians at each phase guides reprogramming, ensuring a user-oriented product. Data will be available by mid-July 2014.

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MOSQUITO VECTOR MICROBIOME DIVERSITY ACROSS HABITATS IN CENTRAL THAILAND ENDEMIC FOR DENGUE AND OTHER ARTHROPOD-BORNE DISEASES

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Anthropogenic environmental change is among the most frequently identified factor linked to emergence of infectious diseases. The proposed mechanism by which anthropogenic environmental changes affect infectious disease spread is through the perturbation of the ecological communities involved in transmission, resulting in changing distribution and relative abundance of the key organisms. The objective of this study is to characterize the diversity of mosquitoes, relative abundance of vectors, and their associated microbial communities along a forest-agro-urban habitat gradient in Thailand to determine how habitat changes affect multi-level communities. Various adult mosquito traps were set-up along a habitat transect to capture components of mosquito communities in each characterized habitat. Over 62,000 female mosquitoes were identified morphologically and selected vector species, *Aedes aegypti*, *Ae. albopictus*, and *Culex quinquefasciatus*, were pooled by species and habitat type. Segments of 16s and 18s rRNA genes were sequenced from the pools using 454 pyrosequencing technology to assess genetic and taxon-based diversity of the non-eukaryote and eukaryote microbiota. Female mosquito abundance was highest in rice fields and lowest in forests. Based on extrapolated species richness estimators (Chao1 and ACE), forest and fragmented forest habitats had the most diverse mosquito communities, followed by the rural, rice field, suburban and urban habitats. Interestingly, the relative abundance of two vector species, *Ae. aegypti* and *Cx. quinquefasciatus*, was negatively correlated with mosquito diversity. Furthermore, the microbiota community assembly and diversity of selected vector species were associated with habitat types suggesting that habitat factors differentially affect mosquito microbiota. In this study, mosquito community and vector microbiota diversity varied across a continuum of habitat types in a pattern reflecting habitat change. Moreover, changes in the abundance of important mosquito vectors tended to follow a predictable pattern. Collectively, these findings help illuminate how mosquitoes and their associated microbial communities vary across habitat types and how these dynamics could contribute to emergence of arboviral diseases in Thailand.

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STUDYING THE EXTRINSIC INCUBATION PERIOD IN DENGUE VIRUS INFECTED MOSQUITOES

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In the past 20 years, dengue has become the most prevalent arthropod-borne virus affecting humans today. This exponential increase in disease incidence has brought with it significant health, social and economic problems. Vectorial capacity, which is a measurement of the efficiency of vector-borne disease transmission, is influenced by a few key factors. Extrinsic incubation period (EIP), which is the interval of time from the ingestation of an infectious bloodmeal to the time of transmission, is a key determinant of vectorial capacity. Here we have used a repeat sampling technique for saliva to study individual variation in EIP in dengue-infected *Aedes aegypti*. We show significant heritability (~40%) for EIP and a strong positive correlation between length of EIP and mosquito lifespan. We also examine the effect of the biocontrol agent, Wolbachia pipientis on EIP under different temperature regimes.

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RIFT VALLEY FEVER OUTBREAK IN SAUDI ARABIA ANTICIPATED FROM AFRICA OUTBREAKS AND TIME-SPECIFIC SATELLITE DATA

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Rift Valley fever (RVF) is an acute zoonotic viral disease that affects domestic animals and humans in sub-Saharan Africa. More recently, a RVF outbreak was first identified outside Africa in the Arabian Peninsula. We used ecological niche modeling to assess the ability of models to identify areas of disease risk in Saudi Arabia using 8-day composite Land Surface Temperature (LST) and monthly Normalized Difference Vegetation Index (NDVI) data from the Moderate Resolution Imaging Spectroradiometer (MODIS) satellite imagery (1 km spatial resolution) for January to December 2000 (i.e., the time of disease outbreak in Saudi Arabia). The model was calibrated based on records from African outbreaks, and transferred to Saudi Arabia to anticipate suitable sites of RVF across Arabia. Results suggested suitable areas in western Saudi Arabia in Gizan region. We evaluated models using occurrence points from the 2000 outbreak in Saudi Arabia. Models calibrated in Saudi Arabia revealed similar spatial patterns. This study revealed the potential of niche modeling approaches to anticipate suitable areas for disease emergence in areas with no previous disease history, opening the possibility of genuine prediction of risk, particularly in future studies that add data on availability of vectors.

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UNDERSTANDING DENGUE TRANSMISSION AND RISK FACTORS IN BANGLADESH

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Southeast Asian countries including Bangladesh have remained hyperendemic for dengue due to unplanned urbanization, overcrowding, poverty and health inequalities. In recognition of the need for an multidisciplinary, scientific research on this problem, we applied an "Ecohealth Approach" to understand dengue virus (DENV) transmission and social-ecological risk factors in Dhaka, Bangladesh. Multiple disciplinary aspects were encapsulated by examination of: i) rates of human exposure to DENV by identifying individuals (via a serosurvey in 1200 households and 47 clinical samples) with IgM and IgG antibodies to DENV; ii) abundance of dengue vector during monsoon and dry seasons in the same households; iii) self-risk perception by the community members; and iv) human organizations responsible for interventions. Data included in the analysis are: a) two vector surveys [i.e., pupal surveys conducted in 847 households (monsoon season 2011) and 459 households (dry season 2012)]; b) two serosurveys [i.e., serosurveys conducted in 1128 households (pre monsoon season 2012) and 1130 households (630 paired sera and 500 replacement sera during post monsoon season 2012)]; c) socio-demographic survey of 300 households; and d) 12 focus group discussions and 12 key informant interviews. Competent dengue vectors were detected in >40% and 12% of households during the monsoon and dry seasons respectively. The monsoon and dry seasonal pupal index were 0.40 and 0.33 respectively for the selected 12 wards. Only 8 types of key containers and two types of ecological clusters are responsible for 72% of pupal distribution. More than 80% IgG and nearly 3% IgM were positive during pre- and post-monsoon serosurvey. Among the IgM positives, in-house PRNTs, using a serial dilution of sera mixed with a DENV serotype, are being carried out. There are significant variations in dengue risk perception between lower and higher socioeconomic groups. Also, experts ranked dengue risk at a much lower level than lay persons and experts emphasized the need for stronger institutional measures to control dengue outbreaks. The overall findings of the study will contribute to the advancement of DENV transmission knowledge, forecast the disease burden as well as socioeconomic burden in the City of Dhaka, Bangladesh.

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INVESTIGATING THE EFFICACY OF MONOVALENT AND TETRAVALENT LIVE-ATTENUATED DENGUE VACCINE FORMULATIONS AGAINST DENV-4 CHALLENGE IN AG129 MICE

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Dengue virus (DENV) causes a rapidly spreading mosquito-borne human viral disease that has major impact on global health and economics. Currently, there is no licensed vaccine against DENV. 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. In a mouse model, we investigated the immune responses to monovalent DENV-4, DENV-2 or tetravalent DENV vaccine formulations, and their efficacy against challenge with a newly mouse adapted DENV-4 strain 703. Single doses of the tetravalent or monovalent vaccines elicited neutralizing antibodies and cellular responses to DENV-2 backbone. Monovalent DENV-2 vaccine also elicited cross-neutralizing antibody responses to DENV-4. When tested against a lethal challenge of DENV-4 sixty days post-primary immunization all mock-vaccinated animals (n=15) died, but all vaccinated animals survived except for 1 of 15 DENV-2 vaccinated mice. Investigation of DENV-4 viremias post-challenge showed that only the placebo-treated animals had high viremias on day 3 post-challenge. Overall, these data highlight the immunogenicity and efficacy profiles of our candidate dengue vaccine in the mouse model.

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EFFECT OF MIGRATION STATUS ON RISK OF DENGUE VIRUS INFECTION IN PUERTO MALDONADO, PERU

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Dengue virus (DENV) affects more than 100 countries worldwide. DENV 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, mainly from non-endemic areas for DENV. The risk of DENV infection may vary between recent migrants (RM) and long-term residents (LTR) due to both biological and sociologic factors. We explored the prevalence of past DENV infection and knowledge, attitudes and practices (KAP) related to dengue disease control and prevention of RM versus LTR, defined respectively as residency in Puerto Maldonado for less than or greater than 5 years. In 2012 we conducted a cross-sectional serosurvey and administered a KAP questionnaire to members of randomly selected households. Sera were screened for antibody to DENV by ELISA and confirmed by plaque reduction neutralization test (PRNT). We created *ad hoc* indices for KAP (KAPi), household infrastructure and services (CFSi) and assets (Ai). PRNT results were analyzed against migration status and the various indices with an ordered logistic regression. Five hundred and five participants from 309 households provided a blood sample and completed a questionnaire. RM comprised 12% of the study population and were more likely to be DENV antibody negative than LTR on bivariate analysis (42% vs. 56%, p=0.043). However, after controlling for the other variables in the multivariate analysis, KAPi (p=0.032), commercial activities (p=0.023) and CFSi (p=0.017) were associated with antibody positivity, while migration status was not (p=0.226). The higher KAPi is likely consequence of experiencing the disease. Commercial activities and CFSi may be related to the location or infrastructure of areas where participants spend long periods of time. We conclude that risk of DENV infection in Puerto Maldonado relates more to socio-economic status, especially infrastructure and services available in the household, than to migration status *per se*. These findings should help tailor specific prevention and control strategies for dengue diseases in the area.

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HOST CELL RESPONSES ATTENUATE DENV-2 PDK53 BUT NOT DENV-3 PGMK30FRHL3

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The global prevention of dengue is challenging without a vaccine, the development of which has been limited by an incomplete understanding of pathogenesis. In particular, efforts to develop a live attenuated tetravalent vaccine encountered problems in balancing immunogenicity with reactogenicity. Such vaccines have been derived through serial passage of dengue viruses (DENVs) in various primary cell cultures followed by selection of strains that meet specific but empirically-derived phenotypic criteria such as small plaque size. Unfortunately, these empirically-derived criteria do not invariably inform on attenuation. The Mahidol University-developed DENV-2 PDK53 and DENV-3 PGMK30FRhL3 strains both fully met these empirically-derived phenotypic criteria. However, while PDK53 was shown to be safe and immunogenic, PGMK30FRhL3 was reactogenic and vaccine recipients developed symptoms consistent with dengue fever. In this study, we asked if a molecular approach might be able to distinguish a more accurate basis of attenuation. We observed that Huh-7 cells infected with PDK53 upregulated the expression of many genes in the innate immune system compared to wild-type DENV-2 16681 from which it was derived. In contrast, the pattern of expression of these same genes in both PGMK30FRhL3 and the parental DENV-3 16562 were similar and mostly low. Functional studies suggest that the innate immune responses restrict the plaque size of PDK53 while that of PGMK30FRhL3 plaque size is limited by its slower replication. We suggest that a molecular definition could be developed for a more accurate identification of viral attenuation.

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DENGUE VIRUS TYPE 3 EVOLUTION AND EPIDEMIC ACTIVITY IN INDONESIA, A HISTORICAL STUDY OF OUTBREAKS FROM 1976-1979

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Dengue viruses are one of today's most significant vector-borne disease agents threatening human health throughout the tropics and subtropics, infecting hundreds of millions of people annually. Dengue is primarily transmitted by *Aedes aegypti* mosquitoes. There are four known serotypes circulating in humans (DENV-1 to -4) all of which can cause a febrile illness known as dengue fever that can progress to more severe and potentially fatal disease involving hemorrhage or shock (DHF/DSS). We report here follow up sequence data on DENV-3 strains isolated during epidemics that occurred in Indonesia between 1976 and 1979. The epidemics began with the detection of fatal DHF/DSS associated with DENV-3 in Jakarta in Jan-Mar, 1976. The virus spread to Bantul, Central Java in Oct. 1976, and to Surabaya, East Java and Pontianak, West Kalimantan in 1977. All of these were explosive epidemics with associated severe disease. A smaller outbreak with more sporadic transmission, milder illness and much lower viremia levels occurred in Sleman, Central Java in 1978. Viruses were isolated by one of us (DJG) from all of these epidemics and stored in infected mosquitoes at -70 C for nearly 40 years. The viruses had not been passaged in mice nor mammalian cell cultures. Full genomic sequence analysis suggests that a single strain of DENV-3 with greater epidemic potential and possibly virulence emerged in Jakarta and spread rapidly along the main transportation routes to Central and East Java, and to Kalimantan. Interestingly, the Sleman DENV-3 viruses were genetically distinct, belonging to a separate clade from the other strains. There were two unique Bantul isolates that also belonged to the Sleman clade, suggesting that the Sleman virus descended from these Bantul viruses. Our findings emphasize the importance of dengue evolution and genetic variation as a contributor to epidemic intensity and severe dengue disease.

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ANALYSIS OF HUMAN DENGUE-IMMUNE SERA USING WHOLE VIRUS PROTEOME PEPTIDE ARRAYS

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Dengue, a mosquito-transmitted viral disease, has become a major worldwide public health burden. Four distinct serotypes of dengue virus (DENV1-4) contribute to the challenge of developing a safe and effective tetravalent vaccine. We have exploited high-density peptide arrays for the readout of serum antibodies from dengue patients from ongoing pediatric hospital-based and community-based cohort studies

in Managua, Nicaragua. First, we selected a set of 3,172 overlapping 15-mer peptides, covering the proteomes of 20 different DENV genotypes (3 DENV1, 7 DENV2, 5 DENV3, 5 DENV4). Then, we generated five peptide arrays applying our novel particle-based peptide synthesis method using a custom-built laser printer, which allows for complete content flexibility on every array. Each array enables simultaneous analysis of a patient's IgG and IgM antibody reactivity to all four DENV serotypes. We first analyzed acute, convalescent, and 12-month samples from primary and secondary DENV2 cases. The results from the arrays identified the acute infecting serotype and revealed time-dependent waning of antibody responses in patients' sera. We also observed an increase in reactivity to a few peptides after 12 months, specifically, to peptides in NS1 and NS3. To confirm these results, we are currently analyzing 46 additional serum samples of primary and secondary DENV infection with a set of 2,000 overlapping 20-mer peptides, derived from 361 different DENV strains representing all known Nicaraguan genotypes in DENV1-3 and 9 Colombian DENV4 strains. In addition, to obtain a profile of the immunogenic DENV proteome map, we incubated one peptide array with a pool of 100 patient sera from adult Red Cross donors from Nicaragua who had experienced one or more DENV infections. From this broad polyclonal response, we identified immunogenic pan-serotype protein regions, as well as serotype-specific regions. In contrast to protein arrays, our peptide-based approach allows us to obtain a fine resolution map of the proteome immunogenicity. These results can help to elucidate structural and functional differences among the DENV serotypes and point to possible diagnostic biomarkers and vaccine targets.

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ECONOMIC IMPACT OF WORK ABSENTEEISM DUE TO NON-SEVERE DENGUE VIRUS INFECTION IN A CITY IN THE PERUVIAN AMAZON

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Dengue fever is the most prevalent mosquito-borne viral disease globally. The public health impact of this disease is compounded by the fact that it occurs primarily in developing countries, adding a significant burden for local economies and individuals that are already under-resourced, undermining both regional and national development. To assess the economic impact at the community-level in Iquitos, the largest city in the Peruvian Amazon basin, we collected information on work absenteeism due to dengue virus (DENV) infection. Clinical and epidemiological data were obtained from outpatients > 18 years-old who sought care for acute febrile disease at one of twelve health facilities in Iquitos during July 2009 to June 2010, when DENV-4 was the predominant circulating serotype. Dengue fever was confirmed by PCR, IFA or by a four-fold rise in IgM titer between acute and convalescent blood samples. Housewives were not included in the study, since they are not formally financially compensated. Daily wage estimates were assigned based on the reported occupation (the average of reported salary ranges in the city), then multiplied by the number of work days reported lost due to illness by each case. The effect on the local economy was estimated using data from the Regional Government. Of the 504 enrolled patients who engaged in wage-earning employment and provided absenteeism information, 199 (39%) had laboratory-confirmed dengue fever. Dengue fever patients missed an average of 3.9 work days translating to a mean income loss of US$45.Twenty-six percent of those sampled earned US$151-199 monthly, significantly less than the Peruvian minimum wage of US$283. The average income loss in this group was US $48, representing 24-32% of their monthly income. Despite the fact that these dengue fever cases were not severe because they were all outpatients, in the context of a local economy where many people are independently employed and have subsistence-level income, DENV infection posed a considerable financial burden.

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IDENTIFYING HOST INNATE IMMUNITY FACTORS CRUCIAL FOR PROTECTION AGAINST DENGUE VIRUS

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Dengue virus (DENV) is a mosquito-borne human pathogen with no existing vaccine. Previous vaccine development efforts have relied on serial passage in cell culture to derive attenuated strains. This approach by Mahidol University led to a tetravalent vaccine with all strains meeting *in vitro* phenotypic criteria of attenuation, such as small plaque size. In clinical trial, DENV-3 PGMK30FRhL3 caused symptoms consistent with dengue fever, whereas DENV-2 PDK53 did not. As such, the traditional phenotypic criteria are not able to accurately predict human response to vaccines. Defining the molecular basis of attenuation could thus provide for more objective means of assessing candidate vaccine strains. We hypothesized that the PGMK30FRhL3 strain maintains its wild-type ability to evade host antiviral defenses, while the PDK53 strain elicits protective antiviral sensors and interferon signaling. To study host innate immunity defenses, we performed RNAi knockdown of key genes of antiviral sensors and interferon signaling cascade--MAVS, IRF3, TRIF, STAT1, and NF-kB--in Huh-7 and BHK-21 cells. Following infection with PDK53 and PGMK30FRhL3, we compared plaque and focus size between cells with normal and impaired antiviral defenses. We then quantified viral spread via plaque size, immunofluorescence foci, and flow cytometry. As expected, plaque size of attenuated PDK53 was smaller than wild type DENV-2 16681. Upon knockdown of IRF3 or p50, the plaque size of PDK53 but not 16681 increased significantly. Similar observations could be made in the human Huh-7 cell line. These findings indicate that the plaque size of PDK53 is because of its inability to overcome the innate immune response, whereas the replication of wild type 16681 does not benefit from the knockdown of these innate immune genes. Our results suggest that the inability of PDK53 to escape innate immune recognition is the molecular basis of attenuation.

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THE SPATIAL PATTERNS AND INFLUENCES OF CLIMATE VARIATIONS ON DENGUE OUTBREAKS IN SOUTHERN TAIWAN THROUGHOUT 1998 TO 2012

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Southern Taiwan has been a hotspot of Dengue Fever (DF) transmission since 1998. The incidence of dengue fever in Taiwan shows strong seasonality. Mosquito ecology and the transmission of dengue fever are influenced by multiple environmental factors, especially for climate variations. Thus, interannual variability in climatic conditions could be important drivers for annual outbreaks. This study explored the spatial patterns of dengue outbreaks in Tainan and Kaohsiung city in Southern Taiwan. Multiple climatic indices generated from weather stations at these two study regions were used to develop a model to evaluate the risk of dengue transmission. In view of disease early warning system, the climate variations in the early season is emphasized in the analysis rather than the conditions during the summer time. Non-linear generalized additive models (GAMs) were used to evaluate the influences of monthly weather

variables, including average temperature (TAVG), maximum temperature (TMAX), minimum temperature (TMIN), precipitation (PREC), and relative humidity (RH), on interannual variations of DF incidence from 1998 to 2012. The significant temporal and spatial heterogeneity of large scale DF outbreaks were shown in the two cities. Kaohsiung experienced significant outbeaks in 2002, 2010, and 2011; however, 2007 and 2012 are two outbreaks years in Tainan. The spatial patterns vaired in different year for both regions. The best-fitting model highlighted the importance of temperature, especially for TMIN, on the transmission of DF. Warmer TMIN during the preceding winter indicated elevated DF risk for the next summer and fall in both regions (p=0.03 for Kaohsiung, p=0.001 for Tainan). The outbreak of DF in Tainan is also associated with TMIN in February (p=0.007); however, DF outbreaks in Kaohsiung could be determined by the TMIN in April (p=0.009). The different responses to temperature variations in the two connected cities might be affected by other environmental factors, such as urban stuctures or land use types. Our modeling approach can provide useful information for establishing dengue early warning system in Taiwan.

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CLIMATE VARIABILITY AND DENGUE EPIDEMICS IN PUERTO RICO

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Inter-annual climate variability is driven by climate systems such as El Niño-Southern Oscillation (ENSO) and the North Atlantic Oscillation (NAO). Warm ENSO events have been found to be associated with dengue epidemics in the Caribbean, whereas the impact of NAO variability on dengue dynamics has not been investigated. These climate systems affect the local climatology (air temperature, rainfall) of Puerto Rico, which in turn may affect dengue virus transmission. This study investigated the influence of inter-annual climate systems at various temporal scales (decadal, yearly, monthly) on local meteorological variables and dengue dynamics in Puerto Rico from 1987-2013. It was found that the impact of El Niño on dengue incidence may be modulated by the phase of the NAO, and that both climate systems can interact to exacerbate or reduce the magnitude of epidemics. For example, the largest dengue epidemic ever registered in Puerto Rico in 2010 occurred after a strong El Niño that peaked in December 2009 / January 2010, bringing anomalous warmer conditions and a strong negative NAO that brought exceptionally wetter conditions. Annual dengue incidence was significantly associated with temperature and dengue incidence during the winter. Warmer winters allow higher than normal dengue virus transmission that may cause epidemics during the rainy season when mosquito populations increase.

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USE OF HOUSEHOLD CLUSTER SURVEYS TO QUANTIFY UNDER RECOGNITION OF DENGUE DURING THE 2013 EPIDEMIC IN LUANDA, ANGOLA

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Dengue is endemic throughout the tropics and is under recognized in Africa. In March 2013, a dengue epidemic was identified in Luanda, the capital of Angola. In total, 1,214 dengue cases were reported, of which 811 (67%), including 11 fatal cases, tested positive with a dengue rapid diagnostic test (RDT). Only dengue virus (DENV)-1 was identified. To estimate the percent of individuals infected during the epidemic and identify risk factors for infection, we conducted household cluster surveys within a 25-meter radius of RDT(+) dengue case-patients' and randomly selected households. All participants reported demographic data, medical history, and individual and household mosquito avoidance practices, and provided a serum specimen for dengue diagnostic testing by RT-PCR and anti-DENV IgM ELISA. Of 453 specimens collected, anti-DENV IgM was detected in 41 (9%); none were positive by RT-PCR. Of 173 individuals from 67 households in 21 RDT+ clusters, 16 (9%) were anti-DENV IgM(+). Of 247 individuals from 90 households in 26 random clusters, 25 (10%) were anti-DENV IgM(+). There were no statistically significant differences in frequency of detection of anti-DENV IgM among individuals, households, or clusters. Of the 41 anti-DENV IgM(+) individuals, 13 (32%) reported fever in the past 30 days, of which 5 (38%) and 1 (8%) reported symptoms consistent with dengue with warning signs and severe dengue, respectively. Seven (54%) of the recently infected febrile individuals sought medical care; 1 (14%) was hospitalized, and none were diagnosed with dengue. Factors associated with protection against recent DENV infection identified in this sample of individuals included bed net use in the past 30 days (p = 0.04) and delivery of household water by truck (p = 0.01). Identification of recent DENV infection among 10% of survey participants and lack of accurate diagnosis of participants that sought medical care suggest under recognition and underreporting of dengue. Clinical awareness of dengue should be strengthened to better define the epidemiology of dengue in Angola.

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COMMUNITY-BASED PARTICIPATORY RESEARCH FOR PREVENTION OF DENGUE FEVER FROM THE APPROACH TO HEALTH COMMUNICATION: THE EXPERIENCE IN THE CARIBBEAN SLOPE OF COSTA RICA

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Dengue fever is the most important tropical vector-borne disease in Costa Rica. In 2013, the country had the largest outbreak in its history when 49 993 new cases were reported. A Community-based participatory research (CBPR) was developed between years 2012-2014 at La Roxana of Pococí, an endemic locality at the caribbean slope. Social, cultural and health public components of two communities (Luis XV and San Antonio) were studied in 2012 with the purpose of promoting community organization for Dengue prevention during 2013. Focal groups and etnomethodology were used as qualitative tools meanwhile knowledge, attitudes and practices survey has been employed as quantitative approach. Qualitative data has suggested that Dengue Fever has not been an important health public trouble since community perception. Communities were more interested in problems as water disposal and availability, and waste management. This has caused difficulties for the implementation and acceptance of vector control and other community actions. Although, quantitative data has suggested that communities have a lot of knowledge about dengue, its vector and specific actions for the disease control. Knowledge has been obtained from mass media, national education system and the neighborhood. Data analyzed were used to promote women organization in Luis XV and young people organization in San Antonio for implementation of a public health strategy for control of dengue based in health promotion and waste management. Indicators have suggested success in implemented actions. CBPR has demonstrated

the importance of community awareness to increase the success of communication strategies for the prevention of dengue. Also, when CBPR is based on the health needs and interests, it can promote the community self-organization. In the case of La Roxana women's empowerment and leadership of community groups were considered essential for success.

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A NOVEL ALLOSTERIC SMALL MOLECULE INHIBITOR OF INDUCIBLE HSP70 REDUCES DENGUE VIRUS INFECTION

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The inducible protein chaperone Heat shock protein 70 (Hsp70i) is important in maintaining protein folding and cellular homeostasis. Hsp70i is utilized throughout the viral lifecycle for replication and propagation of the virus. Dengue virus, HIV, and rotavirus are a few of the viruses that exploit Hsp70i for infection and replication. However, the complete role of Hsp70i in dengue virus pathogenesis remains unclear. Previous studies have shown that Hsp70 may act as a receptor complex for virus internalization. Additionally, Hsp70 siRNA knockdown reduced dengue virus load, and Hsp70 aids in propagating the virus following internalization. Dengue virus is now endemic in over 100 countries and there are currently no approved vaccines or treatments for dengue virus infection. To date, few Hsp70 inhibitors have been identified and characterized, and their efficacy in clinical settings is unknown. We have identified a novel allosteric small molecule inhibitor of Hsp70i, HS-72, using FLECS (fluorescence linked enzyme chemoproteomic strategy). Inhibition of Hsp70i in a monocyte cell lines reduces dengue virus infection, while maintaining cell viability. Additionally, HS-72 leads to a reduction in the binding and entry of dengue virus in monocytes. Hsp70i is expressed at low levels preceding infection, but intracellular Hsp70i expression is rapidly induced upon dengue virus infection, while surface Hsp70i is unaffected. Furthermore, increasing intracellular Hsp70i expression prior to infection through Hsp90 inhibition, leads to an increase in dengue virus infection. This work highlights an essential role for Hsp70 in dengue virus pathogenesis and identifies a potential therapeutic anti-viral agent for dengue virus infection.

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NEUTRALIZATION OF DENGUE VIRUSES IN VIREMIC HUMAN BLOOD; MAPPING THE MOST POTENT VIRUS NEUTRALIZING HUMAN MABS

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A better understanding of the characteristics of antibodies that neutralise dengue viruses *in vivo*, rather than *in vitro*, is a research priority. We established an experimental human-to-mosquito dengue virus (DENV) transmission system for measuring the potency of human mAbs to neutralise dengue virions in DENV viremic human blood collected from Vietnamese dengue cases. In this assay we have characterized the potency of a panel of 15 human mAbs that target a range of epitope regions on all four DENV serotypes. The results demonstrated that selected serotype-specific mAbs, recognizing a quaternary epitope, were the only mAbs able to neutralize DENV in the "*in vivo*" environment of viremic blood. These results identify a class of antibodies that dengue vaccines should aim to elicit.

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ASSOCIATION OF NUTRITIONAL STATUS AND DENGUE COMPLICATIONS IN CHILDREN: RESULTS FROM A PROSPECTIVE COHORT STUDY CONDUCTED IN COLOMBIA

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Undernutrition is an important predictor of complications in communicable diseases; however, the evidence of its role in pediatric patients with dengue remains controversial. We conducted a prospective cohort study (in three recruitment waves between 2003 and 2011) among out-patients aged 5-19 years old from Colombia, who had fever and serologic or virologic evidence of dengue infection. Socio-demographics, warning signs, and nutritional status were ascertained at baseline. Nutritional status was determined by calculating height for age (HFA) and body mass index for age (BFA) z-scores using the software WHO-ANTHRO. Patients were followed for complications defined as new-onset of shock (age-specific tachycardia and pulse pressure <20 mmHg or systolic or mean blood pressure below PAHO's age-specific cut-points for hypotension) or severe hemorrhage (hematemesis, melena, hematochezia or hematuria). We evaluated 330 children free of complications at baseline (mean age: 12.3 years; 57% male; mean disease's duration: 3.5 days). During a median follow-up of 3 days, 75 patients (22.7%) developed complications: 63 (19.1%) shock, 9 (2.7%) severe hemorrhage, and 3 (0.9%) shock and severe hemorrhage. Baseline HFA and BFA were lower among incident than non-incident cases: -0.1 vs. 0.3 (p=0.021) and -0.3 vs. 0.1 (p=0.007), respectively. After controlling for age, gender, and disease's duration, an increase in 1 z-score unit of HFA and BFA were independently associated with 23% (OR=0.77; 95%CI: 0.61, 0.98) and 25% (OR=0.75; 95%CI: 0.61, 0.92) lower probability of complications, respectively. There was no evidence of HFA-by-BFA interaction (p=0.377). Further adjustment for baseline warning signs did not attenuate associations. Our results suggest that, from a public health perspective, tackling undernutrition in dengue endemic countries might reduce the burden of the disease in the pediatric population.

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ESTIMATING CROSS-ENHANCEMENT AND CROSS-PROTECTION OF DENGUE VIRUSES USING TIME SERIES DATA FROM THAILAND

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Using serotype specific case data from Queen Sirikit National Institute for Child Health in Bangkok, previous work estimated the length of cross protection to be 1-3 years. We have extended this work in two directions. First, we have developed models to consider whether there are serotype-specific differences in the cross-protection period and secondly to estimate the population-level impact of any enhancement of susceptibility or symptom severity of secondary infections, concurrently with cross-protection. We estimate whether the length of cross protection depends on the serotype of the primary or secondary infection, or the combination of both. Early results show that it may be difficult to detect any differences in the length of cross-protection by serotype. However our work continues to define what we can estimate, and what the impact of differential infection-to-case ratio or transmissibility by serotype will have on this estimate. We derive estimates of both cross protection and enhancement by combining data from multiple sources, including serotype specific and non-serotype specific case data from multiple locations in

Thailand. Early results show that the estimates of the duration of cross-protection from previous work are robust to the inclusion of susceptibility enhancement in the model. Further work is currently underway to further test these preliminary results. The model framework also provides estimates of the seasonality in transmission. We correlate these estimates of seasonality in multiple places with climatic variables throughout the year. From this we can determine drivers of seasonality in transmission, how this relationship varies depending on serotype, and how seasonality in transmission interacts with immunological processes. Methodologically, the extension of this framework to use multiple data sources will give us more power to estimate parameters that govern transmission, by making use of the similarities and differences observed in multiple locations. Further consideration of these processes of dengue transmission will enhance our understanding of what drives the observed dynamics of dengue cases. This, in turn, could lead to improvements in future dengue control efforts.

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AN EARLY-LIFE DENGUE VACCINE CANDIDATE INDUCES EFFECTIVE IMMUNITY IN A MOUSE MODEL

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Dengue viruses (DENV1-4) cause 50-100 million clinical infections every year, several hundred thousand of which progress to severe hemorrhagic and shock syndromes. Preexisting immunity resulting from a previous DENV infection is the major risk factor for severe dengue during secondary heterologous infections. During primary infections in infants, maternal antibodies pose an analogous risk. At the same time, maternal antibodies are likely to prevent induction of endogenous anti-DENV antibodies in response to current live, attenuated vaccine (LAV) candidates. Any effective early life dengue vaccine has to overcome maternal antibody interference (leading to ineffective vaccination) and poor induction of antibody responses (increasing the risk of severe dengue disease upon primary infection). Here we demonstrate the feasibility of a non-propagating VEE virus replicon vector (VRP) expressing DENV E protein as an early life vaccine platform for dengue. We previously showed that a DENV-VRP vaccine is immunogenic even in the presence of maternal antibodies that otherwise interfere with a live virus vaccination in weanling BALB/c mice. In this report, we observed that a single immunization in 7-day-old neonatal BALB/c mice with a VRP vaccine expressing E ectodomain of DENV induced neutralizing antibody (NAb) titers by 6 weeks, which remained stable until at least 15 weeks post-immunization. DENV-specific cell-mediated immunity was also induced in these immunized mice. Furthermore, the NAb levels induced to each serotype by a tetravalent VRP formulation were equivalent to those of each monovalent vaccine components, suggesting that this vaccine modality can overcome serotype interference. VRP immunization in neonatal mice was durable and could be boosted later in life to further increase NAb and T-cell responses. Although the neonatal immune response was lower in magnitude than responses in adult BALB/c mice, we demonstrate that, both monovalent and tetravalent VRP vaccines generated protective immunity from a lethal intracranial challenge after a single neonatal immunization. In summary, VRP vaccines expressing DENV antigens were immunogenic and protective in neonates, and hence are promising candidates for safe and effective vaccination in early life.

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DEFINING THE TARGETS OF DENGUE VIRUS INFECTION IN HUMAN SKIN

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Dengue is the most important arthropod-borne viral infection of humans causing an estimated 50-100 million cases worldwide every year. Dengue virus (DENV) is introduced into human skin, its replication site, by the bite of infected mosquitoes. While Langerhans cells have been implicated as the main targets of DENV infection the dynamics of infection of human skin remain ill-defined. Here, we exposed skin explants from healthy human donors to DENV to determine the targets of infection. Virus was inoculated into skin explants using a stabbing method with a bifurcated needle, and mock-infected and infected skin was analyzed by immunofluorescence after intervals of incubation using antibodies to cell-specific markers and viral protein NS3. Time course experiments showed that the first targets of DENV infection were basal keratinocytes, with infection first detected within 8 hours. From 12-48 h of infection abundant virus replication was detected in epidermal Langerhans cells and dermal macrophages as well as tubular structures consistent with lymphatic endothelium. Quantitative analysis indicated that exposure to DENV resulted in significant infiltration of macrophages into dermis. These preliminary data suggest that DENV initially infects basal keratinocytes which may release factors promoting the mobilization of infected Langerhans cells out of the epidermis, and the influx of macrophages into the dermis, which subsequently replicate DENV to high levels. Ongoing experiments are designed to determine the mechanism for macrophage recruitment. These studies are revealing that DENV infection of human skin is a dynamic process involving sequential infection and recruitment of distinct cellular targets.

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USE OF INTEGRATED DISEASE SURVEILLANCE AND RESPONSE SYSTEM (IDSR) TO DETERMINE THE EXTENT OF DENGUE FEVER OUTBREAK ALONG THE COASTAL REGION OF KENYA

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Integrated Disease Surveillance And Response System (IDSR) is a comprehensive strategy used for capturing information on communicable diseases by the Ministry of Health (MOH) in Kenya. From January to May 2013, MoH reported laboratory confirmed cases of dengue (DEN) along the Kenyan coast in Mombasa County. The Kenya Medical Research Institute (KEMRI) and the US Centers for Disease Control and Prevention (CDC) confirmed the presence and co-circulation of three DEN serotypes (DEN1, DEN2 and DEN3). On May 29th, 2013, KEMRI and CDC together with MoH initiated enhanced passive surveillance of DEN in six public sites in Coastal Kenya; Likoni, Port Reitz, Lamu, Kilifi, Msambweni and Taveta district hospitals and in 3 private hospitals; Bomu, Pandya and

Mombasa Hospital. The surveillance was from 21st June to 30th September, 2013. All persons presenting at the healthcare facilities with symptoms consistent with the case definition of suspect DEN; temperature ≥38.0°C for up to five days AND did not meet criteria for acute respiratory illness, was to be reported using IDSR outbreak report forms. A blood sample was taken where possible from two suspect DEN cases per day per site for five days a week in the public hospitals and in the private hospitals, as cases were identified. Real-time RT-PCR was performed at the KEMRI/CDC laboratories. Blood samples were collected from all 67 suspected cases identified. Two-thirds (44/67) were from the public hospitals. Of the 67 cases, 14 (21%) tested positive for DEN, 78.6% from private hospitals and 21.4% from public hospitals. Use of IDSR report forms varied across facilities and partially conformed to the MoH guidelines. We found varying rates of positivity and compliance between the public and private facilities. IDSR is the strategy adopted by WHO-AFRO for disease surveillance and outbreak response in the region; however it needs to be strengthened to ensure that the planned objectives are met while ensuring optimal resource use. In Kenya, IDSR implementation following devolvement of government may need to be decentralized to the county level.

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SPATIAL ANALYSIS AND MODELING OF DENGUE SEROPREVALENCE IN VENEZUELA

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Dengue has become one of the most important public health problems of urban areas in Venezuela. Control of dengue and of its mosquito vector has proven challenging in settings of uncontrolled urban growth and unreliable water supply. The ability to identify high-risk areas of dengue transmission can be used to target surveillance and control measures to those locations in a cost-effective manner, particularly in countries where resources are scarce. We used mapping technology and spatial statistics to identify clusters ("hot spots") of transmission within a community-based cohort of 2012 individuals living in 840 households in the hyperendemic city of Maracay, Venezuela. Two spatial analyses of epidemiological and dengue seroprevalence data were conducted: 1) at house level, and 2) at block level. Risk-maps drawn at a fine scale determined that dengue seroprevalence is highly heterogeneous at the studied spatial scale. We detected significant hot spots (Gi*[d] >2.79, P≤0.05), at each spatial scale in all neighbourhoods. Our results also suggest that dengue transmission is very focal (20-80 meters). To better explain what determines dengue spatial clusters, we did comparative analyses of risk factors within and outside of hot spots areas using logistic regression modelling. Finally, we applied spatial regression modelling to identify which variables (demographic, socioeconomic and environmental) were more relevant to explain local dengue dynamics in Venezuela. Results will be presented.

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LONG-TERM EPIDEMIOLOGICAL ANALYSIS OF PEDIATRIC DENGUE IN VENEZUELA (2000-2011)

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Dengue is a major public health problem in Venezuela. It exhibits a variable epidemiological pattern in time, and associates with a significant annual number of cases, ±50% of which, are seen in children. A retrospective morbidity-mortality and disease burden study of dengue in Venezuelan pediatric population, based on official data from the Ministry of Health and National Institute of Statistics from 2000 to 2011, was carried out. Statistical analysis was performed by a SPSS20 program. Significance tests were performed as required. Burden of disease was estimated as DALYs associated with cases and deaths. Of the 632,066 dengue cases registered in the general population, 316,404 (50.05 %) were seen in children <15 Y/O. Sixty nine per cent of the cases occurred in six epidemic years (2001/2005-2007/2009 and 2010), the latter with a historical record of over 110,000 cases. Whereas the average General Morbidity Rate for the period was 194.87, it was 1.7 higher in children (332.19). The most affected age group was that of 5-9 Y/O (364.98). The <15 Y/O experienced 28,065 cases of severe dengue (56.60% of all severe dengue). Mortality Rate in children was 1.42 times higher than in adults (0.27 vs. 0.19, respectively) ($p < 0.05$), and in the group under 1 year it was 4.10 times as much (0.78) ($p <0.01$). Lethality rate was 20% lower in children than in adults (0.08 vs. 0.10, respectively) ($p <0.01$). However, in <1 Y/O it was 2,4 times higher than in adults and 3,4 times more than in all other pediatric age groups ($p <0.01$). Of note, during the last half of the analyzed period both mortality and lethality rates significantly increased for all pediatric age groups, especially in 5-9 Y/O. Disease burden was estimated in 3,793.65 DALYs per clinical cases and 9,796.78 per deaths. In Venezuela, dengue exhibits both endemic and epidemic cycles. Epidemics were frequent in the studied period and associated with majority (±70%) of reported cases, reflecting failures in the control and prevention programs. Lethality in children was lower than in adults and that reported internationally. The marked increase in the mortality and lethality during the last lustrum is unexpected, and requires to be explained. Although the most affected groups were the 5-14 Y/O, mortality and lethality rates were much higher in those less than 1 year. These results may be useful to understand better the epidemiology of the disease in the country and improve the effectiveness of disease control.

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THE HUMAN CD8+ T CELL RESPONSES INDUCED BY A LIVE ATTENUATED TETRAVALENT DENGUE VACCINE ARE DIRECTED AGAINST HIGHLY CONSERVED EPITOPES AND ARE SIMILAR IN MAGNITUDE AND BREADTH TO THOSE FOLLOWING NATURAL INFECTION

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The incidence of infection with any of the four dengue virus serotypes (DENV 1-4) has increased dramatically in the last few decades, and the lack of a treatment or vaccine has contributed to significant morbidity and mortality worldwide. The recent failure of a candidate vaccine to protect

against disease despite induction of antibody responses against all DENV serotypes in most subjects suggests that better correlates of protection are needed. A recent comprehensive analysis of the human T cell response against wild-type DENV suggested an HLA-linked protective role for CD8+ T cells. Here, we characterize for the first time CD8+ T cell responses after live attenuated dengue vaccination and compare them to responses observed in natural infection with dengue virus. We have collected one-unit blood donations from study participants receiving the monovalent or tetravalent live attenuated DENV vaccine (DLAV), developed by the U.S. National Institutes of Health. PBMCs from these donors were screened in IFNγ ELISPOT assays with pools of predicted, HLA matched, class I binding peptides covering the entire DENV proteome. CD8+ T cell responses in vaccinees were readily detectable with a magnitude and breadth similar to natural dengue infection. Interestingly, while broad responses to structural and non-structural (NS) proteins were observed after monovalent vaccination, T cell responses following tetravalent vaccination were, dramatically, focused towards the highly conserved non-structural proteins NS3 and NS5. Epitopes from these proteins are highly conserved in a vast variety of field isolates and are able to elicit multifunctional T cell responses. Live attenuated vaccines against dengue virus are able to induce a CD8+ T cell response comparable to responses seen in natural infection. Detailed knowledge of the T cell response will contribute to the identification of robust correlates of protection in natural immunity and following vaccination against DENV.

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HISTOLOGIC CHARACTERIZATION OF THE LOCAL IMMUNE RESPONSE TO DENGUE VIRUS INFECTION IN INTRADERMALLY INOCULATED MICE

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Dengue virus (DENV) is an emerging arboviral pathogen transmitted primarily by the mosquito *Aedes aegypti*. Approximately 40% of the world's population lives in regions that are at risk for DENV transmission. This virus is associated with a high morbidity of febrile disease and in a small percentage of cases, disease can progress to more severe manifestations. Several models of DENV infection have been developed to study the pathogenesis of this disease in various organ systems. While some experiments have examined the role of dendritic cells following intradermal inoculation of DENV, none have attempted to qualify the local inflammatory response and potential differences between viral serotypes *in vivo* to elucidate the pathogenesis that leads to disease-causing viremia. In this study, mice were intradermally inoculated with either DENV 2 [strain 1232] or DENV 4 [strain 1228] in the hindlimb footpad after allowing mosquitoes to feed at this site. For negative control mice, mosquitoes were allowed to feed, but no viral inoculum was injected. Mice were sacrificed at 3 hours and 18 hours post-inoculation (hpi). The distal hindlimbs, as well as the associated popliteal lymph nodes, were harvested, formalin-fixed and processed for histologic evaluation. At 3 hpi, the dermis was mildly expanded and the inflammatory response consisted predominantly of small numbers neutrophils, fewer mast cells and occasional macrophages. The popliteal lymph nodes contained small numbers of mast cells and fewer neutrophils. There were no observable differences associated with serotype and the inflammatory infiltrates in virus inoculated mice were similar to negative control mice. Histologic evaluations of the 18 hpi mice are pending, as are immunofluorescence assays to correlate location of viral antigen with the inflammatory responses. Characterization of the local immune response following intradermal DENV infection will help elucidate the early pathogenesis of disease and determine any potential differences associated with serotype.

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ASSESSMENT OF INCIDENT DENGUE VIRUS INFECTIONS AMONG FEBRILE PATIENTS IN WESTERN KENYA

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Although dengue occurs in Africa, it is poorly identified and there is uncertainty about its geographic distribution. In many settings, a lack of diagnostic capacity has resulted in use of syndromic guidelines for the management of febrile illnesses. In East Africa, dengue outbreaks have been reported recently and seroprevalence studies suggest dengue virus (DENV) transmission. A retrospective study was conducted to estimate the incidence of DENV infection among febrile patients in western Kenya. Serum was collected from febrile (≥38°C) patients in an ongoing population-based disease surveillance study in Asembo District, Kenya, from Sept.-Nov. 2011 and Mar.-July 2013; rainy seasons when DENV transmission was expected to be high. We excluded patients with alternative clinical or lab diagnoses (e.g., bacteremia, positive urine culture, virus-positive nasal swab), or >5 days of fever. Testing for DENV RNA was performed by real-time RT-PCR and RNA integrity was verified by a human RNAse P control. DENV positive and negative controls performed as predicted. A total of 688 febrile patients met the inclusion criteria and 615 (89.4%) of them had samples available for testing. The median age was 4.5 years and 53% were female. No samples were DENV RNA positive. In this study, no DENV infections were detected using molecular diagnostic testing, despite samples being collected in what would be the viremic period of dengue. Possible reasons include inaccurate reporting of fever onset, a predominantly rural study site resulting in lower vector density than in coastal Kenya where incident dengue has been detected, or a genuine absence of DENV. If subsequent IgM analyses are also negative, the combined results will support the idea of uneven distribution of dengue in East Africa. Such variation, combined with the possible spread of disease in the area, reinforces the need for including dengue diagnostics in febrile illness surveillance to better inform management of fever.

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RISK FACTORS OF BLEEDING IN HOSPITALIZED ADULT DENGUE PATIENTS

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Hemorrhagic manifestations are undesirable clinical outcomes and important criteria of dengue prognosis. Clinical bleeding is required for the classification of dengue hemorrhagic fever. Severe bleeding is a form of severe dengue. However, risk factors for bleeding in dengue patients are not well studied. We conducted a retrospective study of adult dengue patients hospitalized at Communicable Disease Center, Singapore from 2005 to 2008, confirmed by positive PCR or dengue serology with World Health Organization 1997/2009 probable dengue criteria (n=6070). Clinical bleeding was defined as bleeding excluding petechiae and severe bleeding include gastrointestinal bleeding or menorrhagia or requiring blood transfusion. Those with no prior bleeding were included into the analysis (n=4383).Through statistical modelling, we aim to identify factors associated with clinical and severe bleeding. There were 869 (19.8%) and 148 (3.4%) patients who developed clinical and severe bleeding respectively. Variables with a p-value of <0.2 in the univariate analysis were entered into the multiple logistic regression model. The final model was derived using manual backward elimination and adjusted for age, gender,

disease severity, Charlson's score, hematocrit and aspartate transaminase. Fever on admission (aOR:1.4 [1.2-1.7]), absence of rash (aOR:0.8 [0.7-0.99]), anorexia (aOR:1.2 [1.01-1.4]), neutrophilia (aOR:1.01 [1.008-1.02]), leukopenia (aOR:0.86 [0.82-0.9]) and thrombocytopenia (OR:0.996 [0.993-0.998]) were significantly associated with clinical bleeding. Fever on admission (aOR:2 [1.3-2.9]), neutrophilia (aOR:1.02 [1.005-1.03]) and abdominal pain (aOR:1.44 [1.003-2.05]) were significantly associated with severe bleeding. Our findings warrant further validation in different cohorts, including other countries, children and different serotype outbreaks.

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STUDY OF CROSS-REACTIVE ANTIBODIES AGAINST DENGUE VIRUS ENVELOPE PROTEIN FOLLOWING HETEROTYPIC IMMUNIZATION AND SECONDARY INFECTION

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The four serotypes of dengue virus (DENV) are the leading cause of arboviral diseases in humans in tropical and subtropical regions. After secondary DENV infection, multitypic neutralizing (NT) antibodies (Abs) were developed not only against the previously exposed serotypes but also against the serotypes to which they have never been exposed ("non-exposed serotypes". These heterotypic NT Abs are believed to contribute to protection against subsequent infection by the non-exposed serotypes. The nature of these NT Abs remains largely unknown. To study cases of well-documented primary and secondary DENV infections, we examined sera from 10 vaccinees, who received two doses of live-attenuated vaccine in a heterotypic immunization study (Durbin et al. J Infect Dis 2011;203:327-334). Serum samples prior to and at 42 days after primary and secondary DENV immunizations were examined by Western blot analysis, virion-ELISA, modified 8M urea-ELISA, and focus reduction neutralization test (FRNT). Binding studies with virion-ELISA and IgG avidity showed stronger recognition of the primary infection serotype compared to other serotypes after secondary DENV infection, which is consistent with the "original antigenic sin". FRNT revealed multitypic NT Abs to both exposed and non-exposed serotypes. Depletion with West Nile virus antigens resulted in reduction of NT activities, suggesting that group-reactive Abs contributing to NT activities after secondary infection. Similar trend was also observed in sera from patients with secondary DENV infection. Together, these findings resonate with our recently published report of high-avidity and potently NT cross-reactive human mAbs derived from patients after secondary infection.

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DENGUE TRANSMISSION IN A MEDIUM-SIZED CITY FROM BRAZIL AND THE LESSONS WE CAN LEARN TO AVOID IDENTICAL SCENARIOS ELSEWHERE

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Dengue viruses (DENV) have been a public health problem in tropical regions for decades but autochthonous transmission has now been reported in countries like USA, France and Croatia. In 2013, 2.35 million dengue cases were reported in the Americas and Brazil alone was responsible for more than 50%. The country has been presenting outbreaks for more than three decades and important lessons can be learned. Thus, in depth analysis of dengue transmission in Araraquara, a medium-sized city at the central portion of São Paulo, which is the richest state of Brazil and a critical area for DENV transmission, may clarify how random circulation of the virus may evolve to massive outbreaks. DENV has been circulating in the city since 1990s at low incidences. However, the number of cases has increased in recent years and we will be describing the scenario of five years of transmission herein. Official data on dengue reports from 2008 to 2012 were recovered from the Information System on Diseases of Compulsory Declaration. Data from 5,282 reported cases were analyzed. The majority - approximately 60% for the five-year period - was reported in people with up to six years of formal education; this trend is an indication that areas with low living standards may play an important role in DENV dispersion. Another important observation is that dengue transmission has become endemic in the city, with cases being officially reported in all months of the year, with the exception of 2009, which was atypical in the whole state of São Paulo. Severe dengue or dengue with complications was a rare event in the city. The incidence in Caucasians (78,4%) was higher than in other ethnic groups, a pattern that has been described worldwide. Females were more affected (54,5%) than males and further analysis is required to assess this figure. This is part of an ongoing project that is also focused on classical and molecular epidemiology involving the implementation of regular molecular diagnosis in the city, phylogenetic analysis of serotypes in humans and mosquitoes and spatial statistics.

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USE OF ANTIGENIC CARTOGRAPHY TO CHARACTERIZE NEUTRALIZATION OF DIVERSE DENGUE VIRUSES IN THE MONTHS FOLLOWING PRIMARY DENGUE VIRUS INFECTION

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The four dengue virus types (DENV1-4) elicit early neutralizing antibody responses with high titers to homologous as well as heterologous DENV types, which have been observed to narrow to the homologous type in the ensuing months. However, research on changes in the neutralizing response over time often use single representatives of each DENV type, and thus the degree to which changes are due to the quantity of antibodies or the pattern of neutralization is not well understood. Here, we use antigenic cartography to describe how 17 primary-infection African green monkeys (AGM) with antisera drawn one, three and five months after inoculation neutralize a panel of 40 diverse DENV isolates assembled by the Dengue Antigenic Cartography Consortium. The neutralizing antibody response to the DENV panel over the four-month period significantly declined for eight AGMs, was stable for eight AGMs, and dramatically increased for one AGM. We made an antigenic map of the DENV panel titrated against the AGM antisera drawn at one, three and five months, as the fit of the data was comparable to maps made of each time point separately. Like antigenic maps made of each time point, the distance within and between DENV types was comparable: DENV2, DENV3, and DENV4 had similar variation within and between DENV types, while DENV1 isolates were slightly more varied between than within type. Antisera drawn one-month post-infection differed in position on the map by an average of 3.2-fold dilutions from three-month antisera (SD: 2.4-fold), while three-month antisera were only 1.6-fold dilutions from five-month antigenic positions (SD: 1.4-fold). This difference was significant, and suggested moderate changes in reactivity between one and three months, but minimal changes between three and five months. Only four AGMs shifted to the periphery of homologous antigenic cluster, suggesting increasing type-specificity. The remaining antisera moved toward the center of the antigenic map over time, thus transitioning to more cross-reactive responses. We find that only a subset of primary infection antisera become more type-specific over time, and that minimal changes in reactivity are observed between three and five

months after infection. Further, the use of all three time points enables better coordination of the DENV panel, making possible comparison of the relationships between viral genetic and antigenic differences.

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EVIDENCE FOR THE RECENT EMERGENCE OF DENGUE IN BANGLADESH: RESULTS FROM A SEROPREVALENCE STUDY

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Dengue disease is endemic throughout Southeast Asia and has been reported throughout India. Global models of dengue incidence suggest it is widespread across Bangladesh; however, while dengue infections have been reported in Dhaka and other cities, it is unknown if the pathogen has spread to rural communities that make up the majority of the country. To address this gap we conducted a seroprevalence study of dengue in a rural district that borders India in the northwest of the country. We randomly selected 40 villages and visited randomly chosen households within each community. All household residents were asked to provide a blood sample and information about socio-demographics. Indirect PanBio IgG ELISAs were used to identify dengue-specific antibodies as a marker of past dengue infection. In total, 1497 individuals participated in the study with a median age of 26 (range 0 - 90) years. 18% of the study population had serological evidence of past infection. There was significant spatial heterogeneity with virtually no past exposure detected in the north of the district whereas communities in the south near the district capital had over 60% seropositivity, although we found no differences in seropositivity by age (p-value: 0.96). In addition, we found no difference in seropositivity by age (p-value 0.17), suggesting that all individuals had experienced a similar cumulative risk of infection characteristic of recently emergent pathogens. We used a multilevel model to identify risk factors associated with historic dengue infection. Males were 1.4 (95% confidence interval [CI] 1.0-1.9) times more likely to have been infected than females. Having other infected individuals in the household increased the probability of being seropositive by 1.3 (95% CI 1.1-1.5) times. The presence of seropositive individuals in the community (but outside the household) further increased the risk of having been infected by 1.2 (95% CI 1.1-1.3) times. These findings suggest that dengue has only recently emerged in parts of this rural area and underscore the importance of considering rural communities when assessing the burden of dengue. Household and community-specific factors appear key to determining individual risk. Further work exploring differences in the ecological suitability for the vector in this region and the flow of people from dengue endemic communities will help us further understand the observed patterns of exposure.

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ATTITUDES TOWARD HEALTH, VECTOR CONTROL AND DENGUE FEVER IN SEVEN ENDEMIC COUNTRIES: INSIGHTS FROM ETHNOGRAPHIC RESEARCH IN BRAZIL, COLUMBIA, INDONESIA, MALAYSIA, MEXICO, PHILIPPINES AND SINGAPORE

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Perception of dengue and its prevention was assessed in 84 adults from various socio-economic groups and both urban and suburban dengue-endemic areas in 7 countries. Participants aged 18-55y (76 were women), 79 of whom had children <16y, were interviewed at home in 1.5-2h, semi-structured interviews. Prior to interviews, participants prepared scrapbooks to gauge emotional aspects. Interviews were filmed and vector control measures observed. Health was a top priority for all, contributing to family wellbeing and happiness. Work, financial stability and religion contributed to family wellbeing in Asia, and education in both regions. Health prevention was associated with nutrition, hygiene and exercise, and with childhood vaccination in Latin America. Prevention was considered part of hygiene efforts. The top health concerns included: 1/ cancer, heart attacks, and meningitis, 2/ diabetes and obesity, 3/ childhood diseases, 4/ dengue, and 5/ other infectious diseases (IDs). Dengue was spontaneously mentioned in Brazil, Indonesia and Philippines only, and was perceived as a bigger threat than other IDs in all countries. Latin Americans perceived vaccination positively and favored public vaccination, while Asians expressed concerns about vaccines and favored vaccination in private clinics. Knowledge of dengue was high, with gaps around the severity of secondary infection, existence of different serotypes, differences between DF and DHF, and the dangers of self-medication with some classes of NSAIDs. Dengue was associated with negative images, e.g. blood, humidity, pain, disability, dirt, filth, death. Level of concern and intensity of preventive measures were influenced by: personal experience of dengue, time of day, rainy season, national dengue incidence, public awareness campaigns, and concerns about children. Respondents felt safe at home and more exposed outside. They were committed to dengue prevention, yet preventive measures were inadequate and mosquito repellents were often absent. These results shed light on attitudes to dengue and prevention, and may help inform public communication campaigns.

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VIROLOGICAL TEST ALGORITHM FOR DENGUE CASES - OBSERVATIONS FROM A PHASE 2 LATIN AMERICAN DENGUE VACCINE TRIAL

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The CYD tetravalent dengue vaccine candidate is being evaluated for protective efficacy against symptomatic dengue in phase III efficacy trials. The laboratory test algorithm to confirm dengue cases was evaluated prior to Phase III trials. During a Phase II safety and immunogenicity trial in Latin America (Clinicaltrials.gov NCT00993447) a dengue epidemic occurred in the study countries. A total of 72 suspected dengue cases were reported and assessed: virological confirmation comprised qRT-PCR methods and a commercial ELISA kit for NS1 protein (Bio-Rad). The qRT-PCR included a screening assay targeting a dengue-conserved region of the 3'-UTR (Dengue screen assay) followed by 4 individual serotype assays targeting the conserved dengue NS5 genomic region (WT dengue qRT-PCR assays). The NS1 and WT dengue qRT-PCR were the protocol endpoint assays for virological confirmation (PVC). Of the 72 suspected cases, 14 were PVC: 9 by WT dengue qRT-PCR (5 Den-1, 4 Den-3) and 5 positive by NS1 Ag ELISA only. For the 9 PCR positive cases, 8 were also positive by NS1. However, a unique pattern of dengue qRT-PCR results were observed in 5 suspected cases. In these 5 cases, all from Honduras, the dengue screen qRT-PCR assay was positive but both the WT dengue qRT-PCR and NS1 Ag ELISA were negative. To investigate, exploratory data were generated using additional molecular methods: a SYBR Green-based RT-PCR assay, sequencing assays directed at the dengue genomic regions covered by the WT dengue qRT-PCR, and a commercial dengue RT-PCR test (Simplexa™ Dengue, Focus Diagnostics). The exploratory data confirmed these additional cases as dengue. Results indicated the serotype 2 WT dengue qRT-PCR assay was unable to detect a circulating Latin American strain (DENV-2/NI/BID-V608/2006) due to a mutation in the probe-binding region of the isolate. The Simplexa™ Dengue RT-PCR test was able to detect dengue in all samples tested except one. On the basis of these results and additional evaluations, the PVC algorithm was modified for the Phase III efficacy trials and future studies.

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EVALUATING THE UTILITY OF REACTIVE VECTOR CONTROL FOR DENGUE OUTBREAKS

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Dengue has the highest burden of any viral vector borne disease. A major contributor to this high burden is the unpredictable timing and magnitude of dengue outbreaks that often overwhelm healthcare facilities. While some early warning systems have shown local successes, the standard response to such outbreaks remains reactive. With no effective human prevention or cure, control efforts have to focus on the mosquito population with activities such as fogging of adults or larviciding of juveniles. While reactive measures are important, it has not yet been tested whether the timeliness and effectiveness of currently available vector control interventions are appropriate for controlling dengue outbreaks. Here we use a compartmental SIR model of mosquito and human population dynamics to estimate the effects of interventions on eventual outbreak size given the time delays between dengue virus transmission and intervention implementation. In this analysis we evaluate the effects of several commonly used reactive interventions and calculate the threshold timeliness and effectiveness that would be required for vector control interventions to be more appropriately used as response measures than simply applied at random. This is the first time the time delays at various stages of transmission and subsequent intervention have been incorporated into a dengue transmission model and the resultant outcomes will be important for evaluating and supporting effective public health policies regarding dengue outbreaks.

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SEROTYPE-SPECIFIC DENGUE NEUTRALIZING ANTIBODY RESPONSES IN FCΓR-EXPRESSING CV1 CELLS

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A Phase 2b efficacy trial (CYD23; Clinicaltrials.gov NCT00842530) of the live attenuated CYD tetravalent dengue vaccine (TDV) showed clinical protection against dengue. Point estimates of vaccine efficacy differed between dengue virus (DENV) serotype and no measurable efficacy was observed against DENV2. This observation contrasted with results of a Vero cell based PRNT50 assay that showed similar levels of vaccine-induced neutralization antibodies for all four serotypes. We investigated the potential of a seroneutralization assay in FcγRIIa-expressing CV1 cells to assess both neutralization and potential enhancement of dengue infection. Parallel assays were performed in Vero and CV1-FcγRIIa cells to evaluate the neutralizing antibody response against all 4 dengue serotypes on serum samples from a naturally dengue-infected cohort and serum samples collected after three injections of CYD-TDV in a clinical trial (NCT01134263) of "dengue-naïve" subjects. Neutralizing antibody titer GMT values were 2- to 9-fold lower in CV1-FcγRIIa cells than in Vero cells with lowest relative (Vero titer/CV1 titer) decrease amongst the 4 serotypes seen for the anti-DENV2 response. While the presence of an activating FcγR led to a global decrease in neutralizing capacity, the fact that the lowest relative decrease was against DENV2 suggests that anti-CYD2 responses lead to lesser enhancement of infection relative to the other 3 serotypes. Additional CV1 studies are underway with sera from CYD-TDV vaccinees in dengue-endemic areas (NCT01187433) to determine what role, if any, differential levels of dengue pre-immunity have on the generation of neutralizing antibodies against each serotype. Results of these investigations suggest that despite the absence of clinical efficacy observed against DENV2 in the CYD23 trial, *in vitro* neutralizing antibody responses against DENV2 elicited by CYD2 in TDV vaccination were not more enhancing than responses against other serotypes.

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MULTICENTER CLINICAL EVALUATION OF TWO ELISA AND TWO RAPID FORMAT ASSAYS FOR DIAGNOSING DENGUE

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Extensive prospective evaluations using multiple trial sites, a defensible gold-standard reference testing methodology, and quality systems that provide confidence in the study results, are required for reliable performance assessment of diagnostic tests. In this study, we evaluated the SD Bioline Dengue Duo (NS1/IgM/IgG) and the Panbio Dengue Duo (IgM/IgG) rapid diagnostic tests as well as the Panbio IgM and IgG Dengue ELISAs in a prospective, controlled, multicenter study. Paired samples were prospectively collected from 1021 individuals initially presenting on Days 0-7 at study sites in Peru, Venezuela, Cambodia, and the United States. An additional panel of 135 paired retrospective samples from Thailand was also used. A mix of primary and secondary infections and all four dengue serotypes were captured. Reference testing was performed using an algorithm involving virus isolation, IgM capture ELISA, and plaque reduction neutralization tests, in order to fully characterize the dengue status of each subject. Our primary end-points was positive and negative percent agreements of these devices against the reference methodology, but these numbers were also stratified using several factors known to influence overall accuracy including geography, days post onset of symptoms, infecting serotype, primary or secondary infections, and other demographic features. We determined that the SD Bioline Duo Cassette (NS1/IgM/IgG) had an overall sensitivity of 87% and specificity of 87% over the first 14 days post onset of symptoms. The Panbio Duo Cassette had a sensitivity of 92% and specificity of 59% during days 4-14 post onset of symptoms. This study generated reliable performance characteristics for several dengue diagnostic assays using prospectively collected specimens from both Asia and the Americas. Such results facilitate data-driven healthcare product choices for managing patient care during dengue

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HOST RESPONSES AFTER PRIMARY DENV-2 CHALLENGE IN CYNOMOLGUS MACAQUES: IMPACT OF STRAIN, DOSE AND ROUTE OF ADMINISTRATION

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Some non-human primates including Rhesus (*Macaca mulatta*) and Cynomolgus monkeys (*M. fascicularis*) are sensitive to infection by Dengue viruses and reproducively develop viremia when inoculated by SC route with 4-5 $\log_{10}$ PFU. Viremia is modest, usually several orders of magnitude below human viral load in dengue patient, and animals do not develop dengue clinical signs in this model. However classical dengue hemorrage was observed in rhesus monkeys showing high viremia after intravenous challenge with 7 $\log_{10}$ PFU (Onlamoon 2010). We report here the results of 2 challenge studies carried out in Cynomolgus macaques with aim to explore parameters associated to high viral loads. In the 1st study, 4 groups of 5 monkeys were challenged by SC route with 5.0 log10 CCID50 of 4 DENV-2 strains presenting different passages history: 2 laboratory strains (DENV-2 16681 and DENV-2 16803) and 2 low-passage isolates. Serum viremia was followed by qRT-PCR, daily from D1 to day 14, then at D28. Viral RNA was detected in all animals (except 1 in the DENV-2 16803 group) on D2 after injection for laboratory strains, and at various time points between D1 and D6 for viral isolates. Peak titers, viremia duration, and AUC were significantly lower in the DENV-2 16803 group than in other groups. The more homogeneous curves were observed in animals challenged with DENV-2 16681. In the second study, 6 groups of 5 monkeys received 5.0 or 7.0 log10 CCID50 of this virus administrated

by SC, ID or IV route. A clear dose effect was observed whichever the route of administration, with a peak viremia increase of about 1.0 log10. Reduced viremia duration and shorter time to viremia were also associated to dose increase. The IV challenge generated the highest peak titers and the most homogenous viremia curves: peak titer 7.0 ±0.2 on day 2, duration 5.0 ±0.0 days. DENV-2 neutralizing antibody titers and some blood biochemical parameters were also analyzed and will be presented. The implications of these data on the development of new DENV challenge model to measure protective efficacy of vaccine candidates will be discussed

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COST OF DENGUE VECTOR CONTROL: SYSTEMATIC LITERATURE REVIEW

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As the most important vector-borne viral disease, dengue is a serious and growing global public health problem, with 3.6 billion people at risk of infection. Currently, vector control is the only prevention tool to control dengue transmission. Little is known about the cost and the effectiveness of current dengue vector control measures, which rely heavily on outdoor fumigation and use of larvacides. We performed a systematic literature review, searching for studies on the cost of dengue vector control and found 14 articles and reports. Of these, 4 examined only one specific vector control intervention, such as community mobilization or source reduction, while 10 analyzed comprehensive vector control activities. We then separated these 10 studies according to dengue severity in the study year. Of these, 3 analyzed an epidemic year and 7 a non-epidemic year, consistent with dengue epidemiology. As 2 sites were duplicated, the studies cover 8 locations: Brazil, Cuba Malaysia, Mexico, Panama, Puerto Rico, Thailand and Venezuela, representing the regions in which dengue is most heavily endemic: Latin America and the Caribbean, and South East Asia. Finally, the costs of these comprehensive programs were compared to the cost of dengue illness in the same country for the same year. Preliminary results show that, on average, comprehensive vector control cost $1.69 per capita and was 52% of the economic cost of dengue illness in these same countries ($3.28). However, this relationship varied widely among countries. For example, Cuba's per capita cost of dengue vector control activities ($3.03) was 23 times its cost of dengue illness ($0.13). On the other hand, Venezuela's cost per capita of vector control ($0.57) represented only 8% of the country's cost of dengue illness ($7.38). If half the population at risk of dengue infection received comprehensive vector control at the current average cost, the global cost would be $6.1 billion annually. Innovative vector control strategies under development include genetics-based sterile insect methods, infection of mosquitoes with Wolbachia, interior residual spraying, auto-dissemination approach (spreading of insecticides by adult mosquitoes), attractive lethal ovitraps, sticky traps, and new pesticides. If any prove more effective than current measures, billions of dollars on current vector control and dengue illness could be saved or redirected.

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EXPERIMENTAL EVOLUTION OF WEST NILE VIRUS IN WILD-CAUGHT BIRDS

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Wild birds are the most important vertebrates in the West Nile virus (WNV) transmission cycle. Several studies have demonstrated that they generally impose purifying selection on the virus, and have suggested that they may select for novel WNV variants. However, the extent to which different important avian species influence WNV at the population level is poorly understood. Therefore, we evaluated whether different wild birds have distinct impacts on WNV populations. Specifically, we serially passed clone-derived WNV five times in wild birds that and experience varying levels of mortality: American crows (*Corvus brachyrhynchos*), house sparrows (*Passer domesticus*), and American robins (*Turdus migratorius*). After passage, we measured virus replication, pathogenesis and fitness in wild birds, chickens and mosquitoes. We also determined levels of intra-host genetic diversity using next-generation sequencing. Crows infected with crow-passed WNV developed higher viremias and experienced earlier mortality compared to birds infected with the unpassed virus. Sparrows developed an earlier peak viremia with the passed compared to unpassed virus, however the mortality and viremia differences compared to the unpassaged virus were insignificant. Passage in birds resulted in the generation of viruses with increased fitness gains in the same species and chicks compared to the unpassaged virus. Additionally, the bird passaged viruses did not lead to a trade-off of decreased competitive fitness in mosquitoes, which was previously observed in our laboratory. Studies in robins are ongoing. We obtained 20,000 to 60,000x WNV sequence coverage and found that intra-host genetic diversity increases in the early crow passages followed by positive selection of potentially adaptive variants. We did not find consensus changes to the crow passaged virus, suggesting that fitness gains may be achieved through rare mutations. Collectively, these results lend insight into the role of wild birds in selecting novel WNV genotypes.

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FLAVIVIRUS INFECTION INDUCES TRIGGERING RECEPTOR EXPRESSED ON MYELOID CELLS 1 (TREM-1): IMPLICATION FOR A ROLE IN INNATE IMMUNE RESPONSES

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Innate immune responses are essential for the control of flaviviruses, including WNV, which has emerged as a significant cause of viral encephalitis in humans. However, the specific mechanisms that regulate the programming of the innate immune signaling pathways remain unclear. Activation of TREM-1 signaling via the adaptor protein DAP12 is important for inflammation and activation of antigen presenting cells, however little is known about its role in viral infections. Here, we investigated the effect of flavivirus infection on the expression of TREMs and its potential role in the production of cytokines. We show that the expression of TREM-1 was markedly increased in dengue virus-infected THP-1 cells, which correlated with peak viral titers. Similarly, WNV infection significantly increased the mRNA levels of TREM-1 in MEFs, BMDMs and BMDCs. *In vivo* characterization of TREMs in mice demonstrated a significant up regulation in the transcripts of TREM-1, -3 and -4 in the peritoneal cavity cells and brain at day 3 and 8 after WNV infection respectively. Interestingly, serum levels of soluble-TREM-1 also increased significantly at days 2-3 after infection. Further, activation of TREM-1 using an agonist antibody increased mRNA of WNV-induced cytokines such as IFN-α, TNF-α and IL-6 in MEFs, which decreased following blocking of TREM-1. The changes to IFN-α and IL-6 secretion were validated with ELISA and Luminex, respectively. Collectively, our results for the first time document the response of TREMs to flavivirus infection and indicate a novel role of TREM-1 in modulating inflammatory response to WNV. Further studies are ongoing to define role of TREM-1 in WNV disease outcome.

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COMPARISON OF THE EFFICIENCY AND COSTS OF WEST NILE VIRUS SURVEILLANCE METHODS IN CALIFORNIA

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Surveillance systems for West Nile virus (WNV) aim to determine the location and timing of viral amplification early enough to direct mosquito control and prevent transmission to the human population. To ensure an optimal surveillance approach, the sensitivity and timing of each component of the surveillance system must be measured against its efficiency and costs. We evaluated each of the most widely used surveillance methods for WNV (testing of mosquitoes and public-reported dead birds, and seromonitoring of sentinel chickens) using data from three vector control agencies in California for the years 2004 through 2012, encompassing the period following WNV's arrival to California. The methods were compared after standardizing spatial sampling density, frequency of sampling, and costs. At equal spatio-temporal sampling, testing mosquitoes and dead birds typically detected viral activity 2-5 weeks earlier than seromonitoring of sentinel chickens. Viral activity was detected most frequently in mosquitoes during the early season (May-June) and in sentinel chickens during peak season (July-August). Testing dead birds reported by the public was found to be the most cost-effective of the available methods in areas where corvids and other avian hosts with high disease-dependent mortality were abundant. For a given budget, testing of dead birds or mosquitoes provided the greatest early warning and return on costs during spring and early summer (1.3 and 3.5 WNV-positive samples per $1,000 spent), and serological monitoring of sentinel chickens was of most utility during summer and early fall (2.8 WNV-positive samples per $1,000) as viral activity began to wane.

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EVIDENCE OF NEUTRALIZING ANTIBODIES TO WEST NILE AND SAINT LOUIS ENCEPHALITIS VIRUSES IN PERUVIAN HORSES

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Arboviruses are responsible for thousands of human and animal infections worldwide. Saint Louis encephalitis virus (SLEV), West Nile virus (WNV) and Venezuelan Equine Encephalitis virus (VEEV) are zoonotic arboviruses that infect horses and are widely spread in South America. SLEV was first reported in Brazil in 1960, and then reported in human sera in Peru in 1965. Venezuela, Colombia, Argentina, and other Caribbean-bordering countries have reported bird and equine infections. Epizootics and epidemics of VEEV has been reported in many countries throughout Central and South America, including Colombia, Venezuela, Trinidad, Ecuador, Mexico, and Peru. VEEV was isolated in 1942 for first time in Peru. In order to assess the presence of neutralizing antibodies to SLEV, WNV, and VEEEV we tested 3470 horse sera samples from 25 locations in Peru. The Peruvian Animal Health Office (SENASA) collected serum samples in 2011. We screened these samples with IgG indirect ELISA at the Naval Medical Research Unit No. 6 (NAMRU-6) in Lima, Peru. Positives were confirmed by solid Plaque Reduction Neutralization Test with 80% reduction ($PRNT_{80}$). Virus neutralization titers ranged from 1/20 to 1/640. We found 19 (0.5%) positive samples for WNV, mostly from the Cajamarca Region. Fourteen (0.4%) samples were positive for VEEV and 24 (0.7%) for SLEV, mostly from Piura. The samples were equally distributed within ecologically different areas in the coast, highlands, and rainforest. The presence of the neutralizing antibodies in horses against SLEV and WNV suggest prior infections and possible continuing spread of these arboviruses universally throughout Peru. Therefore, continued epidemiologic surveillance in horses is necessary in order to protect human populations against future outbreaks and subsequently confirm the continuing circulation of these viruses.

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CONTEXT-DEPENDENT CLEAVAGE AT THE C-PRM JUNCTION BY THE WEST NILE VIRUS NS2B/3 PROTEASE MODULATES THE EFFICIENCY OF VIRUS ASSEMBLY AND RELEASE

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Flavivirus assembly is governed in part by cleavage of the single viral open reading frame orchestrated by host and viral proteases. The sites recognized by flavivirus proteases have been defined principally through the use of small peptide substrates. Prior studies have shown that it is possible to produce infectious virions composed of the structural genes of one flavivirus and a sub-genomic RNA of a distantly related virus. While the production of "pseudo-typed" virions composed of the structural genes of DENV2 (strain 16681) and a WNV replicon is quite efficient (>106 infectious units/mL), the production of virus particles incorporating the C-prM-E proteins of the DENV2 NGC strain was not possible, despite sharing 97.8% amino acid sequence identity. To understand the underlying mechanism, we constructed chimeras of the structural genes of these two DENV2 strains and identified the capsid (C) protein as the source of incompatibility. Subsequent mutagenesis studies revealed that a single C substitution, T101S, restored the ability to produce infectious virions composed of NGC C-prM-E and the WNV replicon. Residue 101 is the P1' position of the NS2B/3 viral protease cleavage site, and indeed, we found virion production capability mapped to the efficiency of C-prM cleavage. The significant impact of the T101S substitution on the efficiency of cleavage by the WNV protease is surprising in light of published biochemical studies of the requirements for cleavage. We conducted more extensive mutagenesis of the C-prM junction to further define the sequence requirements for cleavage by the WNV and DENV proteases; these studies revealed a context-dependent substrate specificity of the viral protease. Definition of the substrate specificity of the viral protease against the backdrop of the viral polyprotein may facilitate the development of new protease inhibitors and provide insight into associated patterns of drug resistance.

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ECOLOGY OF CULEX FLAVIVIRUS DURING WEST NILE VIRUS EPIDEMIC AND INTER-EPIDEMIC YEARS IN SUBURBAN CHICAGO, USA

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Insect-only flaviviruses have been identified in mosquito populations throughout the world. These insect host-adapted viruses co-circulate with medically important arboviruses, but their ecology and effects on their mosquito hosts in nature are poorly understood. Culex flavivirus (CxFV) is an insect-only flavivirus found in *Culex* species mosquitoes, important

vectors of West Nile virus (WNV) in the United States. CxFV and WNV co-circulate in and co-infect *Culex* mosquitoes at a WNV "hotspot" in suburban Chicago. We previously identified a positive association between CxFV and WNV in mosquito pools collected in 2006. To further investigate the ecology of CxFV and its association with WNV, we compared the spatial and temporal distribution of CxFV in 2011, an inter-epidemic year for WNV, with its distribution in 2012, an epidemic year for WNV. The overall prevalence of WNV in mosquitoes in 2011 was 0.11% (95%CI: 0.04-0.25), whereas in 2012 it was 0.62% (95%CI: 0.42-0.90). The overall prevalence of CxFV in 2011 was 10.21% (95%CI: 9.28-11.26), whereas in 2012 it was 17.02% (95%CI: 15.37-18.86). Both viruses were significantly more prevalent in mosquito pools in 2012 than in 2011 (Wilcoxon signed rank test, WNV: V=41, p<0.02; CxFV: V=164, p<0.001). CxFV was identified at all 37 trap locations that were repeated in both years. During 2012, the trap location with the highest WNV prevalence (2.83%) was also the trap location with the highest CxFV prevalence (79.96%). Among locations positive for both viruses, a positive correlation between CxFV and WNV was observed in 2011 (t= 4.31, df= 3, p < 0.05), but not in 2012 (t = 1.61, df= 14, p > 0.1). These results demonstrate an association between WNV and CxFV on a fine spatial scale in an urban setting that may be driven by similar responses of the two viruses to common environmental drivers.

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SPATIAL ANALYSIS AND EVALUATION OF 2014 PREDICTIONS FOR A WEST NILE VIRUS EARLY WARNING SYSTEM

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Since the introduction of West Nile virus (WNV) to the USA in 1999, numbers of human cases reported to the CDC have varied from fewer than a thousand cases per year to 5,674 cases in 2012. A national model conditioned on weather and other data, including bird species distributions and human population density, has explained the large variations seen over the 2005-2013 period and a prediction was made for 2014. We found the most significant predictors for a human WNV case in a county are the mean minimum temperature in January, the deviation of this minimum temperature from the expected minimum temperature, the total population of the county, the bird population, and if the county had a case of WNV the previous year. Due to aberrant weather patterns in early 2014, the pattern of human WNV was predicted to be equally aberrant, presenting a genuine test of the model and implications for public health in the face of climate change. Predictions are compared to up-to-date case reports and locally conditioned models are examined for regions where more data are available, providing a start toward regional early warning systems.

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AVIAN SPECIES DIVERSITY AND AMPLIFICATION OF WEST NILE VIRUS IN ATLANTA, GEORGIA

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The dilution effect is the reduction in vector-borne disease risk that occurs through the presence of a diverse set of potential host species, some of which are relatively or completely incompetent as hosts. West Nile virus (WNV) is a mosquito-borne disease that is maintained in various avian host species. In Atlanta, Georgia, substantial WNV presence in the vector and host species has not translated into a large number of human cases. In order to determine whether a dilution effect was contributing to the reduced WNV spillover in the area, we conducted comprehensive multi-season, multi-habitat, characterization of the avian species community as well as longitudinal WNV surveillance in avian hosts and mosquito vectors in urban Atlanta between 2010 and 2011. We measured host diversity in two ways: diversity at-large and diversity as experienced by the pathogen. Regardless of how we measured avian species diversity or whether we considered host infection and vector infection as predictor variables or outcome variables, we did not detect a dilution effect. Instead, we detected an amplification effect, in which increased host diversity resulted in increased rates of infection, the first empirical evidence for this effect in a mosquito-borne system. We suggest that the observed amplification effect may primarily be driven by an over-abundance of moderately to poorly competent host species, such as Northern Cardinals, which may cause optimal hosts to be more rare and therefore to be present primarily in more species-rich areas. Other possible mechanisms driving amplification could be increased vector species richness and innate mosquito preference for certain host species over others. We encourage further research to assess the scale and prevalence of amplification effects in the WNV system, as well as the contributions of various host and vector species to its establishment.

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GENETIC DETERMINANTS OF AVIAN PATHOGENESIS OF LINEAGE 2 WEST NILE VIRUS STRAINS

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In 2010, a human outbreak of a West Nile virus (WNV) lineage 2 (L2) in Greece established, for the first time, an association between L2 WNVs and extensive human disease in Europe. Sequence analysis of a L2 WNV virus (NS10) isolated from a pool of infected mosquitoes in Nea Santa, Greece during the 2010 WNV outbreak revealed an NS3-H249P mutation not previously observed in any sequenced L2 WNV strains. Interestingly, an NS3-T249P mutation has previously been demonstrated to increase viremia and virulence of a lineage 1 WNV strain in American crows (AMCRs). In order to assess the virulence potential of NS3-249P substitution in L2 WNVs, AMCRs, thought to be a key reservoir host for WNV in North America, were inoculated with the parental L2 WNV viruses, NS10 and South Africa 1989 (SA89), in addition to mutants generated containing polymorphisms at the NS3-249 site (Pro, His and Thr). The parental NS10 and SA89 strains displayed 100% and 33% mortality with average peak viremias of 9.5 and 7.5 log10 PFU/mL in AMCRs, respectively. The NS10 mutants, NS10 NS3-249H and NS10 NS3-249T, exhibited 80% mortality with peak viremias of 8.7 log10 PFU/ml sera and 20% mortality with peak viremia of 6.0 log10 PFU/mL sera, respectively. The SA89 mutant, SA89 NS3-249H, elicited 100% mortality with a peak viremia of 9.6 log10 PFU/mL and the SA89 NS3-249T resulted in 0% mortality with a peak viremia of 2.6 log10 PFU/mL sera. Viremia and mortality differences in AMCRs between the L2 WNV backbones harboring the same polymorphisms at the NS3-249 site suggest that epistatic interactions of alternative genetic elements are involved in generating the variable phenotypes. The sequence of the SA89 strain was compared with that of NS10 and a total of 16 amino acid differences were identified exclusive to the nonstructural genes. In order to understand the factors related to the emergence of a human disease associated L2 WNV in Europe, and the potential role of alternative genetic factors in epistatic maintenance of the virulence associated with the NS3-249 site, chimeras between NS10 and SA89 were generated and tested in AMCRs and House Sparrows.

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METEOROLOGICAL CONDITIONS ASSOCIATED WITH INCREASED INCIDENCE OF WEST NILE VIRUS DISEASE IN THE UNITED STATES, 2004-2012

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West Nile virus (WNV) is the leading cause of mosquito-borne disease in the United States. Annual seasonal outbreaks vary in size and location. Predicting where and when outbreaks will occur can help direct public health control efforts. Weather can impact several factors associated with WNV transmission, such as mosquito vector and zoonotic host abundance. We developed models for the continental United States to identify meteorological conditions associated with higher incidence of WNV neuroinvasive disease (WNND) from 2004-2012. We used county-level WNV surveillance data reported to CDC and meteorological data from the North American Land Data Assimilation System. Due to geographic differences in WNV transmission, we divided the United States into east and west (defined by ~100 degrees West longitude) and 10 US Environmental Protection Agency regions. Meteorological conditions were evaluated from October of the prior year through September of the given WNV season. For each US county, we calculated standardized z-scores that described annual WNND incidence, average temperature, and total precipitation compared to the mean values for 2004–2012. For WNND incidence, a z-score ≥0.5 was defined as above average. We used fixed effects models to assess independent associations between anomalies in temperature or precipitation and above average WNND incidence within each geographic area. Preliminary results showed warmer than average annual temperature was associated with above average WNND incidence nationally and in all geographic areas. Lower than average total precipitation was associated with higher disease incidence nationally but the effect varied significantly by region. These findings suggest anomalies in temperature and precipitation are associated with above average WNV disease incidence but the overall effects vary by region. Although multiple factors influence WNV transmission, readily accessible meteorological data may be used to develop predictive models to forecast geographic areas with elevated WNV disease risk prior to the coming season.

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PREVALENCE AND RISK FACTORS OF HUMAN CORONAVIRUSES

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Acute respiratory tract infections (ARI) are the leading cause of morbidity and mortality in developing countries, especially in Africa. However, information on the viral aetiological agents are scanty. We therefore conducted a cross-sectional serological study to determine the prevalence of Human Coronaviruses among individuals living in rural areas of Ghana from September 2010 to October 2013. The study areas are Kwamang in the Ashanti Region, Buoyem in the Brong Ahafo Region and Forikrom also in the Brong Ahafo Region. A total of 201 subjects were enrolled in the study. Subjects were tested for IgG antibodies to three HCoVs namely; HCoV-NL63, HCoV-OC43 and HCoV-229E. Of the 201 subjects, 97 (48.3%) were positive for all viruses. The most prevalent virus was HCoV-229E (23%; 95% CI: 17.2 - 29.3), followed by HCoV-OC43 (17%; 95% CI: 12.4 - 23.4), then HCoV-NL63 (8%, 95% CI: 4.6 - 12.6). Of all positive HCoV-NL63 subjects, those in Kwamang had the highest sero-prevalence (68.8%). In contrast, HCoV-229E (41.3%) and HCoV-OC43 (45.7%) were much higher in Forikrom compared to the other study areas. There was however no statistical difference between living in any of the study areas and being positive for HCoVs. The gender distribution for all three viruses was also similar. The median ages of those positive for HCoV-OC43 (47 years, IQR = 33 - 52.5) and HCoV-229E (40 year, IQR = 27 - 54) were higher than negative subjects. The age difference for HCoV-NL63 subjects were similar (p = 0.994). A comparison of the blood group types between subjects positive for HCoVs and those negative showed no significant statistical difference (p = 0.163). This study demonstrates the occurrence of three types of HCoVs in remote areas of Ghanaian rural populations.

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THE LABORATORY AS A TOOL IN THE ENDGAME POLIOVIRUS ERADICATION PROGRAM IN GHANA

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Polioviruses and other enteroviruses cause acute flaccid paralysis, and this is of public health concern. The World Health Organization (WHO) sees the laboratory as being an important tool in the virological investigation of acute flaccid paralysis cases, therefore, the laboratory having a crucial role to play in ensuring that no viruses, especially, wild polioviruses are missed. Until 1999, the Laboratory isolated indigenous wild poliovirus outbreak infections, which led to quick interventions with oral polio vaccines.The country enjoyed close to five years of wild type poliovirus elimination until the laboratory in 2003 and then in 2008 isolated a wild type 1 imported poliovirus in the country. Since the 2008 outbreak, Ghana has been free from wild type poliovirus infections.This paper sought to look at the crucial role the laboratory continues to play in ensuring that no wild poliovirus is missed; even after Ghana, being free of wild poliovirus since 2009. A detailed analyses of data on all AFP stool specimens investigated for a period of 10 years (2004 to 2013), using Epi Info data software; as we look at the performance and progress of the laboratory in polio eradication in Ghana. A total of 4,555 AFP specimens from all ten regions of Ghana were analyzed. Ninety percent(90%) of the stool specimens were from children below the age of 15 years. Males constitute just above half (57.2%) of the total specimens. Over 80% of the specimens were received in good condition.The annual non polio enterovirus isolation rate was above 10%, which was within WHO recommended non polio enterovirus isolation rate of 10% for the laboratory. Two hundred and twenty three(223 -4.9%) of the specimens were positive for poliovirus; and 8(3.6%) of the 223 were wild-type 1 imported polioviruses.Timeliness of reporting within 14days from date of specimen receipt,annually rated not less than 98%.The laboratory over the period have consistently passed annual proficiency test for virus isolation and also for intratypic differentiation (ITD) of polioviruses.The Laboratory's ability and skills in the delivery of accurate results in a timely manner made it possible for the timely intervention when Ghana recorded the two poliovirus outbreak in 2003 and 2008. Indeed the laboratory's continuous involvement in Polio eradication through virological investigations and timely dissemination of results has brought Ghana into the reality zone of wild poliovirus eradication.

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DEEP SEQUENCING AS A TOOL TO IDENTIFY PATHOGENS FROM POOLED RESPIRATORY SAMPLES FROM SOUTH/ SOUTHEAST ASIA

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Emerging and re-emerging respiratory pathogens represent an increasing threat to public health. Asia continues to be the regional epicenter for the emergence of novel pathogens and the site where several pandemics have originated. Etiological determinations during outbreaks have generally relied on clinical information, occasionally accompanied by traditional molecular or serological techniques. Often, the information is inconclusive.

In 2013, the Armed Forces Research Institute of Medical Sciences (AFRIMS) identified one hundred and sixteen nasal-pharyngeal specimens collected from acute influenza-like illness (ILI) patients in several countries in South/South East Asia which were negative by conventional molecular and culture techniques but demonstrated cytopathic effect (CPE) in cell culture. Groups of 8 to 15 CPE-positive samples were organized by the geographic region from where they were initially collected. Deep sequencing was performed on each pool to generate sufficient sequence reads to allow for initial pathogen identification. A total 7.9 Gbases or 22.28 million sequence reads passed quality control with ≥30Q scores. After filtering out host and microbiome background, low abundance sequence reads were analyzed. We were able to identify various respiratory pathogens, which tended to localize in specific regions: (i) parainfluenza 3 in the Bhutan/Nepal pool, (ii) human metapneumovirus and human coxsackievirus A21 strain in the Cambodian pool, (iii) influenza A in the Philippines pool, (iv) human coxsackievirus A21 strain in the Bangkok, Thailand pool, and (v) parainfluenza 4a in the northern Thailand pool. The pools and individual samples with high viral content were confirmed by singleplex PCR, real-time PCR or conventional PCR. Overall, deep sequencing performed efficiently as an initial identification tool for viral pathogens using pooled respiratory samples of unknown etiology but capable of inducing CPE in cell culture.

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ABOLISHMENT OF INDIVIDUAL N-GLYCOSYLATION SITES WITHIN RIFT VALLEY FEVER VIRUS GN/GC ALTERS INFECTIVITY VIA DC-SIGN

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Rift Valley fever is a mosquito-transmitted zoonosis that is characterized by high rates of abortion and fetal malformations in ruminants and causes severe disease in humans. Recently, DC-SIGN (a C-type lectin receptor) has been demonstrated to be a receptor for phleboviruses, including Rift Valley fever virus (RVFV: genus *Phlebovirus*; family *Bunyaviridae*). We hypothesized that N-glycosylation of Gn/Gc plays an important role in the infection of RVFV via DC-SIGN. RVFV glycoproteins (Gn/Gc) encode 5 putative N-glycosylation sites: aa. 438 (Gn), 794 (Gc), 829 (Gc), 1035 (Gc), and 1077 (Gc), but their significance in viral infection via DC-SIGN has not been elucidated. Using a reverse genetics system, we generated recombinant MP-12, which lack one of the five potential N-glycosylation sites of Gn/Gc: N438Q (Gn); N794Q (Gc); N829Q (Gc); N1035Q (Gc); and N1077Q (Gc). To identify which sites are utilized for N-glycosylation, we immunoprecipitated [^{35}S] methionine/cysteine-labeled MP-12 or the mutants in supernatant with anti-RVFV antibody and analyzed the size of the Gn/Gc proteins by autoradiography. The Gn of N438Q, and Gc of N794Q, N829Q, N1035Q, and N1077Q migrated faster than those of parental MP-12, indicating aa.438, 794, 829, 1035, and 1077 are N-glycosylated. To test the infectivity of each mutant, we measured viral RNA copy number using digital droplet PCR with a Taqman probe specific to the MP-12 L-segment. The ratio of PFU in VeroE6 cells per RNA copy was analyzed. Then, Jurkat cells and those that express DC-SIGN were infected with the mutant viruses at the same RNA copy number, and we analyzed the number of infected cells at 6 hpi by flow cytometry (FACS). We are currently repeating the experiments to conclude the statistical differences of infectivity among the N-glycan mutants. Our findings will be useful for understanding of the pathology of RVFV and the rational design of live-attenuated vaccine candidates.

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MOLECULAR ANALYSIS OF INFLUENZA B VIRUSES ISOLATED IN KENYA, 2012

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Introduction: Influenza B viruses belong to two evolutionary lineages (B/Victoria/2/87-like and B/Yamagata/16/88-like) that continue to co-circulate globally in the human population since 1980s. These viruses do not undergo antigenic shifts but drifts as a result of accumulation of amino acid substitutions especially in the HA1 polypeptide have been confirmed. The evolution of surface glycoproteins occurs over time due to selection pressure exerted by host's immunity. Objective: To investigate molecular evolution of influenza B viruses isolated in Kenya by sequence analysis of HA1 hemagglutinin. Methods: Nasopharyngeal specimens obtained from patients meeting WHO definition criterion for ILI were screened by real-time PCR for influenza A and B viruses. Influenza B virus positive samples were inoculated onto Mardin-Darby Canine Kidney cells and HA protein coding gene of selected isolates sequenced and analyzed. Results: Phylogenetic analysis showed all influenza B viruses isolated in Kenya clustered together with B/Brisbane/60/2008 vaccine strain and other viruses of B/Victoria/2/87-like lineage in other regions of the world. All the Kenyan isolates were characterized with D197N amino acid substitution not present in the vaccine strain. This change occurred within 190-Helix antigenic binding site. Majority of Kenyan isolates (except B/Kenya/242/2012, which had I146A) further had I146V amino acid change within the 150-Loop antigenic site, absent in the vaccine strain. Other mutations occurred stochastically in individual isolates. Most notable was isolate, B/Kenya/239/2012 which had an additional V124I amino acid change within the 120-Loop antigenic binding site. Conclusion: There was limited variation among the Kenyan isolates. Kenyan viruses matched closely with the seasons vaccine strain, B/Brisbane/60/2008 despite having mutations at antigenically significant positions in the HA1 subunit. Molecular analysis of influenza B viruses is important for early detection of strains with epidemic potential.

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THE MOLECULAR EPIDEMIOLOGY OF NOROVIRUS IN MILITARY RECRUITS IN IQUITOS, PERU: EVIDENCE OF A NOVEL GENOTYPE

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Norovirus (NoV) is a leading cause of diarrhea in Peru, yet its molecular epidemiology in this region is largely unknown. We therefore explored the genetic diversity of NoV in Peruvian military recruits in Iquitos, a Peruvian city in the Amazon basin. Stools from a random subsample of recruits from a diarrhea surveillance study in Iquitos between 2004 and 2011 were screened for NoV and genogrouped by AgPath-ID One-RT-PCR. The NoV VP1 gene was partially sequenced (330/344 bp, region C) and genotyped using the Noronet webtool. Phylogenetic trees of Iquitos sequences compared with BLAST and Noronet reference taxa were inferred using the maximum likelihood (ML) method with bootstrapping by RAxML software. 360/4234 (8.5%) participants were tested for NoV, 11.1% (40/360) were positive, including 12 for genogroup (GI), 29 for genogroup II (GII) and one GI/GII dual infection. Genotypic and further analysis was able to be performed in 49 % (20/41) of sequences. ML trees demonstrated a wide range of Peruvian GI genotypes with the majority belonging to a GI.4 clade. Spatial clustering of GI Peruvian and Brazilian taxa was noted,

although weakly supported. Genotype diversity of GII NoV in Iquitos was broad with many in GII.4 2006bDenHaag clades. One GII Iquitos sequence was untypable and appears to represent a novel genotype, with a GII.3 Tunisian sequence as the closest typable relative (86% nucleotide similarity) and strong support for clustering with near identical and also untypable Nicaraguan strains. Within most genotype clades there was temporal clustering of Peruvian and reference sequences, consistent with Peru being affected by globally circulating lineages. In conclusion, Noroviruses in the Peruvian Amazon are genetically diverse with evidence of a novel genotype, mixing with global lineages and weak regional spatial structure. Larger studies are needed to clarify the regional phylogeography of noroviruses and confirm and characterise this and perhaps other novel NoV genotypes in Peru

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TIME TRENDS AND CORRELATES OF ROUTINE MEASLES IMMUNIZATION COVERAGE IN ABIA STATE, SOUTHEAST NIGERIA; 2007-2012

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Measles is a vaccine-preventable-viral disease associated with high morbidity and mortality. The national target in Nigeria for routine measles immunization coverage is ≥80%. We conducted this study to assess the variations in annual trends of routine measles immunization coverage rates in Abia State and to identify the factors affecting coverage. A time series analysis was performed on administrative measles immunization data collected by Abia State Primary Health Care Development Agency from 2007-2012. Trends in measles immunization coverage, measles vaccine wastage, measles vaccine supply rates, measles vaccine weekly stock and immunization outreach sessions (vaccination points outside health facilities) were assessed in all 17 Local Government Areas (LGAs) and disaggregated by LGA type (urban or rural LGA). The relationship between measles vaccine wastage, supply rates, weekly stock, immunization outreach sessions, LGA type and an outcome of ≥80% measles immunization coverage was assessed and modeled using step-up logistic regression. The annual birth cohort (study population) was 121,107 in 2007, declined to 110,636 in 2009 and rose to 123,034 in 2012. Both measles immunization coverage and immunization outreach sessions increased at a linear rate ($p < 0.001$) while measles vaccine wastage rate declined linearly ($p < 0.001$). Measles vaccine weekly stock declined at a rate of 3 days stock per month till 2009, increasing afterwards ($p<0.001$). 28% of the LGAs attained ≥80% immunization coverage by 2012; achieved by 46% of urban LGAs. Immunization outreach sessions increased from 1762 in 2007 to 8595 in 2012; 48% were <25sessions/month. 58% of LGAs got ≥80% of their measles vaccine. 38% of LGAs had a vaccine wastage rate of <30%. Having <30% measles vaccine wastage (OR=2.2), ≥80% measles vaccine supply (OR=9.8), ≥25 immunization outreach sessions ($p<0.001$) and being an urban LGA (OR=2.9) was associated with the outcome. The effect of measles vaccine wastage was modified by LGA type ($p= 0.009$). These variables were significant positive predictors for ≥80% measles coverage, following modeling. Routine measles immunization coverage improved over the study period. Only a third of LGAs met the required national target; mostly urban LGAs. Public health resources should be directed at reducing vaccine wastage at service delivery, improving vaccine supply chains and increasing access to immunization, especially in rural LGAs.

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EPIDEMIOLOGICAL CHARACTERISTICS OF ACUTE FLACCID PARALYSIS CASES IN LAGOS STATE NIGERIA, 2001 - 2011

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Globally, Nigeria is one of three countries still endemic for indigenous transmission of polio virus. Acute Flaccid Paralysis (AFP) surveillance is one of four strategies recommended for polio eradication. Data from AFP surveillance guides implementation of immunization activities aimed at interrupting polio transmission. In November 2013, we conducted a study to characterize AFP cases in Lagos State, southwest Nigeria. We analyzed secondary data of AFP cases in Lagos State from 2001 to 2011. AFP was defined as recent onset of floppy weakness or paralysis in a child less than 15 years or any paralytic illness in any person in whom a clinician suspects polio. We reviewed the AFP data and performed univariate and bivariate analyses using Epi-info 3.5.4 software. Altogether, 2,896 AFP cases were reported; 1683 (58.1%) were males. Mean age was 2.9 years (+/-2.7). The most affected age group was 0-5 years (86.1%). Of all AFP reported, 25 (0.9%) did not receive any OPV (Oral Polio Vaccine); 301 (11%) received 1-2 doses. Over 80% of cases had received 3 or more OPV doses. Adequate stool specimen was collected for analysis in 2815 (97.4%) of the AFP cases. Only 30 (1%) were positive for wild polio virus (WPV). WPV type 1 (WPV1) were 23 of which 1 had no OPV and WPV3 were 7(all received OPV). Last confirmed human WPV was in year 2009. Polio virus transmission has been interrupted in Lagos state. The high immunization status would have contributed to population immunity and reduced transmission. Government should continue to strengthen and scale-up routine and supplemental immunizations with OPV.

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EPSTEIN-BARR VIRUS IN PATIENTS WITH ACUTE FEBRILE ILLNESS IN KENYA

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Epstein Barr Virus (EBV) causes infectious mononucleosis and other lymphoproliferative disorders, including endemic Burkitt's lymphoma (eBL). EBV epidemiology is best known from serological studies. This study determined the EBV viremia and prevalence in patients with acute febrile illness (AFI) in Kenya. Patients with AFI were enrolled at 8 out-patient hospitals between September 2008 and April 2013. DNA was extracted from whole blood and BALF5 gene used as target for real-time PCR. Prevalence rates and viremia were determined and correlated by age, region and co-infection with malaria. Of the 2021 patients examined, EBV was detected in 588 (29%) and their viremia ranged from 52 to 7.2 x106 copies/mL (geometric mean 4345 copies/mL). Patient mean age was 5 years (range 1-80 years). Viral prevalence was highest in patients 2000 copies/mL, a cutoff considered clinically important. The <5 year olds constituted the majority (41%) in this group. Regions holoendemic for malaria had the highest prevalence compared to the hypoendemic regions. In addition, patients with EBV/malaria co-infections had higher viremia (geometric mean 5929 copies/mL) compared to those with EBV alone (3793 copies/mL, $p = 0.003$).The study demonstrates how common EBV is among patients with AFI. That malaria is an important determinant of EBV viremia, reinforcing the possibility that increased viremia in EBV/malaria co-infections could be a precursor to the development of eBL.

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ADVERSE EVENTS FOLLOWING IMMUNIZATION WITH NEWLY INTRODUCED MEASLES-RUBELLA VACCINE IN JIRAPA DISTRICT, GHANA

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Background: Vaccines are one of the most cost effective public health interventions. Real or perceived adverse events following immunization (AEFI) can undermine the credibility of a vaccine and an immunization programme. Ghana rolled out a measles-rubella combined vaccine in a mass immunization campaign in September, 2013. We assessed the AEFI associated with the vaccine in Jirapa District to obtain base-line data and appropriately respond to public concerns on safety issues. Method: A risk interval cohort study was conducted. Three hundred and fifty children aged 9 months -14 years followed for twelve weeks. Seventy children in four age groups were selected from each of five communities in Jirapa District using modified EPI coverage guideline. Participants were observed for four weeks before vaccination then eight weeks after vaccination for adverse events in the pre and post vaccination control windows and risk window. An AEFI was defined as any medical incident, which occurred after vaccination with measles rubella vaccine. An AEFI was said to be serious if it was life-threatening and required intervention and/or hospitalization or resulted in disability/incapacity or death. Univariate and bivariate analysis were done using Epi info 3.5. P values less than 0.05 were considered statistically significant. Results: Three hundred and fifty (350) vaccinees, 51.6%(180/350) females and 48.4%(170/350) males were followed for twelve weeks. Overall incidence of adverse events following immunization was 5.1% (95% CI: 3.2-8.2%). Of these fever accounted for 66.7% (12/18), febrile convulsion 5.6% (1/18), headache 16.7% (3/18), skin rashes 5.6 (1/18), and pain at injection site 5.6 (1/18). Only two (11.1%) of the adverse events were serious. Three (16.7%) of the adverse events occurred within 24 hours after vaccination while 11 (61.1%) occurred between the first and seventh day after vaccination. Children aged 9months- 3 years were 6.6 times more likely to develop fever than children aged 10-14 years (RR=6.6, 95% CI: 0.83-52.62; P <0.04). Conclusion: The adverse events following immunization with Measles-Rubella Vaccine were few and generally mild. Continued surveillance for adverse events and investigation of serious ones are recommended.

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MOLECULAR SIGN OF INFLUENZA A AND B VIRUSES IN CUBA DURING TWO CONSECUTIVE YEARS

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The work contributes to a better understanding of influenza virus circulation in Cuba, sub-tropical country located at the Caribbean area. The objective of this work was to determine circulation and molecular characterization of influenza viruses in Cuba. From January/2012-December/2013, Cuban National Influenza Centre received 7783 clinical samples of individuals presenting influenza-like illness symptoms, severe acute respiratory infection or fatal cases. Samples were tested for seasonal A(H3N2), A(H1N1)pdm09 and B influenza viruses by real-time reverse transcription-polymerase chain reaction. Nucleotide sequences from hemagglutinin HA1 region segment were obtained directly from positive cases clinical samples. Genetic distances were calculated using MEGA v.5.05. Phylogenetic tree was constructed using Mr.Bayes v.3.1.2 software. Potential N-Glycosylation sites were predicted using NetNGlyc server 1.0. Of the 1335 samples positives to influenza virus, 48% were positive to influenza A(H1N1)pdm09, 31% to influenza B and 21% to influenza A(H3N2). Year 2012 was marked by low circulation of H3N2 subtype, with only 23 detections. Sequences obtained directly from clinical samples, belong to the clade 6. In year 2013 circulation of H3N2 subtype was highest, all of them grouped into the clade 3C (3C.2 and 3C.3), related to the vaccine strain signed by lineage A/Texas/50/2012. Circulation of influenza A(H1N1)pdm09 was highest in 2013 (26,2%) respect 2012 year (8,3%). Studied sequences distributes into three distinct clades: sequences from 2012 year belong into the clade 7, sequences from January 2013 belong into the clade 7 mainly and one of them into the clade 6C together with sequences from May 2013, last sequences from November 2013 belong into the clade 6B. Influenza B viruses were detected during the two studied years, characterized by the circulation of lineage B/Victoria in 2012 (20.5% of influenza detections) and lineage B/Yamagata in 2013 (7.4% of influenza detections). Strains of B/Victoria lineage grouped with the vaccine strain B/Brisbane/60/2008, while strains of the B/Yamagata lineage belong into the clade 2 represented by the vaccine strain B/Massachusetts/02/2012. It remains to be defined if these viral variants represent an important antigenic drift that would enable viral immune evasion and/or affect influenza vaccine effectiveness.

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EXPRESSION OF INNATE IMMUNE GENES IN HUMAN CELLS INFECTED BY BUNYAVIRUS

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The virulence of the pathogenic Bunyaviruses is directly linked to the roles of viral virulence factors and their capacity to counteract the host pathways. These viruses use cellular proteins to promote their own replication/transcription and, in response, host induces transcriptional reprogramming to activate antiviral effects. In order to verify the early steps of Apeu virus (APEV) and Tahyna virus (THAV) induced innate immune system activation, we performed TaqMan-based qPCR assays using cDNA obtained from mRNA extracted from A549 cells after 4hs or 8hs of infection with APEV, TAHV and VSV virus (ssRNA control virus), besides a mock control. We verified that APEV is recognized by TLR9, differently of TAHV, which follows VSV due to TLR7 recognition. However, APEV follows VSV decreasing TICAM1 expression after 4hs of infection. It is also possible to note an early induction of TLR pathway by VSV when compared to APEV and TAHV. TAHV and mainly VSV, but no APEV, increased expression of IRF5, notably after 8hs of infection. All the viruses were able to increase the expression of TLR3, IRF3 and 7. TRAF3 was slightly more expressed (4hs and 8hs) in cells infected by APEV, but not by VSV and TAHV. The TICAM and IRF3 expression levels were normalized after 8hs of infection. We also observed and 8-fold increase of IRF5 expression after 8hs of incubation with VSV. Also, VSV induced IFNb1 expression just after 4hs of infection, meanwhile TAHV induced IFNb1 increased levels only after 8hs of infection. At this time, IFNb1 expression levels in VSV-infected cells started to diminish, but remained higher than the other viruses. Finally, APEV, even after 8hs of infection, was unable to induce a significant increase of IFNb1 expression. We concluded that these viruses are able to triggers different recognition and intracellular signaling pathways leading to differences in the immune responses and, consequently, determining the pathogenic potential of each tested viruses.

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CYCLICAL OUTBREAKS OF RIFT VALLEY FEVER IN EAST AFRICA: WHY THEY PERSIST AND POSSIBLE SOLUTIONS TO PREVENT OR CONTAIN ITS SPREAD

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Rift Valley fever (RVF) caused by Rift Valley Fever virus (RVFV) is a zoonotic viral disease primarily of domesticated animals that also can cause disease in humans. RVFV belongs to the genus Phlebovirus, family Bunyaviridae and was first isolated during epizootics in Rift Valley Province, Kenya in 1930. RVF outbreaks have occurred in periodic cycles of 4-15 years in East Africa. During 2006-2007, an RVF outbreak that started in Kenya involving 684 cases (case fatality rate (CFR) = 49%), spread to Somalia (114 cases; CFR = 45%) and extended to Tanzania (309 cases; CFR = 46%). In 2008, the outbreak reoccurred in Sudan (698 cases; CFR = 31.8%) and Mozambique (412 cases, 17 deaths). The outbreaks have been associated with flooding from unusually high precipitations in many flood-prone habitats and with significant increases in vegetation cover. Flooding of the dambos (shallow depressions) usually induces the hatching of transovarially infected *Aedes* mosquito eggs that are dormant in the soil. The hatched eggs produce infected adult females which are capable of transmitting RVF virus to high population of domestic of animals and / or human (amplification hosts). Culex mosquitoes subsequently colonize these flooded dambos, feed on the amplifying hosts and produce large populations of infectious mosquitoes which efficiently transmit the virus to non-infected domestic animals and immunologically naïve humans within the environment. Due to these unique ecological and geographical factors involved in RVF transmission cycles, RVF outbreaks may be feasibly predicted and prevented. We review the epidemiological factors associated with outbreaks and their predictability, methods of estimating infection rate based on confirmed or suspected cases, and the importance of entomological/sero- surveys. Ultimately, we conclude that RVF outbreak prevention and outbreak impact mitigation requires timely implementation of appropriately phased activities during inter-epidemic, prediction and outbreak periods.

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DEVELOPMENT OF A TISSUE CULTURE INFECTIOUS DOSE (TCID50) ASSAY AS METHOD FOR QUANTIFYING INFECTIOUS UNITS IN NON-CYTOPATHIC EFFECT CAUSING VIRUSES

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For cytopathic viruses, the gold standard for quantitation of infectious viral particles is the plaque assay; however this method can yield inconsistent results due to variability in plaque quality and the subjective nature of plaque counting. For non-cytopathic viruses, plaque assays are not an option. We report the development of a novel tissue culture infectious dose at 50% assay (TCID50) which is simple to perform, can be utilized for both cytopathogenic and non-cytopathogenic viruses, and does not have the shortcomings of the plaque assay. The TCID50 assay is performed by adding virus dilutions to cells seeded in a 96 well plate. After viral replication in cells, a solution of Presto BlueTM dye (Life Technologies, Grand Island, NY) is added and incubated for 30 min at room temperature. The dye is enzymatically reduced in living cells proportional to the concentration of NADH and NADPH present, causing a color change proportional to cellular changes in metabolism. The results can be either visually read or spectrophotometrically measured. By monitoring of the colorimetric change and comparing this to the non-infected cells, infectious viral concentrations can be determined. Multiple cytopathic and non-cytopathic strains of Crimean Congo Hemorrhagic Fever (CCHF) virus were used to develop this technique. TCID50 assays were tested concurrently with plaque assays and ELISA antigen detection assays that correlated the change in color with the presence of antigen. The assay was also evaluated using Ebola virus, Marburg virus, Middle Eastern Respiratory Syndrome Coronavirus, and Lassa virus. The TCID50 assay developed is a useful tool for the quantitation of both cytopathic and non-cytopathic infectious viruses.

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ARBOVIRUS SURVEILLANCE IN BATS IN UGANDA

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Arboviruses including Rift Valley fever (RVFV), Yellow fever (YFV), West Nile (WNV), Chikungunya (CHIKV) and Zika (ZIKV) viruses have been isolated or detected serologically from various East African bats, however the role of bats in arbovirus transmission cycles is poorly understood. The aim of this study was to investigate the exposure history of Uganda bats to arboviruses as well as attempt virus isolation from bat tissues. Blood, tissues, or both were obtained from 1067 bats from Uganda between 2009 and 2013. Liver/spleen samples were mechanically homogenized in tissue culture media and virus isolation was performed on Vero cells. Virus isolates were identified by either RT-PCR using virus group-specific primers, or next generation sequencing. Serum samples were tested for specific neutralizing antibodies against WNV, YFV, Dengue 2 (DENV2) virus, CHIKV, O'nyong-nyong virus (ONNV), Babanki virus (BABV), ZIKV and RVFV by plaque reduction neutralization test. *Rousettus aegyptiacus* from Maramagambo forest in western Uganda had specific neutralizing antibodies against CHIKV (2/303), ONNV (32/303), YFV (3/303) and WNV (1/303). *R. aegyptiacus* from Mt. Elgon in eastern Uganda also contained neutralizing antibodies against YFV (1/45). *Epomophorus labiatus* from the Entebbe/Kampala area demonstrated specific neutralizing antibodies against BABV (3/52), DENV2 (1/52), and WNV (2/52). DENV2 antibodies were also present in *Chaerephon pumila* (3/123) and *Mops condylura* (1/36) captured around Entebbe, and *Nycteris* spp. (2/10) from Mt. Elgon. One *C. pumila* also had a high neutralizing titer against ONNV. Virus isolates to date include Entebbe bat virus (*Flaviviridae: Flavivirus*) from *C. pumila* in Entebbe. Testing is still in progress and complete results will be presented. Serological and virological evidence suggest that multiple species of fruit and insectivorous bats from Uganda are exposed to and are potential amplification hosts for arboviruses.

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OPTIMIZATION OF A RIBOSOME PROFILING-BASED PIPELINE TO MEASURE GLOBAL CHANGES IN GENE EXPRESSION IN RESPONSE TO VIRAL INFECTION

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Ribosome profiling is a new, powerful technique that enables direct measurement of protein expression at the whole cell level. The technique is based on the deep sequencing of ribosome footprints generated during nuclease digestion of extracted polisomes. During digestion, individual ribosomes protect discrete 30bp fragments (referred to as footprints) that reflect the various positions of ribosomes along all actively translated

mRNAs. Aggregation of footprint sequence data generates all the information needed to build a comprehensive understanding of how global gene expression may delineate particular phenotypes. Ribosome profiling was first developed by Ingolia et al. to run on the Illumina platform. We have adapted the original ribosome profiling strategy to run on the Ion Proton platform, and have optimized it to monitor virus-induced changes in gene-expression profiles. The main modification to the workflow includes a complete re-design of primers and adapters to include the A and P1 sequences required for Ion Proton workflows. Final library lengths were about 151pb (as opposed to 175pb for Illumina libraries) and 13pM of each library were amplified by emulsion PCR. Once amplified, libraries were sequenced using a PIv2 chip. PI chips contain 165M (million) wells. Assuming a 60-70% bead deposition it is expected that runs will generate 99-115M reads. We have achieved deposition rates of up to 93%. We routinely obtain runs of around 145M reads, which after initial quality control processing (elimination of clonal beads, etc) result in runs of about 105M usable reads. These then enter bioinformatics pipelines that make use of Botwie, Cutapad, Tophat and Bioconductor in order to specifically filter process the data and perform differential analyses of expression. To date, we have successfully adapted ribosome profiling for the study of dengue-induced changes in gene expression profiles. Further studies of a variety of infectious diseases caused by various pathogens, including bacteria and parasites, will also be possible provided minor modifications to the already established ribosome-profiling pipeline.

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DISTRIBUTION OF INFLUENZA ANTI-VIRAL RESISTANCE IN SOUTHEAST ASIA IN 2012-13

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The Department of Virology at AFRIMS conducts influenza surveillance in SE Asia. Over 400 acute respiratory specimens collected from Thailand, Bhutan, Nepal, Cambodia and the Philippines in 2012 and 2013 and found positive for influenza virus by RT-PCR were selected for genotypic anti-viral analyses by pyrosequencing. Of these, close to 200 were further analyzed using a functional cell-free neuraminidase inhibition assay to determine their oseltamivir Inhibitory Concentration (IC50). Pyrosequencing analyses found a wide-spread prevalence of genome markers associated with resistance to M2-inhibitor adamantane derivatives in pdmH1N1 and H3N2 specimens. Resistance to M2 inhibitors, associated with the S31N mutation, was present in 100% of 93 pdmH1N1 and 155 H3N2 specimens tested. However, pyrosequencing showed wide-spread susceptibility to neuraminidase inhibitors (NAI) among influenza A specimens, with 98.9% of all tested pdmH1N1 lacking the NAI resistance-associated H275Y marker. Only one pdmH1N1 specimen was found to carry the H275Y marker. Of the H3N2 specimens tested, 78% were found to be susceptible to NAI. One H3N2 specimen had the E119V NAI resistant marker. Thirty-three H3N2 specimens had indeterminate results since they showed mixed populations at the D151N mutation. Two of these H3N2 specimens also showed the E119V mutation with varying distribution. There were no NAI-associated R292K or N294S mutations among the tested H3N2 specimens. NAI-associated resistance markers were more common among the 168 influenza B specimens tested, with nearly 20% displaying the E117A and R374K resistance-associated mutation at varying degrees. Phenotypic testing of NAI resistance to oseltamivir carboxylate showed widespread susceptibility to oseltamivir, most of which correlated with lack of genotypic NAI resistance-associated markers. However, several influenza A and B specimens showed reduced susceptibility to oseltamivir (several-fold higher IC50 than negative controls), some of which did so despite lacking NAI resistance-associated genotypic markers.

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COMPARISON OF MICROSCOPY, NESTED AND REAL-TIME PCR-BASED ASSAYS WITH HIGH-THROUGHPUT POOLED SAMPLES FOR SCREENING ASYMPTOMATIC MALARIA CARRIERS FROM ENDEMIC AREAS OF MYANMAR

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Asymptomatic infection is an important obstacle for controlling disease in malaria-endemic countries. Because asymptomatic carriers do not seek treatment for their infection, asymptomatic carriers can have high levels of gametocytes and constitute a reservoir available for new infection. Herein, we employed a sample pooling/PCR-based molecular detection strategy for screening malaria infection in residents from endemic areas of Myanmar. Blood samples (n = 1,552) were collected from residents in three malaria-endemic areas (Kayin State, Bago and Tanintharyi regions) of Myanmar. Two nested PCR and real-time PCR assays showed that asymptomatic infection was detected in about 1.0%-9.4% of residents from surveyed areas. The sensitivities of the two nested PCR and real-time PCR techniques were higher than that of microscopy examination (100% vs. 26.4% sensitivity; kappa value = 0.2-0.5). Among the three regions, parasite-positive samples were highly detected in subjects from the Bago and Tanintharyi regions. Active surveillance of residents from regions of intense malaria transmission would reduce the risk of morbidity and mitigate transmission to the population in these endemic areas. Our data demonstrate that PCR-based molecular techniques rather than microscopy are more efficient for nationwide surveillance of malaria in endemic countries.

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AWARENESS OF EXISTENCE OF MALARIA DIAGNOSTIC SERVICES AND PATTERN OF PRE-HOSPITAL TREATMENT, MAKARFI, NIGERIA

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Malaria is the leading cause of childhood mortality in Nigeria. Averagely, children <5years (U5) are prone to three episodes annually. In 2005, the national malaria policy recommended Artemisinin-based combination therapy (ACT) due to established resistance to Chloroquine (CQ). It provided for the presumptive treatment of suspected malaria cases in U5. In 2011, the policy was revised to ensure parasite-based diagnosis before treatment of malaria irrespective of age. However, treatment remains largely presumptive. We conducted a hospital-based cross-sectional study in a low malaria prevalence setting to determine factors associated with awareness of existence of malaria diagnostic services (MDS) among caregivers of febrile U5 (FU5) and pattern of pre-hospital treatment practices for FU5. We interviewed consecutively selected caregivers of 295 FU5, attending Makarfi General Hospital, Kaduna state, Nigeria; from December 2010 to August 2011. We included all eligible FU5 without rash. Information on factors influencing awareness of MDS and pre-hospital treatment (PHT) was collected. We examined the Giemsa-stained blood smear of FU5 for malaria. Fifteen (5.1%) caregivers have ever heard about MDS. Eleven (3.7%) caregivers were ever offered MDS by physicians. Being formally educated (Prevalence Odds ratio (POR): 0.05, 95% Confidence Interval (CI): 0.01-0.20), living <5km from a health facility (POR: 4.21, CI: 1.39- 12.55), being a government staff (POR: 9.18, CI: 1.74- 39.93) and ever being offered MDS (POR: 35.09, CI

10.13-134.00) were positively associated with awareness of MDS. Overall, 201(67.9%) children had received any PHT, 121 children (41.0%) at patent medicine stores. Of the 31(10.5%) FU5 diagnosed with malaria and 264 (89.5%) without malaria diagnosis, 13 (41.9%) and 65 (24.6%) have had PHT with CQ respectively. Awareness of MDS remains low. Treatment of FU5 against malaria is predominantly inappropriate within the community despite widespread deployment of affordable ACTs. There is a need to sensitise caregivers and health staff on use of ACTs and adherence to confirmatory malaria diagnosis.

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IMPROVING MALARIA DIAGNOSIS THROUGH MICROSCOPY COMPETENCY ASSESSMENT PROGRAMS IN RESOURCE LIMITED SETTINGS

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Malaria remains a major public health problem in Uganda. Early case detection is fundamental for the reduction of mortality and morbidity. Following the WHO recommendation for Parasitological confirmation of suspected cases, Uganda adopted this guideline with scale-up of Microscopy and Rapid Diagnostic Tests. Although microscopy-based diagnosis remains the standard, its quality is frequently inadequate to ensure good treatment outcomes and accurate epidemiological and surveillance data. Our aim in this program was to identify expert blood smear readers through WHO competency assessment scheme that could be utilized to improve Microcopy capacity in Uganda. A total of 33 experienced microscopists working as trainers and performing routine blood smear examination were selected to participate in the competency assessment. Participants were subjected to read standardized blood slides from the WHO slide bank under an "examination" environment. Scoring and grading was done for parasite detection, species identification and counting in accordance with WHO guidelines. Performance data was analyzed to generate sensitivity, specificity, level of agreement and if there was improvement at significant level of 5%.Participants were aged 20-50 years with 5 to over 10 years of experience in malaria microscopy. The mean score was 79%, 32% and 19% for parasite detection, species identification and parasite counting respectively in the baseline pretest and scores were 83%, 52% and 27% for parasite detection, species identification and parasite counting respectively in the final assessment. Performance improved significantly between pre-assessment baseline and final assessment 79% to 83 %, p=0.006 for parasite detection**,** 43% to 65%, p=0.003 for species identification and 28% to 27%, p=0.648 for parasite quantification. The Mean sensitivity at baseline and final assessment were 80% and 88.4%, p=0.314 and specificity 67.5% and 87%, p=0.817 respectively. Based on results obtained in this assessment, performance for parasite counting and species identification was below the WHO recommended levels of >50% and 90% respectively. Performance in parasite detection was better for all participants. In light of these results, we recommend that competency assessment schemes be conducted for all persons involved in microscopy training, reference expert slide readers and those involved in clinical trials and therapeutic efficacy trials.

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C-REACTIVE PROTEIN IN DIAGNOSIS OF MALARIA IN HYPERENDEMIC RURAL AREA OF LAKE VICTORIA IN UGANDA AND TANZANIA

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There is increasing discussion on the role of C-reactive protein (CRP) in differentiation of malaria from other (bacterial) infections especially in regions with hyperendemic malaria. The aim of this study was to assess if CRP is confirmative to the classic microscopic diagnosis of malaria and if it helps in differentiating malaria from other infections. Altogether, 68 patients with positive blood smear for malaria from two rural hospitals (Kibara, Tanzania and Buikwe, Uganda) close to Lake Victoria in hyperendemic malaria region were assessed for CRP levels during acute phase of infections. All of the 68 patients had CRP levels measured within 48 hours of onset of symptoms when seeking medical advice. Their levels were within 8-184 mg/l. Only 7 patients (10,3%) had CRP values >100 (high level of CRP patients - HLCP) and 2 (2,9%) had even more than 200 mg/l, with suspicion of additional bacterial super-infection (e.g. meningitis). However, 33 cases (48,5%) had values below the reference level (8-10 mg/l). According to our results, CRP values can neither predict, nor exclude malaria in 51% of all microscopically positive cases which had CRP less then reference level (< 8 mg/l). Given this, also negative CRP does not exclude acute falciparum malaria, however positive CRP with levels >100 mg/l may suggest cerebral malaria or severe bacterial infection. Therefore, the yield of CRP detection in hyperendemic malaria region of great lakes in sub-Saharan Africa remains to be low.

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LOOP MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) - EVALUATION OF A NEW KIT FOR DETECTION OF ASYMPTOMATIC LOW-DENSITY MALARIA INFECTIONS IN ZANZIBAR

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Loop mediated isothermal amplification (LAMP) provides an opportunity for improved, field-friendly detection of malaria infections in low-endemic settings but data on its accuracy for detection of asymptomatic low-density parasitemias in pre-elimination settings are lacking. We therefore evaluated the performance of a commercial LoopampTM MALARIA Pan/Pf detection kit (Eiken Chem., Japan) compared with PCR using DNA extracted from dried blood spots from 465 asymptomatic individuals in Zanzibar. All samples were analysed both for Pan (all *Plasmodium* species) and Pf (*P. falciparum*) specifically. The amplification was performed at 65° and results were interpreted after 40 minutes in a real-time turbidometer. A total of 49 (10.5%) and 38 (8.2%) samples were Pan and Pf-LAMP positive, respectively, whereas 54 (11.6%) were positive by PCR, i.e. 33 *P. falciparum* and 13 P. malariae mono-infections and 8 mixed *P. falciparum*/P. malariae infections (mean parasite density 10/µL, range 0-4972). The sensitivity of Pan-LAMP for *P. falciparum* mono-infections (33) was 97% (95%CI 84.2-99.9) and Pf-LAMP for all *P. falciparum* infections (33+8) was 92.7% (95%CI 80.1-98.5), respectively. The sensitivity of Pan-LAMP for P. malariae detection was 76.9% (95%CI 46.2-95) (range 0-5 p/µL). The corresponding specificities were 100% for both Pan and Pf-LAMP. The LoopampTM MALARIA Pan/Pf kit was further evaluated in a field pilot study of 1026 asymptomatic individuals in three malaria hot

spot villages in Zanzibar. Screening was done with Pan-LAMP and Rapid Diagnostic Test (RDT). LAMP was performed using a simple DNA extraction method (boil and spin) followed by LAMP reaction in a heat block and results interpreted under UV-light. LAMP detected 18 (1.8%) and RDT 10 (1,0%) infections. LAMP results were ready within two hours and positive individuals received treatment the same day. In conclusion, the LAMP kit revealed high diagnostic accuracy for detection of asymptomatic low-density parasitemias and performed well under field conditions and detected 80% more parasite carriers than RDT.

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ARE EXIT INTERVIEWS RELIABLE? ANALYSIS OF THE HAWTHORNE EFFECT IN A STUDY OF ADHERENCE TO MALARIA TREATMENT GUIDELINES IN TANZANIA

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Interviewing patients exiting health facilities is a commonly used way to assess consultation practices. It is however unclear if health professionals change their practices when they are aware of such interviews taking place, possibly paying more attention to follow recommended practices. This so-called "Hawthorne effect" could have important consequences for interpreting research and for monitoring program performance, but has rarely been assessed. A cluster-randomised trial of interventions to improve adherence to national guidelines for the use of anti-malarial drugs was conducted in Tanzania. As part of the evaluation, patient interviews were conducted outside participating health care facilities on two randomly-selected days per week for a one year period. Health workers in these facilities were also routinely documenting each consultation in their ledgers. A possible Hawthorne effect was investigated by comparing routine data recorded in ledgers on days when exit interviews were conducted with data from days when no exit interviews were conducted. Routine data were collected in 34 facilities on over 38,000 consultations. No statistically significant differences were found on survey versus non-survey days on any of three pre-specified primary outcomes, after adjusting for geographic region and season. The odds of having a rapid diagnostic test (RDT) result was 7% higher on survey days (Odds Ratio 95%CI: 0.94-1.21, p=0.31), 11% lower for prescribing an anti-malarial drug to a RDT negative patient (0.70-1.14, p=0.36), and 10% lower for prescribing anti-malarial without an RDT result (0.71-1.14, p=0.38). We found no strong evidence of a Hawthorne effect in a study using exit surveys of primary care clinics with data collected from locally trained assistants. This is likely to support such methods in other studies.

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OVER-DIAGNOSIS OF MALARIA USING A RAPID DIAGNOSTIC TEST IN A HIGH MALARIA TRANSMISSION SETTING IN UGANDA

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The introduction of rapid diagnostic tests (RDTs) has provided a means for improving the diagnosis of malaria so as to minimize overuse of treatment and thereby delay development of resistance to ACTs. RDTs based on *Plasmodium falciparum* histidine-rich protein 2 (PfHRP2) have been rolled out across Uganda and several types of RDTs are now available, both in the public and private healthcare sectors. However, important questions remain as to whether all approved RDTs perform well compared to microscopy. The accuracy of SD Bioline Malaria Ag P.F (HRP2/PLDH) RDT was assessed in an area of high malaria transmission in Uganda. The study was conducted at Malaba Health Centre where children aged 0-12 years with a history of fever/ axillary temperature ≥37.5°C in the past 48 hours were recruited. Those with recent antimalarial use were excluded. A total of 217 febrile children were tested using the RDT and the results compared to microscopy as the gold standard. Patients were treated on the basis of the RDT results alone and follow up was done on day 3 and subsequently at 7-day intervals for 28 days. Ninety-four of the 217 patients tested had a positive blood smear for asexual forms of *P. falciparum*. An additional 45 patients tested RDT positive and received antimalarial treatment. Malaria Ag P.F (HRP2/PLDH) RDT had an overall sensitivity of 97%. However the specificity was significantly low at 63%. A negative predictive value (NPV) of 97% and a positive predictive value (PPV) of 68% were observed. A proportion of positive HRP2-based test results that were categorized as false-positive when compared with microscopy may have been due to the presence of subpatent parasitemia and thus PCR testing is being carried out. Reports on specificity of RDTs should be interpreted with caution as there may be wide variations in these measurements depending upon endemicity, season and the age group of patients studied. As RDTs become increasingly available there is a need in Uganda to recognize that 'one size does not fit all'.

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POSITIVE CONTROL WELLS (PCW) FOR MALARIA RAPID DIAGNOSTIC TESTS (RDT): TRAINING EFFECTIVENESS, IMPACT ON RDT USE AND HEALTH WORKER PERCEPTIONS IN LAO PDR AND UGANDA

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Malaria rapid diagnostic tests (RDT) are widely used in health facilities and in community-based care settings in endemic countries. To maintain health worker (HW) and patient confidence in RDT and to optimize their utility, RDT must have consistently reliable results; tools to assess the quality of malaria RDT at the point-of-care are unavailable. Prototype positive control wells (PCW), plastic tubes containing critical concentrations of lyophilized recombinant antigens (HRP2, pLDH, aldolase) that are reconstituted with water, have been developed for HWs to test RDT stocks at their health facilities, to ensure RDT validity and accuracy. HWs routinely using RDTs in Lao PDR (n=269) and Uganda (n=289) underwent standardized half-day training on the use of PCWs; >70% were village health volunteers. After training, HWs were supplied with PCWs for 6 months, and recorded frequency and reason for PCW use and action taken. HW competence in PCW use was measured immediately after training and 3 and 6 months later. Data on RDT use during the study period were extracted from HW logbooks in control and intervention areas. Focus group discussions and interviews were conducted to capture HW preferences for PCW implementation as well as feasibility, acceptability and value of use. Initial analysis shows that on strict observation immediately following training, 241 (90%) participants in Lao and 244 (84%) in Uganda performed all critical PCW steps correctly; performance was generally maintained after 6 months. Most common errors were failing to fill the water dropper provided exactly to the measured mark, and failing to transfer exactly one drop of PCW solution to the RDT well. Overall, ≥91% of participants could correctly identify 'good' and 'bad' RDT and ≥89% could report appropriate action. 784 PCW were reportedly used during the study period in Lao PDR and 1679 in Uganda. The most common reasons cited for performing

PCW during routine work were receiving a new stock of RDT, and wanting to check on RDT stock quality. Initial field reports of negative RDT with PCW were not confirmed upon repeat testing. Data on RDT usage and adherence to RDT results will be available in May 2014. PCW training was effective and in general, PCW appear to improve HW confidence in RDT results.

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PREVALENCE OF ASYMPTOMATIC MALARIA AND GLUCOSE 6 PHOSPHATE DEHYDROGENASE (G6PD) DEFICIENCY IN WESTERN PROVINCE, SOLOMON ISLANDS

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The Solomon Islands has experienced a significant decrease in malaria cases over the last decade. In Central and Temotu Provinces, the estimated malaria prevalence by microscopy is up to 5% while PCR-based diagnostics have indicated local prevalences between 4 and 40%. In Temotu Province, more than 84% of the malaria-positive cases were asymptomatic, with *Plasmodium vivax* being the predominant species. The prevalence of glucose-6-phosphate dehydrogenase (G6PD) deficiency in Isabel and Guadalcanal Provinces were up to 20.3%. Malaria prevalence and G6PD deficiency in other provinces is not described in the literature. The objective of this study is to determine the prevalence of malaria and G6PD deficiency in Western Province, Solomon Islands, toward baseline data for malaria intervention studies and characterizations of the province for the Solomon Islands' malaria elimination program. A total of 3,837 blood spots on filter paper were collected from 19 selected villages located in five island regions of Western Province between August and October 2013. G6PD deficiency was assessed with a NADPH-fluorescent-based diagnostic kit. A total of 2.4% (range 0-6.4% by village) and 9.3% (range 1.9-24% by village) of the population have the deficient and intermediate G6PD phenotype. The samples were then screened for malaria parasites based on amplification of the 18S rRNA gene using a direct PCR approach (sensitivity of 1.6 parasites/ul). Based on successful genus-specific PCR, the average malaria prevalence was 16% (range 5.13-44% by village). In contrast, when *Plasmodium* species-specific primers were used, the total prevalence was 5% (range 0-13% by village), with *P. vivax* accounting for 95% of cases, and 99% of malaria-positive subjects being asymptomatic. Genus-specific positives that did not amplify with species-specific primers are being sequenced to identify species. We will discuss our results in the context of the performance of malaria diagnosis PCR in areas with extremely low parasitemia and implications for the malaria elimination program in the islands.

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ASSESSMENT OF A THREE-BAND (HRP-2/PLDH) RAPID DIAGNOSTIC TEST FOR THE IDENTIFICATION OF SEVERE MALARIA AT A PERIPHERAL HEALTH FACILITY IN WESTERN UGANDA

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The use of rapid diagnostic tests (RDTs) for the diagnosis of malaria has rapidly proliferated at peripheral health facilities in resource-limited settings where there is often no laboratory capability. In this observational study, we examined the utility of a three-band, HRP-2/*pan*-pLDH (SD Bioline FK60) RDT as a tool for the early identification of patients at risk for severe malaria. A total of 1,509 patients underwent RDTs with 637 (42%) positive for malaria. 326 (21.6%) exhibited a single HRP-2 band, 307 (20.3%) exhibited both HRP-2 and pLDH bands, while only 4 (0.3%) exhibited a single pLDH band. The rate of three-band positive results was twice as high among patients <15 years compared those ≥15 years of age (26.3% vs. 13.2%, χ^2=39.9, p<0.001). Notably, the absolute number and proportion of three-band positive RDTs declined over the study period with the transition from the dry to rainy season. The trend of declining three-band positivity in the setting of relatively stable HRP-2 positivity was significant (OR 0.51, 95% CI 0.39 to 0.66). The vast majority (92.1%) of smears from patients with three-band positive RDT results demonstrated *Plasmodium falciparum* mono-infections. The mean parasite density was approximately 42,000/µl (95% CI 6,827 to 77,349) for HRP-2 positive RDTs and 72,300/µl (95% CI 54,859 to 89,727) for three-band positive RDTs (p=0.072). The mean hemoglobin (Hb) was 8.9g/dL (95% CI 8.3 to 9.3) in patients with a negative RDT, 7.3g/dL (95% CI 6.6 to 8.1) in patients with a HRP-2 positive RDT, and 6.3g/dL (95% CI 5.6 to 7.1) in patients with a three-band positive RDTs. The difference in Hb levels between HRP-2 and three-band positive RDTs, however, was not significant (p=0.17). These results require further investigation, but suggest that a HRP-2/pLDH RDT may help identify patients with higher parasite densities and more severe anemia, both risk factors for severe malaria.

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MALARIA IN PREGNANT WOMEN LIVING IN AREAS OF LOW TRANSMISSION OF THE SOUTHEAST BRAZILIAN COAST: MOLECULAR DIAGNOSIS AND HUMORAL IMMUNITY PROFILE

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Pregnant women and children are the main groups under risk of acquiring malaria worldwide. Although *Plasmodium falciparum* in pregnant women has been widely addressed in the literature, the interaction of this cohort with *P. vivax* and *P. malariae* was poorly explored to date and requires a more comprehensive approach. In Brazil, 99% of the infections occur in the Amazon Region. Although malaria is not considered endemic outside this region, autochthonous cases are registered in areas covered by the Atlantic Forest biome. Studies related to malaria in these low transmission areas have acquired scientific and epidemiological relevance, since they suggest continued transmission and potential outbreaks. The Southeast of Brazilian coast has been focus of studies on the occurrence of *Plasmodium*, with reports of asymptomatic cases. In this region, where the transmission of *P. vivax* was established from several studies, our group detected for the first time the occurrence of *P. malariae*, using molecular tools. Data on the occurrence of the disease or presence of antiplasmodial antibodies in pregnant women living in this area of low endemicity had not been described previously. This study monitored quarterly the circulation of *Plasmodium* in pregnant women attended in five health facilities located in Juquitiba, State of São Paulo. We performed diagnosis by thick blood film and sensitive molecular protocols for parasite gDNA detection, as well immunological assays in order to investigate humoral immune parameters. For the first time *P. vivax* and *P. malariae* were detected in pregnant women living in this low endemicity area, with positivity (95% CI) of 5.6% (1.7-9.0). It was possible to detect the two species through sensitive molecular tools, once the cases were asymptomatic. We also found a high prevalence of IgG antibodies showing a significant exposure of this

population to *Plasmodium*, with 44.0% (35.6-52.7) for ELISA-*Pv* and 18.4% (12.6-26.1) for IFA-*Pm*. In regions with a similar profile presented in this study, the diagnosis of malaria might be indicated in prenatal care.

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A QUANTITATIVE NANOPARTICLE-BASED HISTIDINE-RICH PROTEIN 2 ASSAY FOR THE MALARIA PARASITE *PLASMODIUM FALCIPARUM*

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Diagnosis of severe malaria is particularly important in highly endemic regions since most of the patients are positive for parasitemia. Accurate diagnosis is increasingly important to avoid overprescribing antimalarial drugs, minimize drug resistance, and minimize costs. Microscope does not reflect the pathogenic-sequestered parasite burden. HRP2 levels associated with severe malaria are typically greater than 100 ng mL^{-1}. Rapid diagnostic tests (RDTs) are qualitative and because of that they cannot be used for diagnosis of severe malaria. Here we report on a magnetic bead - quantum dot (MBQD) assay for measurement of levels of HRP2 antigen. The assay is relatively straightforward with magnetic beads for capture and concentration of the target protein, and quantum dots for efficient quantitative detection. Magnetic beads containing surface epoxy groups were coupled to a mouse IgG monoclonal antibody anti-HRP2 (clone 3A4). Human urine samples spiked with HRP2 were incubated with antibody-bead conjugates and then analyzed by Western Blot. Western Blot shows detection of captured protein down to a concentration of 5 ng mL-1. Without concentration, the detection limit was about 100 ng mL-1, suggesting a protein concentration of about 20-fold. To demonstrate the complete sandwich assay, magnetic beads conjugated with antibody were incubated with different amounts of HRP2 spiked in serum samples. After magnetic isolation and washing, the magnetic beads were resuspended and incubated with Quantum Dots 525 coupled to the same monoclonal antibody. Using this assay, we were able to detect HRP-2 concentrations in serum as low as 1 ng per test. The correlation between intensity and HRP-2 concentration is linear at higher concentrations with a slope of 1.0 (from 1 ng to 1000 ng). Here we have demonstrated an assay for capture, concentration, and quantitative detection of HRP-2 using magnetic beads and quantum dots that can be easily adapted for point-of-care diagnostics for classification of severity of malaria caused by *Plasmodium falciparum*.

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ADVANCED QUANTITATIVE MICROSCOPY FOR *PLASMODIUM FALCIPARUM* DIAGNOSIS DURING PFSPZ CHALLENGE AND OTHER CONTROLLED HUMAN MALARIA INFECTION STUDIES: RESULTS OF AN AFRICAN TRAINING WORKSHOP

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Sanaria has developed aseptic, purified, cryopreserved infectious *Plasmodium falciparum* (Pf) sporozoites (SPZ) called PfSPZ Challenge as a tool for Controlled Human Malaria Infections (CHMI) to study protective efficacy of anti-malarial drugs and vaccines, to allow refinement of the method of administration of the highly protective PfSPZ Vaccine, and to study innate and acquired immunity to Pf. A critical component of the CHMI studies with PfSPZ Challenge is the diagnosis of malaria parasites in the blood. Diagnosis needs to be highly sensitive in order to detect parasites before the onset of major clinical symptoms, and needs to be highly specific in order to prevent misdiagnosis (i.e. false positive results), which could alter the outcome of the study. False positive results must be avoided in PfSPZ studies where misdiagnosis after vaccination would have potential safety implications and where misdiagnosis after CHMI would alter the estimates of protective efficacy. The European and Developing Countries Clinical Trials Partnership has funded a 7 country African consortium of institutions working with PfSPZ Challenge to optimize CHMI studies in Africa. Technical staff from across the network were hosted by the Ifakara Health Institute in Bagamoyo, Tanzania, for a 1 week course to develop expert level technical expertise in Advanced Quantitative Microscopy for rapid, sensitive, and specific diagnosis of Pf. Here we describe the results of the intensive training sessions and the subsequent establishment of a quantitative thick smear microscopy certification center at KEMRI, Nairobi, Kenya.T

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STIMULATING A PRIVATE SECTOR MARKET FOR MALARIA RAPID DIAGNOSTIC TESTS (RDTS): BASELINE RESULTS FROM KENYA, MADAGASCAR AND TANZANIA

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Key baseline results and formative insights from a multi-country project to stimulate the creation of a private sector market for malaria Rapid Diagnostic Tests (RDTs) will be presented and discussed. Since 2010 the WHO has recommended confirmatory diagnostic testing for suspected malaria, followed by treatment with ACT for positive cases. However, in the private sector, where over 40% of the population in endemic countries seeks care and treatment for febrile illness, RDTs are either non-existent or no cheaper than ACT. The UNITAID Private Sector RDT Project aims to increase both access to and demand for quality-assured RDTs, while improving private providers' fever case management skills and implementing a public-private roadmap that will guide policy and regulation. The Project is led by Population Services International, together with its collaborating implementers, Foundation for Innovative New Diagnostics, Malaria Consortium and the World Health Organization; it is being implemented in Kenya, Madagascar, Nigeria, Tanzania (mainland) and Uganda. In 2011, RDTs were available in fewer than 1 in 10 private health facilities in Kenya and Nigeria, and in Kenya the median price for a test was approximately $1.00, much more expensive than (subsidised) ACT treatments for children ($0.46). Although RDTs were available in over 90% of public facilities in Madagascar, in 2011 only 9% of private doctors and clinics had tests available. Within this context, results will be presented from the 2013 household survey of over 1,300 fever cases on the Kenyan coast, and exit interviews among private-sector fever patients in Kenya, Madagascar and Tanzania (results due July 2014). Operational insights from qualitative research with participating providers will also be shared. The project will contribute to the ongoing discussion on how best to scale-up access to affordable, quality-assured RDTs offered by trained and supervised providers who are incentivized to correctly manage febrile illness.

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MALARIA TESTING AND TREATMENT IN TANZANIA AFTER INTRODUCTION OF RAPID DIAGNOSTIC TESTS IN ACCREDITED DRUG DISPENSING OUTLETS

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In Tanzania, the private sector provides 30% of all treatment for febrile illness to children under 5 years receiving it, but the availability of diagnosis is more limited than in the public sector. The reliance on presumptive malaria treatment in this sector means that many patients receive an incorrect diagnosis and inappropriate treatment. One challenge is that drug outlets comprise 70% of the private sector, but are not allowed to sell or perform malaria tests. This study assessed how patient testing and medication-purchasing behaviors evolved following a pilot study that allowed Accredited Drug Dispensing Outlets (ADDOs) in two districts to sell and perform malaria rapid diagnostic tests (RDTs). A baseline survey was completed in March 2013 to assess testing practices, RDT availability, and medication sales in ADDOs in Morogoro Region. In May 2013, dispensers in 270 intervention ADDOs were trained on how to properly stock and perform RDTs, and given access low cost RDTs. Dispensers from 91 control ADDOs were not given access to the RDTs. Over the next year, ADDO patient register books were used to evaluate the proportion of febrile patients that received an RDT and an artemisinin combination therapy (ACT). Surveys found RDTs were available in 77.5%-87.0% of ADDOs in the intervention region following training. Of 12,730 patients that sought treatment for fever or malaria in the accredited ADDOs during the nine months following training, 79.4% (95% CI: 78.7%-80.2%) elected to purchase an RDT. Of those for whom an RDT test result was recorded (n=9,872), 57.0% (95% CI: 56%-58%) tested positive, and 79.1% (95% CI: 77.9%-80.1%) of those who tested positive received an ACT. Only 3.1% (95% CI: 2.5%-3.7%) of those who tested negative purchased an ACT. In this study, introducing RDTs in ADDOs resulted in high availability and uptake of testing, as well as adherence to test results. The study highlights the potential for improving appropriate use of anti-malarials and preventing overtreatment with ACTs by placing RDTs in ADDOs and training staff in their use.

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EVALUATION OF THE EFFECTIVENESS OF A TRAINING PROGRAM FOR SCHOOL TEACHERS IN PERFORMING AND INTERPRETING MALARIA RAPID DIAGNOSTIC TESTS SAFELY AND ACCURATELY IN ZOMBA, MALAWI

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With increasing levels of attendance, schools present a pragmatic opportunity to improve the access of school children to timely diagnosis and treatment of malaria, increasingly recognised as a major health problem for this age group. The expanded use of malaria rapid diagnostic tests (mRDTs) by community health workers has led to an interest in whether teachers can provide similar services for school children. We investigated the ability of school teachers to constitute safe, accurate and acceptable providers of malaria diagnosis and treatment using mRDTs and artemisinin-based combination therapies (ACTs) following training, as well as the retention of these skills during implementation of a school-based malaria case management programme. A comprehensive, skill-focused, pilot training was conducted in Zomba District, Malawi. Teachers were trained in the use of first aid kits including instruction on the principles and use of mRDTs by facilitators from the Ministry of Health. The four-day training workshop consisted of theoretical and practical sessions, with manuals and job aids adapted from a range of developed materials pre-tested with community medicine distributers and health surveillance assistants. Feedback from this pilot was used to design the full seven-day training workshop conducted prior to implementation of the intervention in schools. We present results on both the effectiveness of the pilot and full training workshops, in relation to increased knowledge and skill sets, and the retention of these through pre and post evaluation questionnaires, and checklist evaluations. Additionally, we report on the acceptability to teachers of carrying out such a role, and their confidence in providing this service, assessed through focus group discussions. To our knowledge, this is the first study in which teachers have been trained to use mRDTs. The results provide important evidence on the feasibility of using teachers to diagnose malaria using mRDTs in terms of safety, accuracy and confidence and to make appropriate treatment decisions based on the results.

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MALARIA DIAGNOSTICS QUALITY IMPROVEMENT AND ASSURANCE PROGRAM FOR TANZANIAN MILITARY HEALTH FACILITIES

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Malaria is responsible for over 100 million reported cases annually and 1-2 million deaths, especially in children. Within Tanzania, malaria diagnostics continues to be challenging, especially in resource-challenged settings. Amethyst Technologies LLC (ATL) partnered with the US Army, Tanzania People's Defense Force (TPDF), and Tanzania National Service Program (JKT) to develop and implement a malaria diagnostics quality assurance program to support the Tanzanian military's malaria measurement and control efforts at 21 TPDF/JKT training camps throughout Tanzania in 2011-2013. The approach of the strategy was to 1) develop Quality Improvement tools, which included assessment checklists (for evaluating camp health facilities), standardized laboratory SOPs, and a malaria diagnostic training course, 2) conduct baseline assessments of the 21 camps using the QI tools and provide malaria diagnostic training at all sites, 3) develop a QI and lab strengthening plan based on individual site assessments, 4) provide feedback to the health facilities and execute QI plans, and 5) execute a quality management plan to provide on-going quality improvement of malaria diagnostics. The QI assessment checklists evaluated sixteen quality criteria, including laboratory safety, human resources, personnel training, supply/stock management, results recording, results reporting, implementing of quality assurance procedures, external quality assessment (EQA), electrical supply, and staining capabilities (thick or thin smears). The quality management plan involved continuing quarterly re-assessment visits using QI tools, performing quality assurance of malaria diagnostics (by collecting malaria blood smears for cross-checking by expert microscopists), and supporting corrective actions. An overall improvement in the diagnostic services performed and strong increases in initial site assessment scores were observed, though sustaining the quality improvements proved challenging. We will present data captured from the baseline assessments and reassessments, showcase areas of success and challenges, demonstrate the utility of the QI tools, and discuss the sustainability of the program.

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MALARIA MICROSCOPY QUALITY ASSURANCE AND SLIDE CROSSCHECKING AT RUVU JKT LABORATORY OF TANZANIA

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The purpose of this exercise was to provide malaria microscopy External Quality Assurance (EQA) at JKT Ruvu where Walter Reed Program - Tanzania (WRP-T) is conducting malaria attack rate study. This EQA consist of 3 elements of (1) On-site Evaluation to assess the performance of the laboratory against the available SOPs, (2) Proficiency testing, and (3) Slide cross-checking.This exercise was also to assess the effect of microscopy error on estimating the malaria attack rate. Overall the performace at JKT Ruvu is satisfactory on the parameter of documentation, accommodation, electrical power supply, laboratory safety, equipment, stain reagents, and training. However the performance of blood film preparation and staining need to be improved as well as re-estimating the workload. Of 583 slides that had been randomly selected, 28 (5%) were judged to be unreadable, 67 (51%) were readable with difficulty due to artefacts, 22 (4%) were of poor quality due to poor preparation, 136 (23%) had poor staining quality, and 330 (57%) were in good quality and easily readable. The technician serving as microscopist at the study site was categorized as expert malaria microscopist for reading *Plasmodium falciparum*. The sensitivity and specificity scores of the microscopist were 90% and 95% respectively. The agreement of initial results between microscopist and expert confirmation is 99.14%. Agreement on positive slides was 95% (76/80), and for negative slides 99.8% (502/503). Those disagreements are due to poor preparation and workload. In conclusion, the commonest cause of inaccurate results was the quality of the slides, correction of which is likely to be achievable within existing Standard Operating Procedures on site. An expert malaria microscopist may report inaccurate results when the workload is too high.The laboratory workload should be measured, recorded and monitored. Microscopy error (false positives, false negatives, and species identification errors) may mis-lead estimation of malaria attack rate results.

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CLINICAL PERFORMANCE EVALUATION OF THE FYODOR URINE MALARIA TEST (UMT)

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Effective case management of malaria requires prompt diagnosis and treatment within 24 hours. Despite current policy guidelines that mandate confirmed parasitological diagnosis before treatment, access to diagnostic testing remains low in sub-Saharan Africa. Today, malaria diagnosis is only by blood-tests (microscopy and rapid diagnostic tests, RDTs), which are invasive, multistep and therefore relatively complex to perform, require technical expertise, and not available in most public and private sector healthcare settings where more than 65% of the population seek care. Here, we report the results of a multicenter pivotal clinical trial of Fyodor Urine Malaria Test (UMT) - a simple (one-step, no blood, no reagents, no equipment) dipstick test that detects *Plasmodium falciparum* parasite proteins shed in the urine of febrile malaria patients. A total of 1,893 participants (≥2 years) with fever (axillary temperature ≥37.5°C) or history of fever in the last 48 hours were enrolled at 6 primary healthcare centers in rural and suburban communities in Lagos State, Nigeria, over a 7-month period that covered both rainy and dry seasons. Matched patient urine and fingerprick blood samples were tested using the UMT, Binax NOW (Inverness) (HRP-2/pLDH) test, and microscopy. A total of 358 participants (18.9%) had confirmed malaria by microscopy; Fyodor UMT, 450 (23.8%); Binax NOW (pLDH), 386 (20.4%) and Binax NOW RDT (HRP-2), 731 (38.6%). Statistical data analyses to determine test performance characteristics are ongoing and will be made available within a month. The UMT has the potential of expanding access to malaria diagnosis especially in settings where blood test is not possible.

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MALARIA INFECTION IN CHILDREN AGED 0 TO 5 YEARS IN THE KASSENA NANKANA DISTRICT, NORTHEASTERN GHANA. A GROUP BASED TRAJECTORY ANALYSIS

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Information about progression of *P.falciparum* risk infection in children over time is limited. This study aimed to assess the pattern of infection events over time in a cohort of children under five years, and to explore the association to maternal factors such as education, ethnicity or preventive care behavior during pregnancy. A cohort study analysis of health surveys with on-site temperature, thick blood smear and hemoglobin measurements. Subjects were from Kassena-Nankana District of northern Ghana, who were previously enrolled at birth into a five-year prospective study. Assessment visits were done twice a year in high and low seasonal transmission periods. A Based Group Trajectory Analysis approached was used to allow identification of sub-groups over time, and a multinomial logistic regression to assess risk factors. A total of 2,107 children were recruited at birth, 4,892 events of infection were identified. Individuals belongs to three patterns of infection, a low-risk group "LoR" (47.5%) which had a prevalence of infection <20% over the whole observation, a intermediate risk "InR", ascending group (44.0%) which reached to 50%-60% of prevalence in the 3-4 years of age, and a high risk group "HiR" (8.5%) with a rapid ascending pattern reaching over 80% of prevalence by the third year of age. The risk of infection increased with less years of mother education especially in those under elementary school (InR OR 1.70: HiR: OR 3.10); and decrease with: each additional antenatal care visit (InR: OR 0.94; HiR: OR 0.80), use of bed net during pregnancy (InR: OR 0.69: HiR: OR 0.60), and receiving antimalarial drugs during pregnancy for the HiR group (OR 0.59). Populations are not homogenous in patterns of infection over time, subgroups can be identified based in social characteristics which may allow preventive health intervention in those individuals.

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ECONOMIC EVALUATION OF INTERVENTIONS TO IMPROVE HEALTH WORKERS' PRACTICE IN DIAGNOSING AND TREATING UNCOMPLICATED MALARIA IN CAMEROON

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Malaria rapid diagnostic tests (RDTs) are a valid alternative to malaria testing with microscopy and are recommended for testing of febrile patients before prescribing an antimalarial. There is the need for interventions to support the uptake of RDTs by health workers. This study evaluates the cost-effectiveness of introducing RDTs with basic or enhanced training in health facilities where microscopy was available, compared to current practice. A three-arm cluster randomized trial was conducted in 46 facilities in Centre and North-west Cameroon. Basic training had a practical session on RDTs and lectures on malaria treatment guidelines. Enhanced training included small-group activities designed to change health workers' practice and reduce consumption of antimalarials among test-negative patients. The primary outcome was the proportion of febrile patients correctly treated: febrile patients should be tested for malaria, artemisinin combination therapy should be prescribed for confirmed cases, and no antimalarial should be prescribed for patients who are test-negative. Individual patient data were obtained from facility records and an exit survey. Costs were estimated from a societal perspective using project reports and patient exit data. Results showed that the incremental cost per febrile patient correctly treated was $8.40 for basic and $3.71 for enhanced arms. Upon scale-up it was estimated RDTs with enhanced training would save $0.75 per additional febrile patient correctly treated. Introducing RDTs with enhanced training was more cost-effective than RDTs with basic training, when each was compared to current practice.

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IMPROVED TARGETING OF ANTIMALARIAL TREATMENT IN COMMUNITY-BASED MANAGEMENT OF MALARIA: EVIDENCE FROM CLUSTER-RANDOMIZED TRIALS IN UGANDA

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Universal access to diagnostic testing for malaria is now recommended by WHO, to encompass all levels of health care, including community-based treatment programmes. Rapid diagnostic tests (RDTs) provide a simple means of confirming malaria diagnosis in locations lacking electricity and qualified staff, however data on the impact of diagnostic testing on treatment and referral practices by community health workers remains limited. A cluster-randomised trial to evaluate the impact and cost-effectiveness of RDTs in community case management was conducted in two areas with contrasting malaria transmission in Rukungiri District, Uganda. A total of 120 communities [379 community medicine distributors (CMDs)], were randomised to training either in use of RDTs or presumptive diagnosis of malaria. All CMDs were trained on how to give antimalarial treatment with ACTs, rectal artesunate pre-referral treatment, and when to refer. Supporting interventions included activities to raise community awareness, and close support supervision to CMDs for the first six months of implementation, after which supervision was scaled back to mimic levels typically seen in health systems in rural Africa. Nonetheless, adherence to RDT results by CMDs remained high, with over 95% of ACT treatments given being consistent with the RDT test results. When treatment decisions by providers were validated by expert microscopy on a reference blood slide collected at the time of consultation, the proportion of patients receiving appropriately targeted treatment was significantly higher in villages where community health workers used RDTs, compared to presumptive treatment: 79% vs 31% ($p<0.001$) and 90% vs 8% ($p<0.001$) in the high and low transmission areas respectively. Data on the impact of RDTs on referral practices will also be presented. In conclusion, diagnostic testing with RDTs in community case management can reduce over-diagnosis and substantially increase the proportion of patients receiving appropriately targeted malaria treatment.

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DISPENSER PERFORMANCE ADMINISTERING MALARIA RAPID DIAGNOSTIC TESTS IN THE PRIVATE RETAIL SECTOR OF TANZANIA

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In 2010, the World Health Organization (WHO) recommended that all suspected malaria cases be tested. In Tanzania, the low confirmatory diagnosis rate (approximately 25% for children under 5) means that many febrile cases receive clinical diagnoses and potentially inappropriate treatment. While approximately 30% of febrile cases seek treatment in the private sector, the informal shops comprising 70% of this sector are not allowed to stock or perform malaria tests. We evaluated whether the gap in accessibility to testing could be addressed by allowing Accredited Drug Dispensing Outlet (ADDO) shop dispensers to sell and perform malaria rapid diagnostic tests (RDTs). Dispensers from 270 ADDOs were trained on RDT administration and safety in Morogoro Region in May 2013. If they passed the training, they were certified and allowed to purchase RDTs at a negotiated low or subsidized price. Certified dispensers were monitored quarterly from May 2013 to 2014. RDT safety, administration, and interpretation were assessed using a list of indicators adapted from the checklist published by the WHO in "Universal Access to Malaria Diagnostic Testing: an Operational Manual". Data on ADDOs' safety and hygiene were also collected. RDTs were stocked in 87% of enrolled shops at the first and second visits and 78% of shops at the third visit ($p=0.006$ for change in stocking across surveys). At least 83% of dispensers performed an RDT correctly on all of the 17 indicators. More than 98% of dispensers correctly interpreted results at each visit. Regarding ADDO's safety and hygiene practices, over 95% of ADDOs kept the area around the shop clean and free of used RDT products across surveys. However, the fraction performing the test in a private area decreased between the first and third visits from 31% to 19% ($p<0.001$ for change across surveys). Following training, ADDO dispensers competently stocked and safely administered RDTs, demonstrating that placing RDTs in certified private shops may be a feasible solution to increase malaria diagnostic access.

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FATTY ACID SYNTHESIS AND PYRUVATE METABOLISM PATHWAYS REMAIN ACTIVE IN DIHYDROARTEMISININ INDUCED DORMANT RING STAGES OF *PLASMODIUM FALCIPARUM*

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Artemisinin (ART) based combination therapy (ACT) is used as the first line treatment of uncomplicated falciparum malaria worldwide. However, despite high potency and rapid action there is a high rate of recrudescence associated with ART monotherapy and recrudescence is not uncommon even when ACT is used. This is independent of the recent observation of ART resistance. ART induced ring stage dormancy and recovery has been implicated as possible cause of recrudescence; however, little is known about the characteristics of dormant parasites including whether dormant parasites are metabolically active. We investigated the transcription of 12 genes encoding key enzymes in various metabolic pathways in *Plasmodium falciparum* during dihydroartemisinin (DHA) induced dormancy and recovery. Transcription analysis showed an immediate down regulation for 10 genes following exposure to DHA, but continued transcription of 2 genes in apicoplast and mitochondria. Transcription of several additional genes in apicoplast and mitochondria, particularly genes encoding enzymes in pyruvate metabolism and fatty acid synthesis pathways, were also maintained. Additions of inhibitors for biotin acetyl CoA carbozylase and enoyl-acyl carrier reductase of the fatty acid synthesis pathways delayed the recovery of dormant parasites by 6 and 4 days, respectively following DHA treatment. Our results demonstrate most metabolism is down regulated in DHA induced dormant parasites. In contrast fatty acid and pyruvate metabolic pathways remain active. These findings highlight new targets to interrupt recovery of parasites from ART-induced dormancy and to reduce the rate of recrudescence following ART treatment.

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EFFECT OF TRANSMISSION SETTING ON THE SULFADOXINE-PYRIMETHAMINE RESISTANT HAPLOTYPES AND SELECTIVE SWEEP CHARACTERISTICS IN MALAWI

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The decline of antimalarial resistant parasites, and the re- expansion of drug sensitive parasites, after the removal of drug pressure, raises the possibility that previously abandoned drugs might once again find clinical utility. Here we investigate the role of transmission setting on the dynamics of sulfadoxine-pyrimethamine (SP) resistance alleles and the characteristics of their associated selective sweeps. High transmission settings are associated with more clinically immune hosts who may serve as a reservoir for drug-sensitive parasites. High transmission intensity leads to higher parasite recombination, with greater genetic diversity. Samples from patients who presented to health care facilities and were found to have malaria were collected from three transmission sites in Malawi: urban (Ndirande), rural-high transmission (Chikwawa), and rural-low transmission (Thyolo). Pyrosequencing was used to determine the predominant haplotypes of dhfr and dhps in each infection. A ~96% prevalence of dhfr 51I/59R/108N, and ~96% prevalence of dhps 437G/540E was found at all three sites. There was no significant difference in haplotype prevalence found between any of the transmission settings, regardless of season. A single SP-sensitive parasite was found in Thyolo. Neutral and flanking microsatellite analysis was used to calculate expected heterozygosity (He) and estimate diversity ratios between the three sites. At markers flanking dhfr and dhps significant differences in He were found between Thyolo and Chikwawa and between Thyolo and Ndirande, however no significant difference in diversity ratios was found between Ndirande and Chikwawa for either dhfr or dhps. Our data indicate that the differences in transmission between these sites, given the high level of SP resistance, were not sufficient to effect change in haplotype prevalence. Differences in sweep characteristics between Thyolo and the other transmission settings will be pursued in future analyses..

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CHANGES IN OCCURRENCE OF DRUG RESISTANCE POLYMORPHISMS TO SULPHADOXINE - PYRIMETHAMINE IN THE *PLASMODIUM FALCIPARUM* IN SOUTHERN ZAMBIA

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Malaria remains one of the major global diseases affecting humans and drug resistance is a major concern for effective treatment. In Zambia, a change from Sulphadoxine Pyrimethamine to artemether lumefantrine as first line treatment for malaria was made in 2004 due to evidence of increasing levels of resistant parasites. However, it is still used for intermittent preventative treatment in pregnancy. Mutations in the dhfr and dhps genes are associated with resistance to pyrimethamine and sulphadoxine. *Plasmodium falciparum* antifolate drug resistance polymorphisms are detected using nested PCR and restriction enzyme digestion. This study aimed to determine and compare the prevelance of dhfr and dhps polymorphisms in a low endemic area at two time points 2008-2009 and 2012-2013. Finger-pricked blood samples (Dried Blood Spots) were collected on filter paper from 993 consenting participants from communities in Macha, Choma District between 2008 and 2009, and 1303 consenting participants between 2012 and 2013. Parasite DNA was extracted and a nested PCR run on these samples from which the positives were genotyped for dhfr and dhps mutations. Restriction enzyme digestion was done on the positives and restriction fragments analysed by gel electrophoresis and visualized under UV transillumination. The number of individuals with *P. falciparum* resistance mutations in the dhfr gene and dhps was 11 for 2008 and 2009 and 5 for 2012 and 2013 respectively. Malaria infections in Southern Zambia have declined due to combined interventions and efforts. The prevalence of mutations in the dhfr and dhps genes has decreased from 2009 to 2013, resistance alleles are still present in the general population. The impact of resistant parasites on the efficacy of SP for IPTp needs to be assessed.

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FORWARD GENETIC CHEMICAL PROFILING OF PIGGYBAC MUTANTS OF *PLASMODIUM FALCIPARUM* REVEALS NEW DRUG TARGETS AND INSIGHTS INTO MECHANISMS OF RESISTANCE TO ARTEMISININ

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The emergence and spread of *Plasmodium falciparum* multidrug resistance highlights the urgency to discover new targets and chemical scaffolds with reduced potential for emergence of resistance. Achievement of this goal will be enhanced by developing a better understanding of mechanisms of action and resistance of available drugs and inhibitors on vulnerable metabolic pathways. In this study we conducted a novel forward genetics, chemical profiling screen of 80 *P. falciparum* piggyBac (pB) mutants to

identify, validate and prioritize anti-malarial drugs, targets, and resistance mechanisms. Mutants carrying a single transposon insertion were profiled for altered responses to standard antimalarial drugs and inhibitors of known metabolic pathways. The results of the screen provide proof of concept and yield new insights into mechanism of action and resistance. For example we found that drugs targeting the same pathway have a significantly higher connectivity to each other than to drugs that inhibit other pathways. In addition we made novel observations about important drug resistance mechanisms. One of the mutants profiled in our study contains an insertion in the intergenic region between the kelch protein 13 (K13) gene implicated in artemisinin resistance (PF3D7_1343700) and a gene encoding a conserved protein of unknown function (PF3D7_134800). This mutant exhibited 2-7 fold increased susceptibility to artemisinin drugs and was one of 10 pB mutants that based upon hierarchical cluster analysis had similar enhanced responses to artemisinin and other inhibitors. We demonstrate that K13 and 3 other genes (PF3D7_0727100, PF3D7_0619800 and PF3D7_1126100) in its chemogenetic cluster are tightly linked in co-expression networks. This implies that these genes are functionally related to K13 and mediate *in vitro* artemisinin response. Our data demonstrate that chemical-genetic profiles can reveal unexpected drug relationships and connect them to gene functions, including hypothetical genes in the malaria parasite.

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NOVEL K13 PROPELLER (KELCH PROPELLER) MUTATIONS IN BANGLADESHI *PLASMODIUM FALCIPARUM* CLINICAL ISOLATES

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The K13 propeller protein of *Plasmodium falciparum* is associated with artemisinin resistance. K13 propeller is a kelch motif containing protein, related to KEAP1 protein of its human host. Its functional role in artemesinin resistance is unknown. On the other hand, few clinical studies carried out in Bangladesh have not concluded any case of artemisinin resistance. In the current study, *Plasmodium falciparum* positive blood samples (n=238) were collected from seven endemic districts of Khagrachari (n=131), Rangamati (n=3), Cox's Bazar (n=68), Bandarban (n=14), Mymensingh (n=2), Netrokona (n=9) and Moulvibazar (n=11) in Bangladesh. K13 was bidirectionally sequenced. ClustalW and Jalview analysis have revealed five different mutations present in these clinical isolates. Two of these were synonymous mutations originating from Mymansingh and Rangamati. A578S mutation was found in two different samples collected from Khagrachari district and W470C and Y604H were found in Rangamati. Mutations observed in this study are different to those reported from Cambodia. W470C and Y604H mutation are located in the highly conserved β-sheet structure. These mutations may alter the integrity of the sheet. A578S is located in the loop structure close to the C580Y mutation - the major one in Cambodia associated with treatment failure and resistance. We report several novel mutations in Bangladesh in the K13 gene associated with artemisinin resistance. Further clinical studies are required to confirm any relationship to delayed parasite clearance

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RAPID TURNOVER OF *PVMDR1* HAPLOTYPES IN *PLASMODIUM VIVAX* POPULATIONS

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Plasmodium vivax is the most widely distributed malaria parasite and causes a serious public health burden. The emergence of chloroquine (CQ) *P. vivax* resistance - the first line treatment in most of the world - represents a hurdle to malaria control. Due to difficulties to *in vitro P. vivax* culture systems, a data base of the mutations in target genes could help to set a baseline for CQ drug-resistance surveillance. For this purpose, 97 *P.vivax* blood samples collected from patients attending for PCR diagnosis at the Brazilian Ministry of Health Extra-Amazonian Malaria Reference Laboratory, between 2010 and 2013, were direct DNA sequencing from PCR products containing *pvmdr1* Y976F and F1076L SNPs. We observed that, between 2010 and 2012, the great majority of the 64 samples tested presented a single mutation, showing the FF (62 / 97%) or the FL (1 / 1.5%) profiles, while only one sample presented the FL double mutant (1.5%). Interestingly, this pattern of haplotypes inversely changed when samples collected in 2013, where analyzed: in this case all the samples presented double mutants showing the FL profile, and single mutations were not detected anymore. Thus, this first report showing the turnover of *pvmdr1* haplotypes in *P. vivax* parasites could supply a baseline to monitor *P. vivax* CQ-resistance. The turnover of *P. vivax* parasites may reflect the introduction of new parasites carrying *pvmdr1* alleles associated to drug-resistance by mosquitoes. To explain this finding additional studies on molecular epidemiology are required.

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EFFICACY OF ARTEMETHER-LUMEFANTRINE AND AMODIAQUINE-ARTESUNATE FOR TREATMENT OF UNCOMPLICATED MALARIA IN CHILDREN: RANDOMIZED CLINICAL TRIAL AT THREE SITES IN UGANDA

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Plasmodium falciparum resistance to artemisinin derivatives is emerging in Southeast Asia, and resistance to artemisinin partner drugs may be increasing. In Uganda, artemether-lumefantrine (AL) is the first-line therapy and artesunate-amodiaquine (AS/AQ) the alternative for the treatment of uncomplicated malaria. We are conducting a randomized, single-blinded trial comparing AL and AS/AQ to treat uncomplicated falciparum malaria at 3 sites in Uganda with different transmission intensities and a range of malaria intervention coverage. A total of 600 children aged 6-59 months will be enrolled (100 per treatment arm per site), randomized to treatment with AL or AS/AQ, and followed for 28 days. The primary outcome is the risk of treatment failure unadjusted and adjusted by genotyping at day 28. Recruitment and follow-up have been completed at two sites (Apac and Mubende), and are ongoing at the third site. No serious adverse events have been reported to date. Preliminary results show no early treatment failures. The uncorrected 28-day risk of treatment failure was significantly lower for children treated with AS/AQ than for those treated with AL at Apac (15.0% vs 31.2%; p = 0.008) and Mubende (33.6% vs 53.7%; p = 0.003). Two recrudescences were observed in Apac in the AL treatment arm compared to none in the AS/AQ arm (corrected: 2.1% vs. 0%; p = 0.16). Parasite clearance was rapid at Apac (37.0 vs. 37.6 hours; p = 0.74) and Mubende (42.6 vs. 39.4 hours; p = 0.06) in AS/AQ and AL treatment arms, respectively. Our results show that the corrected treatment success rates were not different and were very high for both AS/QA and AL. However, AS/AQ appeared to have a better prophylactic effect.

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PLASMODIUM FALCIPARUM GENOTYPES FROM GAMBIAN CHILDREN WHO FAILED TREATMENT WITH ARTEMETHER-LUMEFANTRINE; RECRUDESCENCE OR RE-INFECTIONS?

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The growing risk of resistance to artemisinin derivatives calls for regular monitoring of the efficacy of artemisinin-based combination therapy locally used in endemic countries. Artemether-lumefantrine, the first line ACT in the Gambia shows high but variable efficacy in sites across the country. We conducted a 28-day follow up study to investigate the consistency of this variability in public health facilities in the country. DNA was extracted from filter paper blood spots of eight participants with treatment failures (by microscopy) using the QIAmp DNA blood kit (Qiagen) with DNA concentrations determined on the nanodrop. Each isolate was genotyped by PCR amplification and analysis of MSP1 and MSP2 gene loci and amplified products separated by capillary electrophoresis. Band sizes were determined against a 50-800bp DNA ladder. Isolates were further analysed in duplicates in two sequential assays by MSP1 & MSP2 amplification with Dye labelled primers and fragment analysis employing the GenScan1200*LIZ* size marker for size calling. Fragment size analysis was done with GeneMarker software (Sofgenetic). PCR band and fragment sizes were compared between consecutive PCR runs and scored according to WHO/WWARN prescriptions for distinguishing recrudescence from re-infection. This is based on the presence of at least one PCR band with identical size between samples from different time points and band sizes scored to be similar within a sensitivity margin of ±5 basepairs. Five of the eight samples analysed showed presence of identical-size PCR fragments for MSP1 and MSP2 either by Qiaxcel capillary electrophoresis, Fluorescent based fragment analysis or both. Two samples did not yield *Plasmodium* specific amplification products for the Post Day 0 samples and hence remain undetermined, and one sample was likely a re-infection as all loci analysed between Day 0 and subsequent timepoints were different. Further analysis will help verify the genetic identities of the five samples that indicated possible treatment failure.

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THERAPEUTIC EFFICACY OF DIHYDROARTEMISININ-PIPERAQUINE FOR TREATING UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA AND K13-PROPELLER POLYMORPHISMS ALONG THE CHINA-MYANMAR BORDER

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The World Health Organization (WHO) recommends Artemisinin-based Combination Therapy (ACT) for treating uncomplicated *Plasmodium falciparum* malaria, but resistance to artemisinin derivatives in Southeast Asia threatens malaria control and elimination activities worldwide. Mutations within a *P. falciparum* kelch protein, K13, are associated with both *in vitro* and clinical measures of artemisinin-resistant malaria. Validation and mapping of this marker throughout the malaria endemic world will be helpful to track the emergence and spread of artemisinin resistance. Yunnan, China's only province with endemic *P. falciparum* malaria transmission, borders Myanmar, Vietnam and Laos, and is the key focus of the national malaria elimination program. Patients from two sites (Yingjiang and Tengchong) bordering Myanmar received antimalarial treatment with either dihydroartemisinin-piperaquine or artesunate by directly observed therapy as part of therapeutic efficacy studies conducted from 2009 until the present. After a 28 or 42-day follow-up, treatment efficacy was estimated according to the WHO protocol for assessing and monitoring antimalarial drug efficacy. The *P. falciparum* K13 gene was sequenced in samples collected from approximately400 patients including almost 120 clinical trial participants, using Sanger sequencing. The prevalence of K13 mutations was estimated by study site and year. Linear regression was used to assess the association between K13 mutations and parasite clearance half-life, while adjusting for confounding variables. Haplotype networks of SNPs surrounding the K13 gene were used to assess origins of K13 mutations. Preliminary results indicate a high prevalence (34%) of the K13 F446I mutation, which was significantly associated with the presence of parasitemia 72 hours after treatment. These results suggest that K13 mutations are responsible for artemisinin resistance in Yunnan Province, China, although the predominant K13 mutation is different than in other areas of Southeast Asia, suggesting independent emergence rather than spread of resistance.

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A BETWEEN-HOSTS AND WITHIN-HOST COMBINED MODELING FRAMEWORK FOR THE EVOLUTION OF RESISTANCE TO ANTIMALARIAL DRUGS

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The emergence and spread of drug resistance in *Plasmodium* parasites has long been and is still a significant problem for chemotherapeutic approaches to malaria control. Evolution of drug resistance in mosquito-borne parasites is a complex process, influenced by transmission dynamics between hosts and vectors as well as within-hosts competition among parasite strains. We present here a theoretical model of the evolution of drug resistance that combines both between-host and within-hosts scales, aiming to understand how the dynamics at each level and the interaction between scales influences drug resistance evolution. Our model combines an epidemiological model of malaria transmission between hosts and vectors and a within-host model of the course of infection for multiple competing strains. The latter includes the effects of immunity, treatment and cost of resistance. The epidemiological level can reflect various epidemiological settings and transmission intensities. The model shows that in high transmission environments, where co-circulation of sensitive and resistant strains is more frequent, resistant strains are less likely to spread, particularly when within-host costs of resistance are high. We illustrate how treatment impacts the spread of resistance, and show a general trade-off between disease prevalence reduction and resistance management. We show however that treatment coverage has a stronger impact on disease prevalence, whereas treatment efficacy primarily affects resistance control. We conclude therefore that a primary focus on coverage over efficacy would have the strongest impact on disease control while minimizing selection for resistance. More generally we underline the importance of modeling the evolution of drug resistance across biological scales for a better understanding of the evolutionary dynamics in a variety of eco-epidemiological settings, providing valuable insights for both disease control and drug resistance management.

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EFFICACY, SAFETY AND PHARMACOKINETICS OF ARTEMETHER-LUMEFANTRINE FOR THE TREATMENT OF UNCOMPLICATED MALARIA IN HIV-POSITIVE ADULTS RECEIVING FIRST-LINE ANTIRETROVIRALS IN MUHEZA, TANZANIA

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Although there is concern of drug interactions between artemisinin-based combination treatment (ACTs) for malaria and antiretrovirals (ARVs) for treating HIV/AIDS, very limited information is available about the clinical importance of such interactions. The aim of this study was to examine the efficacy and safety of artemethemter-lumefantrine (AL), the most commonly used ACT, for the treatment of uncomplicated falciparum-malaria in HIV-positive adults recieving first-line ARVs in Tanzania. The InterACT Study was conducted from July 2009 to September 2012 at Muheza District Hospital in northern Tanzania. HIV-positive adults (>15 years of age) receiving either nevirapine- or efavirenz-based ARVs were enrolled and followed-up for 42 days using WHO standard protocols. Three additional groups of patients were included for comparison: 1) HIV-positive malaria patients not receiving ARVs but treated with AL, 2) HIV-negative malaria patients treated with AL, and 3) HIV-positive patients receiving ARVs but without malaria. Blood levels of lumefantrine were measured on day 7 in all patients receiving AL. A total of 17,269 patients were screened for malaria, amongst whom 385 HIV-positive patients with confirmed malaria were enrolled into the study and followed-up successfully for 42 days. The therapeutic efficacy of AL after parasite PCR-correction was 99% in HIV-positive patients receiving ARVs (total n=193; 106 on nevirapine, 87 on efavirenz), 100% in HIV-positive patients not on ARVs (n=43) and 98% in HIV-negative patients (n=149). Rates of malaria re-infection within 42 days of AL treatment was low and did not differ significantly between the groups. Mild adverse events were commonly recorded in all four patient groups. Severe adverse events were more commonly observed in HIV-positive versus HIV-negative patients, regardless of receiving ARV treatment or not. Day 7 levels of lumefantrine were found to be elevated in patients receiving nevirapine, but were reduced in patients receiving efavirenz. Our results confirm the presence of drug interactions between AL and nevirapine- and efavirenz-based ARVs; however, these interactions were not found to be clinically significant. Our findings thus support the current treatment guidelines for malaria and HIV co-infection in adults.

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VALIDATION OF A TOOL FOR PREDICTING ANTI-MALARIAL DRUG MECHANISM OF ACTION

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Malaria mortality has decreased over the last decade, but these gains are threatened by the emergence of drug resistance to artemisinin in South East Asia. There remains an urgent need to develop new classes of effective anti-malarial drugs. High-throughput screens have identified many drugs and drug-like compounds with anti-malarial activity. However, the mechanisms of action of these potential new drugs as well as for many established anti-malarial drugs is unknown or poorly understood. Systems biology tools that could predict the mechanisms of action of candidate drugs would be valuable for prioritizing drugs for further development. Work in our lab indicates that drug perturbations of *Plasmodium falciparum* provoke discernible transcriptional signals. These signals contain information about the pathway(s) that a drug targets and can be used to relate drugs by the extent to which their targets overlap. To validate and expand on our capacity to detect subtle drug-specific response signatures in the face of broad biological and experimental variation, transcription profiles were generated for parasites in which the purported pathway targets are genetically disrupted by *piggyBac* transposon insertions. Transcriptional profiles of a drug-perturbed parent line were compared to lines carrying a genetic perturbation of a related target. We find that genes that are differentially expressed in each genetic perturbation relative to the wild-type control are significantly enriched for biological pathways related to the perturbed genes. Transcriptional signals obtained from drug perturbations of a given pathway overlap with signals observed in genetic perturbations of the same pathway, indicating that a genome wide effort to catalog and relate drug target(s) by their transcriptional response profiles is feasible.

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REFINING A TOOL TO PREDICT ANTIMALARIAL DRUG MECHANISMS OF ACTION

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High-throughput screens have identified many drugs and small molecules with antimalarial activity, usually with no knowledge of their molecular targets. Cost effective prioritization of these potential drugs would be valuable to avoid pathways that have already developed drug resistance and to highlight compounds with novel mechanisms of action. We measured gene expression profiles in *Plasmodium falciparum* to construct a transcriptional response database for 31 drug perturbations. New drugs of interest can be queried against these gene expression patterns, identifying shared targets by similar response signatures. So far, successfully identifying drug-specific response signals has required that each gene's value be normalized across many drug perturbations from the same experiment to compute a drug's specific 'response index' from myriad other experimental and biological sources of transcription variation. However, this is a cumbersome task, and we are therefore exploring modifications to our approach that minimize experimental complexity while still filtering out nonspecific responses. We find that conventional, one drug vs control experiments with replication do not allow for normalization of nonspecific drug responses; consequently, we developed new protocols that can leverage existing data to specify a generalized stress response signature to be used as a standard normalization with each candidate drug. Deeper analysis of the 31 drug response gene expression profiles identified a small subset of drugs with highly diverse mechanisms of action. Normalization with this panel allowed removal of nonspecific culture and perturbation stress and accurate prediction of drug mechanism of action. By optimizing this analysis, we hope to build a standardized community web-based search tool for predicting drug mechanism of action.

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PREVALENCE OF THE DHFR AND DHPS MUTATIONS AMONG PREGNANT WOMEN IN RURAL BURKINA FASO SEVEN YEARS AFTER THE INTRODUCTION OF INTERMITTENT PREVENTIVE TREATMENT WITH SULFADOXINE-PYRIMETHAMINE

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The spread of drug resistance is one of the major challenges for malaria control in endemic areas. Intermittent Preventive Treatment of malaria in pregnancy (IPTp) with Sulfadoxine-pyrimethamine (SP) is currently recommended by the World Health Organization for preventing the adverse effects of malaria during pregnancy in mothers and their offsprings. In order to assess the evolution of SP resistance in Burkina Faso, we analyzed the prevalence of dihydropteroate synthase (dhps) and dihydrofolate reductase (dhfr) mutations among pregnant women in samples collected in 2010 and compared our results with retrospective data from 2003. Asymptomatic and symptomatic pregnant women attending Antenal Clinics (ANC) in Nanoro District were invited to participate in the study. All enrolled women were interviewed and examined clinically before taking a blood smear for microscopy examination and a filter paper blood sample for genotyping. Mutations at codons 51, 59, 108 and 164 of the Pfdhfr gene, and at codons 437 and 540 of the Pfdhps gene were examined using PCR-RFLP. Logistic regression was used to calculate odds ratios and confidence intervals for the comparison between 2003 and 2010. The dhfr and dhps genes were successfully genotyped in most samples, respectively 99.6% (255/256) and 90.2% (231/256). The dhfr C59R mutation was the most prevalent (61.2%=156/255) followed by the S108N mutation (55.7%=142/255). No isolate had the I164L mutation. For the Pfdhps gene more than one third of the samples had the A437G mutation (34.2%=79/231), while none carried the K540E mutation. The dhfr double and triple mutations were found in 36.5% and 11.4% of the isolates, respectively. Compared to 2003, the prevalence of the A437G mutation was significantly lower [OR=0.1 (0.1-0.3)]), and double and triple dhfr mutations were slightly less frequent in 2010 than in 2003 (13.2 and 16.8%). Mutations in the Pfdhfr and Pfdhps genes associated with resistance to SP were relatively common among pregnant women in the study area. Nevertheless, the prevalence of the triple dhfr mutation was very low suggesting that SP may be still efficacious for IPTp.

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CHARACTERIZATION OF GENETIC POLYMORPHISMS RELATED TO ANTIMALARIAL PHENOTYPES IN *PLASMODIUM FALCIPARUM* SAMPLES COLLECTED IN BRAZIL

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Plasmodium falciparum has shown a major ability to evolve resistance to nearly all antimalarials. Monitoring candidate mutations of drug resistance and clarifying their role in treatment outcome may contribute to optimizing malaria control measures. This study aimed to analyze resistance-associated genes over a twenty-seven year period. *P. falciparum* samples collected from 1984 to 2011 from patients enrolled at Brazilian health facilities were assessed. The infections were mainly from South American and African countries. All South American samples harbored the 76T mutation in the *pfcrt* gene, in agreement with their chloroquine-resistant *in vitro* response; African samples presented the wild type and mutant alleles. With regards to the *pfmdr1* gene, the emergence of the 86Y mutant was observed in the last decade in South American samples, whilst African samples presented both mutant and wild type alleles. Analysis of the 1246 codon revealed a mutant frequency of 100% in South American samples and the wild type variant in African samples. As to the *pfdhfr* gene, 100% of Brazilian samples presented the 51I and 108N mutants. The 59R mutant was not observed in the period of 1980-1990 but occurred in 22.6% of samples from 2000-2010. The 437G mutant of the *pfdhps* gene was observed in 100% of Brazilian samples in all decades and the 540E mutant decreased in the period 2000-2010 when compared to 1980-1990. In relation to artemisinin (ART) resistance candidate mutations, DNA sequence analysis of the *pfATPase6* gene showed previously described mutations. Two novel mutations were observed in the *pfAP2-μ* gene. A new molecular marker for ART resistance (Kelch-13 propeller) is being sequenced in several isolates. No association between the polymorphisms studied and *in vivo* or *in vitro* responses to mefloquine, quinine and ART derivatives was observed. This study established the genetic profile of *P. falciparum* regarding resistance-associated mutations over twenty-seven years, when the parasite was exposed to the selective pressure of several therapeutic schemes adopted in Brazil.

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PARASITE CLEARANCE TIME AND *IN VITRO* SENSITIVITY OF *PLASMODIUM FALCIPARUM* TO ARTEMETHER-LUMEFANTRINE IN SOTUBA, MALI

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In Mali, previous studies show high frequency (30-40%) of recurrent parasite after treatment with artemether-lumefantrine. We hypothesize that recurent parasites after treatment have a lower sensitivity to artemether-lumefantrine. We conducted a longitudinal study with 250 field isolates. *In vitro* field isolates sensitivity to Artemether and Lumefantrine was assessed using hypoxanthine isotopic test. A total of 250 *P. falciparum* isolates were successfully cultured *in vitro* and the sensitivities of 25% are available to date. All of the isolates tested *in vitro* were 100% sensitive to Artemether and Lumefantrine. The mean IC_{50} values are 3.08 nM and 3.54 nM for Artemether and Lumefantrine, respectively. Malian field *Plasmodium falciparum* isolates are sensitive *in vitro* to Artemether and lumefantrine.

903

THE POLYMORPHISMS IN KELCH AND FALCIPAIN-2 ASSOCIATED WITH ARTEMISININ RESISTANCE ARE NOT PREVALENT IN *PLASMODIUM FALCIPARUM* ISOLATED FROM UGANDAN CHILDREN

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Artemisinin resistance, manifested as delayed clearance of *Plasmodium falciparum* following treatment with artemisinins, has emerged in Southeast Asia. SNPs in the *PF3D7_1343700* kelch propeller (K13) domain were recently associated with artemisinin resistance after *in vitro* resistance selection and in clinical isolates, providing a molecular marker to monitor the spread of resistance. The cysteine protease falcipain-2

(FP2; *PF3D7_1115700*) contributes to artemisinin action via hemoglobin degradation, and it also was mutated after *in vitro* selection with artemisinin. Although delayed parasite clearance after artemisinin therapy has not yet been noted and artemisinin-based combination therapy remains highly efficacious in Uganda, it was important to characterize the diversity of these genes. We therefore sequenced the K13-propeller domain and FP2 genes in 104 samples collected in 2011-2012 from Ugandan children with malaria emerging after recent exposure to ACTs for treatment (within 28 days of treatment with artemether/lumefantrine) or chemoprevention (monthly DHA/piperaquine). Using 3D7 as the reference genome, we identified polymorphisms resulting in 5 amino acid substitutions in the K13 gene, none of which were among the markers of resistance seen in Asian isolates. No single SNP was found in more than 2 isolates. For FP2, we identified polymorphisms resulting in amino acid substitutions at 29 loci, 17 in the pro and 12 in the mature domain of the protease; these did not include the SNP reported after *in vitro* selection for resistance. The prevalence of K13 and FP2 polymorphisms did not increase over time, and no SNPs were associated with malaria episodes in which parasite clearance was relatively delayed (persistence ≥2 days after the onset of treatment). These results indicate that the K13-propeller and FP2 coding polymorphisms associated with artemisinin resistance are not prevalent in Uganda. Thus, we see no evidence of artemisinin resistance in Ugandan parasites at present, but continued surveillance for resistance-mediating genotypes is warranted.

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RELATIONSHIPS BETWEEN K13-PROPELLER ALLELES AND ARTEMISININ SUSCEPTIBILITY IN CAMBODIAN AND SENEGALESE *PLASMODIUM FALCIPARUM* ISOLATES

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Plasmodium falciparum resistance to artemisinin and its derivatives (ART) has emerged in Southeast Asia. ART resistance manifests *in vivo* as a long parasite clearance half-life after ART monotherapy or ART-based combination therapy (ACT), and *in vitro* as increased survival of young ring-stage parasites in the ring-stage survival assay ($RSA^{0\text{-}3h}$). In Cambodia, several mutations in the PF3D7_1343700 kelch propeller domain (K13-propeller) were recently associated with ART resistance *in vitro* and *in vivo* (Ariey *et al*., 2014). To investigate the role of these and other K13-propeller mutations in ART resistance, and to screen for emerging ART resistance in Africa, we have initiated studies of Cambodian and Senegalese parasite isolates. In collaboration with the Tracking Resistance to Artemisinins Collaboration (TRAC), we are presently adapting Western Cambodian isolates (half-life range, 1.7 – 11.8 h) to *in-vitro* culture, PCR re-sequencing their K13-propeller domains, and testing them in the $RSA^{0\text{-}3h}$ as reported previously. Preliminary data from this study have identified parasites carrying the C580Y, Y493H, R539T, I543T, and D584V mutations, and have associated these K13-propeller mutations with long half-life and elevated % survival values in the $RSA^{0\text{-}3h}$. Among Senegalese samples, we have identified a parasite isolate carrying a novel K13-propeller V637I mutation which has not yet been observed in Southeast Asia. We are now developing genotyping and sequencing assays to screen for these and other K13-propeller mutations in Africa.

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TRENDS IN ANTIMALARIAL MEDICINE AND MALARIA DIAGNOSTIC AVAILABILITY IN CAMBODIA BETWEEN 2009 AND 2013

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The advancement of artemisinin resistance (AR) in the Greater Mekong Subregion threatens the international community's ability to combat malaria. Ensuring consistent and accurate diagnostic testing, better access to artemisinin-based combination therapy (ACT), and removal of oral artemisinin monotherapy (oAMT) are key activities to contain AR. As such, a thorough understanding of the anti-malarial market is a pre-condition for guiding containment efforts. In 2009, 2011 and 2013, ACTwatch conducted nationally representative outlet surveys in Cambodia. Data from the 2013 survey is currently being analyzed and will be presented at the ASTMH conference. Preliminary results indicate that among outlets stocking antimalarials, nearly all public sector outlets had ACT available and very few had OAMT available in 2009 (ACT: 96%, OAMT: 2%), 2011 (ACT: 97%, OAMT: 0%) and 2013 (ACT: 99%, OAMT: 0%). In comparison, antimalarial availability has been less stable in private sector outlets stocking antimalarials, with an increase in ACT availability (63% to 83%) and a decrease in OAMT availability (20 to <1%) between 2009 and 2013. The percentage of outlets stocking rapid diagnostic tests (RDTs) has increased by 20% in public sector outlets (75% to 95%) and 26% in private sector outlets (37% to 63%) between 2009 and 2013. Logistic regression will be used to compare the rate of change for antimalarial and malaria diagnostic availability between outlet types, urban/rural status and artemisinin tolerance zones. Additional analysis will be performed to identify factors associated with changes in ACT, OAMT and RDT availability in private sector outlets in order to better understand the improved private sector market profile. Several interventions are being implemented in Cambodia to contain artemisinin resistance. The current study demonstrates increasing ACT and decreasing OAMT availability in the private sector, suggesting that the regulation of antimalarial medicine sales and other AR containment efforts may have been successful in improving the antimalarial market profile.

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POOLED SEQUENCING OF MALARIA PARASITES FOR IDENTIFICATION OF DRUG RESISTANCE GENES

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The rapid spread of drug resistance genes through malaria parasite populations distorts the allele frequencies of flanking mutations. Such "selective sweeps" can be rapidly uncovered by pooled population sequencing, but this approach has yet to be applied to malaria parasites. We explored the potential of pooled sequencing to swiftly and economically identify selective sweeps due to emerging artemisinin (ART) resistance in a South-East Asian malaria parasite population. ART resistance is defined by slow parasite clearance from the blood of ART-treated patients and mutations in the kelch gene (chr. 13) have been strongly implicated to play a role. We constructed triplicate pools of 70 slow-clearing (resistant) and 70 fast-clearing (sensitive) infections collected from the Thai-Myanmar border and sequenced these to high (~150-fold) read depth in an Illumina HiSeq lane. Allele frequency estimates from pools

showed almost perfect correlation (Lin's concordance = 0.98) with allele frequencies at 93 SNPs measured directly from individual infections, giving us confidence in the accuracy of this approach. By mapping genome-wide divergence (FST) between pools of drug resistant and drug sensitive parasites we identified three large (>150kb) regions (on chrs. 11, 13 and 14) and 18 smaller candidate genome regions. To identify individual genes within these genome regions we re-sequenced an additional 38 individual parasite genomes (22 slow and 16 fast-clearing) and performed rare variant association tests. These confirmed kelch as a major molecular marker for ART resistance (p=6.03x10-6), and provide suggestive associations for the involvement of several other genes. This two-tier approach is powerful because pooled sequencing rapidly narrows down genome regions of interest, while targeted rare variant association testing within these regions can pinpoint the genetic basis of resistance.

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SEQUENTIAL EVOLUTION OF DRUG RESISTANCE: EVIDENCE THAT EPIGENETIC REGULATION PRECEDES GENETIC ADAPTATION IN THE ACQUISITION OF HALOFUGINONE RESISTANCE

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Understanding mechanisms of drug resistance is essential for the design of future antimalarial therapies and rational design of drug combinations. To understand the multi-step nature of drug resistance acquisition in *Plasmodium falciparum*, we performed metagenomic sequencing of six time points in two *in vitro* drug resistance selections over 60 generations. For these experiments we used the *P. falciparum* cytoplasmic prolyl tRNA synthetase (cPRS) inhibitor halofuginone. We found that non-genetic adaptation to halofuginone precedes mutation or amplification of the target cPRS gene. Part of this non-genetic adaptation occurs through regulation of cellular amino acid homeostasis. Upon exposure to halofuginone, *P. falciparum* increases cytosolic proline and overexpresses PSAC determinants cytoadherence-linked asexual gene (clag) 2 and clag 3.2. Using an allelic exchange approach, we found that both cPRS halofuginone resistance mutations HFGRI (L482H) and HFGRII (L482F) only confer halofuginone resistance when clag genes are also overexpressed. Furthermore, by tracking the evolution of two drug resistance selections by whole genome sequencing, we demonstrate that the cPRS locus accounts for the majority of genetic adaptation to halofuginone in *P. falciparum*. Thus, we provide evidence for a three-step model of multi-locus evolution of drug resistance via genetic and non-genetic adaptations in *P. falciparum*: first, non-genetic adaptation predominates and permits later acquisition of target-site mutations; second, either target-site mutations or amplifications develop; and third, wild type target-site amplifications take over and out compete less fit target-site mutations. This sequential model of drug resistance evolution has greater implications for malaria drug-resistance surveillance and combination drug development.

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A COLLECTION OF CLONED PARASITES FROM *PLASMODIUM FALCIPARUM* INFECTIONS SHOWING SLOW OR FAST CLEARANCE FOLLOWING ARTEMISININ TREATMENT

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A collection of laboratory adapted parasites with carefully defined clearance rates following artemisinin (ART) treatment would provide a valuable resource for understanding the mechanism of ART-resistance, the role of kelch, the involvement of other loci and for understanding the costs of resistance. We therefore dilution-cloned 45 infections from hyper parasitaemia patients visiting clinics run by the Shoklo Malaria Research Unit on the Thailand-Myanmar border (2008-12) that showed either very slow (T1/2 > 5) or very fast clearance (T1/2 < 3), determined by 6 hourly measures of parasite density following ART-treatment. The clones isolated were genotyped using 93 polymorphic SNPs to verify their matches to the original infections. Clones identical to the original infection were obtained from 33/45 infections. These 33 genotype-verified parasite clones from a single location, and from the extremes of the clearance rate distribution, will be made available to the research community.

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RESISTANCE MARKER PREVALENCE IN *PLASMODIUM FALCIPARUM* IN RESPONSE TO RECENT INTRODUCTION OF AL ANTIMALARIAL DRUG

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Due to increasing drug resistance in the malaria parasite *Plasmodium falciparum*, chloroquine (CQ) was replaced by sulphadoxine-pyrimethamine (SP) in 1998 as first-line treatment for uncomplicated malaria in Kenya. However, after less than a decade following the policy changes, artemisinin-based combination therapies (ACTs) have replaced SP as the most effective anti-malarial option due to widespread SP-resistance. In 2006, the new malaria policy implemented artemether-lumefantrine (AL) as the first-line treatment for uncomplicated malaria and quinine for complicated and severe malaria. Monitoring and understanding the genetic factors underpinning drug resistance is imperative to maintaining effective antimalarial drug administration policies. This study aims to examine the resistance marker prevalence of *P. falciparum* and determine if there is a transition toward widespread *P. falciparum* resistance to the recent introduction of AL as compared to previously used drugs in Western Kenya. Sequences of five candidate genes were compared among *P. falciparum* samples to examine targeted mutations associated with resistance. They are (1) the chloroquine resistant transporter (crt) and multidrug resistance 1 (mdr1) genes for CQ/quinine resistance; (2) the dihydropteroate synthase (dhps) and dihydrofolate reductase (dhfr) genes for SP resistance; and (3) the sarco/endoplasmic reticulum Ca2+-ATPase (atp6) gene for AL resistance. Two comparisons were made to investigate the level of selective pressure on the various resistance genes. The first was between high and low transmission areas. In areas of high malaria transmission, we expect that selective pressure could be greater in parasite populations due to frequent use of drugs. This would be reflected by a higher frequency of the selected codon as compared to parasite populations in low transmission areas. The second compared different resistance genes based on parasite samples from the same area. This comparison allows us to infer the degree of resistance based on genetic

information with respect to different antimalarial drugs. Despite its recent implementation in Western Kenya, AL has already demonstrated reduced efficiency in treating falciparum-malaria in other parts of East Africa.

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PERSISTENT RESISTANCE TO DISCONTINUED ANTIMALARIAL AGENTS IN *PLASMODIUM FALCIPARUM* CLONES OF SOUTHWEST INDIA

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Malaria control in India comes with significant challenges, including variations in accuracy of differential diagnosis, the frequent co-occurrence of *Plasmodium falciparum* and *P. vivax*, varying drug-treatment preferences for the different species in different localities, and migrations of people across the sub-continent. The impact of this, both on the emergence as well as persistence of drug resistance in this environment, is of interest. As a part of the US NIH International Center of Excellence for Malaria Research (ICEMR) program activities, we have collected and culture-adapted malaria parasites at Goa Medical College, Bambolim, Goa. This is a tertiary care center with patients presenting uncomplicated as well as severe malaria. This site is of particular interest because Goa stopped using chloroquine for *P. falciparum* treatment, as national policy favored artemesinin-derived combinations about seven years ago. Five years ago, Goa stopped using the nationally recommended first line artesunate-pyrimethamine-sulphadoxin combinations to treat *P. falciparum* in favor of artesunate-mefloquine. Given the well-known fitness costs of chloroquine-resistance and fansidar-resistance, we expected emergence of sensitivity to these traditional antimalarials. Our studies from 2012-2014, based on DNA sequencing of drug-resistance markers as well as direct phenotypic assays of freshly cultured parasites, point to persistent and frequent occurrence of chloroquine and fansidar resistance. This suggests that there must be unintended but sustained drug pressure from old antimalarials on modern parasite populations in this locality. So far, resistance is not seen to mefloquine and artesunate, the preferred drug combination in Goa. Similar background surveys are planned across multiple ICEMR sites across India, with patient parasite samples collected over a period of five years.

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MONITORING ANTIMALARIAL DRUG RESISTANT PARASITES IN WESTERN KENYA

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Artemether-lumefantrine was implemented as first-line therapy for treatment of uncomplicated *Plasmodium falciparum* malaria in Kenya in late 2006. Amodiaquine-artesunate is registered as an alternative treatment. *In vitro* drug sensitivity assay and molecular surveillance for markers associated with resistance are useful surrogates to monitor trends in the susceptibility to *P. falciparum* parasites to anti-malarial drugs. We report findings from a study conducted in hospitals in western Kenya between 2010 and 2013. A total of 204 samples were enrolled. *In vitro* sensitivity to chloroquine (CQ), mefloquine (MQ), amodiaquine (AQ), artesunate (ART) and dihydroartemisinin (DHA) was determined. Samples were also genotyped for single nucleotide polymorphisms (SNPs) associated with CQ, MQ, AQ, and sulphadoxine-pyrimethamine (SP) resistance. No significant changes in the mean IC_{50} response to ART or DHA were observed during this study period. A significant decline in the prevalence of the CQ resistant CVIET genotype and the *Pfmdr-1* 86Y mutation (both associated with CQ and AQ resistance), was observed between 2010 and 2013. A decrease in the mean IC_{50} to these drugs was also observed. No increase in the copy numbers of the *Pfmdr-1*gene and mean MQ $IC_{50,}$ both associated with MQ resistance, was observed. On the contrary, the prevalence of parasites with the combined *Pfdhfr/Pfdhps* quintuple mutation (51I/ 59R/108N and 437G/540E), associated with SP resistance, was above 90%. The rare 164L and 581G mutations were observed in 4/204 and 3/204 samples, respectively and a 2.3% increase in the prevalence of the previously described 436H mutation was recorded during this study period. The prevalence of the *Pfdhps* 436H/ 437G/ 540E genotype is on the rise in this region of East Africa. Further microsatellite analysis around the *Pfdhps* gene demonstrates that it is under selection. In conclusion, the *in vitro* drug sensitivity pattern for ART and DHA is consistent with *in vivo* drug trials data conducted by other groups in this region indicating that artemisinin-based combination therapies are efficacious in western Kenya. CQ sensitive strains are increasing, likely due to decreased use of CQ. High prevalence of SP resistant quintuple mutations in this population is an important concern, because SP remains the only recommended drug for intermittent preventive treatment in pregnant women.

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EVALUATION OF DIHYDROARTEMISININ-PIPERAQUINE WITH AND WITHOUT SINGLE-DOSE PRIMAQUINE: AN OPEN-LABEL RANDOMIZED, CONTROLLED TRIAL IN ANLONG VENG, CAMBODIA

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Evidence of dihydroartemisinin-piperaquine (DP) resistance has recently emerged outside of western Cambodia. We are currently evaluating the safety and therapeutic efficacy of DP for uncomplicated *Plasmodium falciparum* or mixed malaria infections, as well as its transmission blocking potential in combination with single dose primaquine (PQ) in northern Cambodia. Up to 150 volunteers, including those with mild-moderate G6PD deficiency, are being enrolled in a randomized, open-label clinical trial of 3 consecutive daily oral doses of 120/960mg of DP with or without a single 45mg dose of PQ. Transmission blocking is assessed with a mosquito membrane-feeding assay while cardiac safety is evaluated with serial electrocardiograms and time-matched drug levels. In the 107 patients completing follow-up to date, the 42-day per protocol PCR-unadjusted failure rate was slightly higher (45%) that the previously reported PCR-adjusted rate of (36%) among the first 50 volunteers. Mean piperaquine drug levels in the terminal elimination phase were lower than mean *in vitro* parasite piperaquine IC50 among treatment failures. Mean piperaquine QT-interval prolongation was lower (428ms) than those we previously reported with a compressed 2-day course of therapy (455ms) at Cmax (4 hours after the last dose). Gametocytemia among P.f.-infected patients was uncommon, though limited data suggests that 45mg single-dose primaquine was effective in preventing transmission based on oocyst counts. DHA-piperaquine is rapidly failing in Northern Cambodia, though single dose primaquine (45mg) may help to halt the spread of resistant parasites and should be implemented where adequate safety assessment is

feasible. The standard 3 day course of DP appears to carry a lower cardiac safety risk than a 2 day course and is consistent with previously reported safety data.

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DIHYDROARTEMISININ-PIPERAQUINE TREATMENT FAILURES IN *PLASMODIUM FALCIPARUM* IN NORTHERN CAMBODIA ARE NOT MEDIATED BY KNOWN ACT RESISTANCE MARKERS

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Dihydroartemsinin-piperaquine (DP), one of the last remaining effective drugs for multidrug resistant *Plasmodium falciparum,* has been the first line artemisinin combination therapy (ACT) in Cambodia since 2012. However, clinical trials in northern Cambodia in 2010 and 2013 show rising rates of DP failure, reaching a 42-day PCR-corrected recrudescence rate greater than 40%. With nearly 60% of patients remaining parasitemic at 72 hours and increasing piperaquine IC_{50}s, treatment failures are likely due to decreased susceptibility to both artemisinin and the partner drug. We are investigating whether these failures are associated with polymorphisms in known or candidate molecular markers of antimalarial resistance. Isolates from approximately 140 Cambodian patients with *P. falciparum* infections treated with DP are being analyzed. Preliminary analysis of over half of the isolates shows fixation of the CVIET haplotype at *pfcrt*, near fixation of the 184F mutant (96%) in *pfmdr1*, and wild type alleles only at codons 86, 1034, 1042, and 1246 of *pfmdr1*. Increased *Pfmdr1* copy number (CN) is present in 14% of isolates and is associated with greater *in vitro* susceptibility to piperaquine (mean PPQ IC50 18.3nM vs. 36.7nM in samples with CN>1 and CN≤1, respectively, p=0.02). Sequencing of the recently described kelch propeller gene (K13) associated with artemisinin resistance reveals that, as in western Cambodia, mutant K13 alleles are highly prevalent, with only 7.5% showing the wild type allele. Finally, we measured CN of the Xr5 locus, where a duplication on Chr 5 has been associated with *in vitro* piperaquine resistance in drug-pressured parasites. Using digital droplet PCR, we found no increase in CN at four genes within the Xr5 domain. In sum, none of the molecular markers analyzed correlate with DP treatment failure in these patients. These findings suggest that escalating ACT resistance in northern Cambodia is incompletely described by current molecular markers. Research to elucidate as yet undiscovered mechanisms of DP resistance are urgently needed for molecular surveillance worldwide.

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RESIDUAL MALARIA INFECTIONS IN PRE-ELIMINATION SCENARIOS: HOW DOES DETECTABILITY RELATE TO PREVALENCE?

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Recent empirical and modeling studies have implicitly made conflicting claims about the relationship between *Plasmodium falciparum* malaria parasite densities in humans and transmission intensities, and about the consequences for the importance of sensitivity of diagnostic methods to detect blood stage parasitemia. Evidence that there are disproportionate numbers of sub-patent infections at low transmission appears to be at variance with suggestions that parasites are more likely to lead to clinical attacks at low levels of naturally acquired immunity. An existing mathematical model, parameterized using parasite densities from malaria therapy patients, was used to simulate a successful program to reduce *P. falciparum* transmission. The relationships between prevalence and parasite densities were further investigated in field data. The simulations suggested that after halting transmission, the proportion of infections detectable by standard diagnostic tests will initially drop, but then increase as acquired immunity wanes. In field data the relationship between parasite densities and prevalence of patent parasitemia can vary over short distances in time and space, reflecting transmission heterogeneity and seasonality. In pre-elimination transmission landscapes, cases are indicative of local active transmission. Conversely, areas with a disproportionate number of sub-patent infections are likely to represent sink areas where these lingering old infections have little vector contact, and contribute little to transmission. Hence, detection of these infections is of little relevance for elimination programs. The use of more sensitive diagnostic methods that lower the limit of detection of asexual blood stage parasitemia would not address the key limitations of mass screening and treating as a strategy for long-term reduction in malaria transmission. These limitations are the short term impact of the intervention, and inefficiency owing to lack of targeting in space and time of infections that are particularly likely to transmit.

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COSTS OF ELIMINATING MALARIA AND THE IMPACT OF THE GLOBAL FUND IN 34 COUNTRIES

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International financing for malaria increased more than 18-fold between 2000 and 2011; the largest source came from The Global Fund to Fight AIDS, Tuberculosis and Malaria (Global Fund). Countries have made great progress, but achieving elimination requires sustained financial resources to interrupt transmission and prevent reintroduction. Since 2011, global financing for malaria has declined, fueling concerns that progress in reducing malaria may be impeded, especially for the 34 malaria-eliminating countries that face a particular risk of malaria resurgence if programs are disrupted. This study aims to 1) assess past total and Global Fund funding to the 34 malaria-eliminating countries, and 2) estimate future funding needs to achieve malaria elimination and prevent reintroduction through 2030 in the 34 countries. Historical funding is assessed against trends in country-level malaria annual parasite incidences (APIs) and income per capita. Following Kizewski et al. (2007), program costs to eliminate malaria and prevent reintroduction through 2030 are estimated using a deterministic model. The cost parameters are tailored to a package of interventions aimed at malaria elimination and prevention of reintroduction. The majority of Global Fund-supported countries experiencing increases in total funding from 2005 to 2010 coincided with reductions in malaria APIs and also overall GNI per capita average annual growth. The total amount of projected funding needed to achieve elimination and prevent reintroduction through 2030 is approximately US$8.5 billion, or about $1.84 per person at risk per year (PPY) (ranging from $2.51 PPY in 2014 to $1.43 PPY in 2030). Although external donor funding, particularly from the Global Fund, has been key for many malaria-eliminating countries, sustained and sufficient financing is critical for furthering global malaria elimination. Projected estimates of costs through elimination should help countries identify funding gaps and mobilize resources to obtain adequate financing to achieve their goals.

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A CASE STUDY OF MALARIA ELIMINATION IN THE ECUADOR-PERÚ BORDER REGION

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In recent years malaria has been successfully controlled in the Ecuador-Peru coastal border region. The incidence of disease declined from a high of 2,117 per 100,000 in 1999 in El Oro Province, of southern Ecuador, to 0 as of 2011. The rate declined concomitantly in Piura Province of northern Peru during this same period. Our aim is to document this control effort and identify the best practices and lessons learned that are broadly applicable to malaria control and other vector borne diseases. We conducted a proximal outcome evaluation of the robust elimination program in Ecuador, using El Oro Province as a case study. We conducted semi-structured group discussions with public health experts who played central roles in the elimination effort, reviewed epidemiological records by the Ministry of Health, and reviewed national policy documents to produce a detailed timeline of events, a list of the crucial programmatic and external factors of success and the barriers faced, and to identify the important lessons learned. We found that the binational Ecuador-Peru collaboration was the most important component of the elimination program. This unique relationship created a trusting, open environment, allowing for flexibility, rapid response, innovation and resilience in times of crisis, and ultimately a sustainable control program. Strong community involvement, an extensive microscopy network and horizontal, intersectoral collaborations at the local level were also identified as key programmatic strategies. The results of this study provide key principles of a successful malaria control program that can be looked to by other nations and regions currently working to control malaria infection. These principles should be disseminated to the next generation of public health professionals in the region and serve as a guide to ongoing and future control efforts of other emerging vector borne diseases in this region and elsewhere.

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TOWARDS MALARIA ELIMINATION IN MPUMALANGA, SOUTH AFRICA: A METAPOPULATION MODELING APPROACH

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Mpumalanga in South Africa is committed to eliminating malaria by 2018 and efforts are increasing beyond that necessary for malaria control. Malaria elimination strategies may be unsuccessful if they focus only on the biology of vector and parasite, and ignore the mobility patterns of humans, particularly in Mpumalanga where the majority of infections are imported. A metapopulation model is developed to assess the impact of proposed elimination-focused policy interventions in Mpumalanga. This is the first study designed for this purpose in Mpumalanga. A stochastic non-linear ordinary differential equation model fitted to Mpumalanga and Maputo (Mozambique) malaria data, is used to predict the impact of the scale-up of vector control, mass drug administration, a focused mass screen and treat campaign and foreign source reduction. Scaling up vector control is predicted to lead to a minimal reduction in local infections and mass drug administration and screening and treating at the Mpumalanga-Maputo border is predicted to be impacting but short-lived. Source reduction in Maputo is predicted to generate large reductions in local infections through stemming the flow of imported infections. The model also predicts that if malaria were to be eliminated in Maputo, malaria would also be eliminated in Mpumalanga, highlighting the need for and importance of regional collaboration. To eliminate malaria by 2018, the government of South Africa will need to design and implement an elimination strategy tailored for a country with a high level of imported infections. A regionally focused strategy may stand a better chance at achieving elimination in Mpumalanga and South Africa compared to a nationally focused one in the face of high visitation rates from neighbours in higher transmission areas.

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ACTIVE CASE DETECTION OF MALARIA AND URINARY SCHISTOSOMIASIS IN PUPILS OF KOTTO BAROMBI, SOUTHWEST CAMEROON USING THE CYSCOPE® FLUORESCENCE MICROSCOPE

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Malaria and urinary schistosomiasis (U.S.) remain important public health issues in Kotto Barombi, South-west Cameroon even though the village benefits from free distribution of treatment of both diseases by health authorities. Accurate diagnosis and prompt treatment of malaria and U.S. are necessary for their elimination. Novel tools for rapid and mass diagnosis of malaria and other parasitic infections have recently been developed. This study was aimed at assessing their use in the field to determine the prevalence of malaria and U.S. in Kotto Barombi. Blood and urine samples were collected from 94 boys and 122 girls aged 3 to 14 years. Malaria and U.S. were diagnosed with a rapid test using pre-stained slides for fluorescence microscopy (CyScope®, Partec GmbH, Goerlitz, Germany). Furthermore, Malaria parasite detection and speciation were done using Giemsa-stained blood films. Lugol direct examination was performed for U.S. Performance characteristics of CyScope® for malaria and U.S. were determined. Overall prevalence of malaria was 19.0% and 41.2% for light and fluorescence microscopy respectively (X2=15.33, p-value=0.00019). Malaria prevalence and density were similar in the different age groups, sexes, socio-economic class (SEC) and nutritional class. Overall prevalence of anaemia was 18.5%. Sensitivity was 68.3% and specificity was 64.9% for malaria. Overall prevalence of U.S. was 43.4% and 48.5% for light and fluorescence microscopy respectively. Many cases of co-infection (19.9%) were recorded. Mean intensity of US was 8.1±27.02. Prevalence and intensity of U.S. were significantly higher in the Kotto Barombi Island (78.3%, 33/42) than mainland (33.8%, 52/154). U.S. prevalence was similar in the different age groups, sexes and SEC. Sensitivity of the test was 90.6% and specificity was 83.8%. The CyScope® could be a good tool for active case detection of both diseases especially in areas that lack electricity since it can be battery operated. Drastic measures need to be taken for the elimination of these diseases.

919

IDENTIFICATION OF THE MOST EFFICIENT INTERVENTION PACKAGES ACROSS DIFFERENT EPIDEMIOLOGICAL STRATA: AN APPLICATION TO *PLASMODIUM FALCIPARUM* MALARIA TRANSMISSION AND MORBIDITY IN AFRICA

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Reducing the burden of major endemic infectious diseases is a global priority, but financial constraints require available resources to be allocated rationally to maximise impact. This poses particular challenges

for infections such as malaria, where transmission intensity exhibits a high level of spatiotemporal variability. Here we incorporate financial data into a mathematical model of malaria transmission to evaluate the most efficient (minimum cost) combinations of interventions to achieve disease and transmission milestones across sub-Saharan Africa. We make the optimization problem computationally tractable by classifying each geographic location into a finite set of epidemiological strata according to the average intensity of transmission, pattern of seasonality in transmission and vector species mix. We show that the optimal set of interventions can vary substantially depending on whether disease reduction or elimination of infection is the primary goal. Our analysis indicates that malaria elimination is possible using existing interventions in 64% of the area of sub-Saharan Africa in which P.falciparum malaria is endemic, representing 63% of the population at risk, but that the optimal combination of interventions required shows substantial geographic variation at a range of spatial resolutions. By considering how the degree of this variation differs at different administrative scales, we show the extent to which the additional complexities of implementing more localised control strategies are offset by the fewer resources they require to achieve elimination. Our results provide a rational framework for the global health community to consider the feasibility, cost and resource requirements of different targets for malaria control over the coming decades.

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MATING COMPETITIVENESS OF STERILE MALE *ANOPHELES COLUZZII* IN SEMI-FIELD CAGES

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The renewed interest in the development of control strategies using sterile insects raises hopes of being able to control the disease by cutting down the high reproductive rate of mosquitoes. Specifically, investigations into factors that account for male mating competitiveness are critical to the development of genetic control strategies. In this study, we assessed the effects of partial irradiation with 75 Gy on *Anopheles coluzzii* sexual competitiveness when allowed to mate in different ratios of sterile/fertile males for 2 nights in field cages. Moreover, to determine the dynamics of this competition, competitiveness was compared between males allowed 1 night vs 2 nights of contact with females. Sterilized (S) and fertile (U) males between 4 and 6 days of age were released in field cages (1.70mx1.70mx1.70m) with females (F) of similar age and left for 2 nights at the following ratios (S:U:F): (100:0:100) (100:100:100) (300:100:100) (500:100:100) (0:100:100). Each treatment was replicated 3 times. Competitiveness was determined by assessing the hatching rate of eggs laid en masse and the insemination rate, determined by dissecting recaptured females. An additional experiment with a ratio of (500:100:100) has been done with a mating period of either 1 or 2 nights. For the first experiment, the egg hatching rate was significantly affected by the release ratio and we thus observed that the Fried competitiveness index of sterile males was between 0.29 and 0.55. A similar insemination rate was recorded after 2 nights of contact in experiment 1, while significant difference was observed in the (S:U:F) (100:0:100) ratio between the males left to mate for 1 and 2 nights. However, a similar hatching rate was observed when mating occurred for 1 or 2 nights. The results suggest a release ratio of at least 2 sterile males for 1 fertile male and that *An. coluzzii* mating competitiveness experiments in field cages should be run for 1 instead of 2 nights.

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EVALUATION OF THE NATIONAL MALARIA SURVEILLANCE SYSTEM OF BHUTAN, 2006-2012: A CASE STUDY

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Once a leading cause of morbidity and mortality in Bhutan, malaria has drastically reduced following aggressive control efforts over the last two decades. Bhutan is now embarking on malaria elimination. We examine the role of surveillance in Bhutan's pivot to elimination, assessing the ability of the current malaria surveillance system to meet the objectives of the Bhutan Vector-borne Disease Control Programme (VDCP), highlighting the priorities of the surveillance system as the nation transitions into an era of elimination, and identifying areas that require attention for this goal to be achieved. An evaluation of the national malaria surveillance system of Bhutan from 2006 to 2012 was conducted using the Center for Disease Control guidelines for evaluating public health surveillance systems. In addition, a formal assessment of blood slide quality assurance and control in 2011 was performed and data will be presented. From 2006-2012, *Plasmodium vivax* accounted for one half to two thirds of infections, depending on the district location. The national malaria surveillance system in Bhutan was found to be reasonably flexible, representative, simple, and stable. The quality of data produced is good, but its usefulness and interpretative insight could be improved by the computation of additional assessment measures. Timeliness could be improved by telephone reporting and increased health worker training and accountability is needed going forward. Nationally, non-residents comprised between 6.2% (in 2010) and 22.6% (in 2012) of all cases. Thus, more rigorous case identification and investigation will facilitate targeted interventions, while more attention is required to address re-introductions of infections through migrant workers and cross-border prevention initiatives in the coming years. Currently, the malaria surveillance system of Bhutan generates data that is useful and of good quality, but the pivot to elimination will require system function enhancement focus and intensify malaria prevention efforts.

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SHIFTING THE BURDEN OR EXPANDING ACCESS TO CARE? ASSESSING MALARIA INCIDENCE TRENDS FOLLOWING SCALE-UP OF COMMUNITY HEALTH WORKER MALARIA CASE MANAGEMENT IN SOUTHERN PROVINCE, ZAMBIA

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Malaria infections constitute a large proportion of outpatient services in malaria endemic areas. Health centers are often understaffed, and the frequency of malaria infections contributes to a decreased quality of care for non-malaria illnesses. Health centers in rural areas can be difficult to access; distance traveled to a health center is often cited as a reason why individuals do not seek treatment for an illness. Zambia has trained community health workers to diagnose and treat malaria using rapid diagnostic tests and artemether-lumefantrine. Community health workers (CHW) operate out of their home or a nearby health post located within the community. Has this extension of malaria services shifted the burden of care from the health center to the community health worker, or has it simply expanded the access to care within the community? Improvements in the reporting of malaria at health centers and from CHWs provide robust health facility data. We used random effects regression to estimate trends in the outcome of outpatient attendance and the outcome of

health facility malaria incidence before and after the scale-up of malaria diagnostic and treatment services at the community level in Southern Province, Zambia. We controlled for environmental drivers of malaria incidence using remotely sensed variables, and adjusted for temporal autocorrelation. After accounting for environmental factors, implementing CHW case management of malaria was associated with an 8.2% (95% CI = 4.1% - 12.2%) reduction of outpatient attendance at the health center. When combining the additional malaria cases actively and passively identified through CHW case management with monthly confirmed health center incidence, implementation of CHW case management was associated with a 45.5% increase in monthly confirmed malaria incidence (95% CI = 33.5% - 58.6%). The implementation of CHW case management has both shifted the burden of outpatient care away from the health center into the communities as well as expanded the access to malaria treatment.

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REDUCING MALARIA AMONG MIGRANTS AND MOBILE WORKERS IN THE GREATER MEKONG SUB-REGION BY BROADENING OPPORTUNITIES FOR MALARIA SERVICES AND PREVENTION

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Migrant and mobile populations are a difficult to reach population and often coined a 'hot population' in malaria control programs in the Greater Mekong Sub-region. Engaging in construction and agricultural production in remote areas where malaria is common, these workers often lack malaria knowledge or immunity, and information on and access to preventive measures and curative services.They could serve as a source of infection introducing malaria to historically low- or non-transmission areas. The PMI Control and Prevention of Malaria (CAP-Malaria) Project has implemented innovative approaches to reach migrant and mobile populations in remote border areas of Thailand, Cambodia, and Myanmar where malaria is concentrated. The project utilizes a multi-pronged approach that provides malaria information and services at multiple points that migrants typically visit or pass through while in the region and returning home. For instance, malaria information is provided by transportation services taking migrants to their employment destinations. Migrants may also obtain information and services during arrival or departure at malaria posts located at border crossings or border malaria clinics. Mobile malaria workers (community volunteers) and mobile clinics with medical staff frequent locations with high concentrations of migrants. Large multinational companies as well as smaller agricultural employers in both Cambodia, Thailand and Myanmar are participating in a lending scheme through which migrants can borrow a long-lasting insecticidal net (LLIN) for the duration of their employment. In Cambodia, a radio show reaches residents and mobile populations in remote areas, providing malaria information and promoting a malaria hotline for one-on-one malaria discussions. Engagement of taxi and bus drivers in providing information on malaria prevention and health seeking behavior are vital for mobile and migrant populations prior to reaching highly malaria endemic zones. During the past 2 years, the project has reached over 50,000 migrant workers in Cambodia, 439,345 in Myanmar, and 3,600 in Thailand.

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USING INCIDENT MALARIA CASES TO FIND ASYMPTOMATIC MALARIA INFECTIONS IN SOUTHERN PROVINCE, ZAMBIA: WHICH CASES INCREASE THE PROBABILITY OF FINDING INFECTIONS?

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As malaria transmission declines it becomes heterogeneous, characterized by pockets of sustained transmission. Finding and removing the pockets of sustained malaria transmission is of utmost importance to malaria elimination programs. Incident malaria cases may be indicative of malaria transmission, and returning to the household of incident malaria cases may be one way in which to target pockets of residual malaria transmission. In Southern Province, Zambia reactive case detection (RCD) has expanded to included >1500 community health workers (CHWs) operating out of >260 health centers. CHWs return to the household of a rapid-diagnostic test (RDT) confirmed incident malaria case and screens individuals within 140 meters for a malaria infection using RDTs, treating those positive. Detailed records of each reaction have been kept by CHWs in Southern Province, and are currently being digitized for further data analysis. These records contain information on the incident malaria case demographics as well as the demographics of individuals screened during reactions. Preliminary analyses of aggregated RCD data suggest that RDT positivity during reactions exceeds RDT positivity during passive case surveillance more often in the dry season, when mosquito habitat as measured through remotely sensed enhanced vegetation index is limited, and when the ambient temperature as measured through remotely sensed land surface temperature is lower. These results suggest that incident malaria cases during the dry season may be more indicative of pockets of asymptomatic malaria infections than incident malaria cases during the wet season. Further analyses with the digitized CHW records will investigate what effect different demographics of incident malaria case affect the ability to find pockets of asymptomatic malaria infections using each individual reaction as the unit of analysis. A more robust analysis of the environmental factors affecting the probability to find pockets of asymptomatic malaria infections will also be conducted. Full analyses will be completed by June 2014.

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SPATIO-TEMPORAL CHARACTERISTICS OF MALARIA INCIDENCE IN LUSAKA, ZAMBIA: TOWARDS MALARIA ELIMINATION IN AN URBAN ENVIRONMENT

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Malaria elimination may be more feasible from within urban areas than rural areas, due to differences in *Anopheles* habitat. Lusaka, Zambia has documented zero malaria infections in children under the age of five in Malaria Indicator Surveys conducted in 2010 and 2012 suggesting elimination of malaria transmission from within Lusaka is near. However, health facilities in Lusaka during this time period continue to record confirmed malaria incidence. Enhanced surveillance of incident malaria cases in Lusaka, Zambia was initiated in 2011. All confirmed incident malaria cases are questioned about history of travel outside Lusaka and previous malaria episodes. Case investigations are undertaken for incident malaria cases without a history of travel, wherein all members of the 9 adjacent households are screened with a rapid diagnostic test

and treated with artemether-lumefantrine if positive. We describe the temporal characteristics of health facility malaria incidence with and without a history of travel in relation to remotely sensed estimates of mosquito habitat. We use spatial scan statistics to estimate the location of spatial clusters of health facility malaria incidence without a history of travel within the catchment areas of 5 health centers in Lusaka using randomly generated population-weighted controls. Eighty percent of confirmed incident malaria cases report a history of travel outside Lusaka in the previous month, with travel being most common in December and January. Incidence rates of malaria without a history of travel follow the suspected seasonal pattern and are highly correlated with those of malaria reporting a history of travel (rho = 0.82). Significant clusters of malaria incident cases were found within each health center catchment area assessed. In all but 1 of the catchment areas, greater than 75% of incident cases without travel were located within clusters. The heterogeneous spatial pattern of malaria incidence in Lusaka is clear - identifying and targeting clusters of malaria transmission will be critical in the pursuit of elimination.

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IMPLEMENTATION AND APPLICATION OF A MULTIPLEX ASSAY TO DETECT MALARIA ANTIBODIES: A PROMISING TOOL FOR ASSESSING MALARIA TRANSMISSION IN PRE-ELIMINATION AREAS

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In countries that move towards pre-elimination, malaria is concentrated in hotspots. To identify these hotspots for targeted and effective interventions it is crucial to improve the understanding of the effectiveness of malaria control tools. However, in areas with a low prevalence and incidence of malaria, such as Cambodia, detection of parasitological indicators is very insensitive. Seroprevalence is based on the detection of antibodies against antigens of malaria parasites, which offers an advantage as anti-*Plasmodium* antibodies can persist for months after infection. Therefore, a multiplex assay has been recently developed for the simultaneous detection of multiple antibodies. An assay based on 14 *Plasmodium* specific peptides, 1 peptide specific for *Anopheles gambiae* saliva protein and 5 *Plasmodium* specific recombinant proteins was developed for the MAGPIX system and applied on blood spots from 2000 individuals collected in the Ratanakiri province, Cambodia. This project fits within the framework of a research project that aims to evaluate the effect of mass use of safe and effective mosquito repellents on the malaria transmission, in addition to the use of impregnated mosquito nets (MalaResT). For all antigens the assay performed equally well in monoplex and in multiplex formats ($p<0.001$). High specific antibody levels and a high seropositivity were detected for antibodies against Pf-LSA3-RE, Pf-CSP, Pf MSP1-19, Pf-GLURP, Pf-SALSA2 and Pf-GLURP-R2 with the comparison between the different serological markers. Blood samples positive for malaria by PCR showed a higher response to some of the antigens as compared to PCR negative samples. An increase in seropositivity was observed with increasing age. Differences in seropositivity were observed between different districts, indicating the heterogeneity of malaria transmission within the most endemic province of Cambodia.

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MALARIA ELIMINATION - NOT YET ACCOMPLISHED IN ZANZIBAR

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Zanzibar represents a unique case study for potential malaria elimination in a previously high endemic area in sub-Saharan Africa. The Zanzibar Malaria Elimination Programme (ZAMEP) has implemented modern malaria control interventions since 2003. These interventions included combined vector control with long lasting insecticidal nets (LLIN) and indoor residual spraying (IRS) as well as improved malaria case management with rapid diagnostic tests (RDT) and artemisinin-based combination therapies (ACT) in all public health facilities. We have studied temporal trends of different malaria indices such as incidence of parasitologically confirmed malaria infections in public health facilities, crude child mortality and community parasite prevalence in two districts of Zanzibar, North A and Micheweni, between 1999 and 2013 in a population of about 200,000 people. Malaria transmission declined already after introduction of ACT and dropped further after addition of intensified vector control. Health facility data showed a 96% reduction in positivity rates (from 38.2% to 1.6%) of parasitologically confirmed malaria cases in the two districts between 2002 and 2012 with a relative shift towards older age groups and an increasing seasonability of the malaria incidence. In North A district, all cause <5 years mortality rate decreased by 70%. A population survey in 2013 revealed a malaria prevalence of 0.3% determined by RDT representing a 97% reduction compared to 2003 (10.3%). However, the parasite prevalence as determined by PCR was 10-fold higher, indicating a remaining reservoir of asymptomatic low-density parasitemias. Moreover, signs of minor increase of reported malaria infections from public health facilities occurred in Micheweni district in 2012 compared with 2010-2011. Malaria control in Zanzibar has reached a level equivalent to malaria pre-elimination. However, a new malaria epidemiological context has emerged necessitating new tools and strategies as well as re-orientations of ongoing control activities in order to further reduce malaria transmission.

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STOCKS AND SHOPS: THE IMPORTANCE OF HEALTH SYSTEMS STRENGTHENING WITHIN PRIVATE SECTOR OUTLETS FOR THE DELIVERY OF ARTEMISININ COMBINATION THERAPIES (ACTS) TOWARDS MALARIA CONTROL AND ELIMINATION

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There remains debate regarding the inclusion of Informal sources of antimalarials, ranging from drug shops and pharmacies to general village stores, within national malaria control planning. The Affordable Medicines facility for malaria (AMFm) has been effective in increasing stocks of subsidised quality-assured Artemisinin Combination Therapies

(ACTs) within drug shops but the impact of this initiative remains unclear. We extended an existing mathematical model of malaria transmission to include health systems factors: 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 interventions in the private sector on malaria mortality and parasite prevalence (i.e. transmission risk). We compared the effect in three settings, which differed by epidemiology and prevailing health systems. Model outcomes suggest in areas where there is high private sector preference, strengthening of public facilities has less impact than anticipated. Improving stocks of ACTs in the private outlets, as per AMFm, was predicted to be more effective than in public clinics in all three regions considered, with a greater relative impact on parasite prevalence than mortality; likely due to high levels of NMFI overtreatment thereby opportunistically treating asymptomatic and sub-patent infections. Modelling the rollout of diagnostic led therapy in drug shops reduced missed cases and thus mortality without increasing prevalence, but only if adequate stocks of ACTs were present. At low and medium transmission settings, pharmacy accreditation schemes to limit the diversity of informal private sources, and improve ACT stock and dispensing practices may have the potential to interrupt transmission, reducing parasite prevalence by up to 86% in scenarios with private sector preference. However investment would be required across the spectrum of case management to improve the quality of care delivered. Innovative methods need to be found to harness the private sector cost-effectively in the push for control and elimination.

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IDENTIFYING AND CHARACTERIZING ASYMPTOMATIC MALARIA INFECTIONS IN ZANZIBAR: THE RESIDUAL PARASITE RESERVOIR

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Asymptomatic *Plasmodium* infections are an important reservoir for continued malaria transmission and must be targeted to achieve malaria elimination. Sensitive molecular methods have shown that asymptomatic infections are often of very low-density in low endemic settings, frequently falling below the detection limit of both rapid diagnostic tests (RDT) and microscopy. This study aimed to identify and characterise asymptomatic low-density malaria infections in Zanzibar by active case detection employing community-based screening for *Plasmodium* infections by real-time PCR. Dried blood spots on filter paper (Whatman 3mm) were collected from asymptomatic individuals participating in cross-sectional surveys conducted in 2005, 2009, 2011 and 2013. For each respective year 548, 2324, 2978 and 3038 samples were screened for parasite DNA using a highly sensitive Cytochrome B SYBR green real-time PCR for Pan-*Plasmodium* detection and restriction fragment length polymorphism assay for species determination. The PCR determined asymptomatic parasite prevalence was 25.7% (CI95% 21.7-29.0), 3.3% (2.6-4.0), 2.2% (1.6-2.8) and 2.3% (1.7-2.8) in 2005, 2009, 2011 and 2013, respectively. The corresponding yearly microscopy/RDT positivity rates were 10.9% (CI95% 9.2-12.7), 0.0% (0-0.3), 0.7% (0.4-1.3) and 0.3% (0.13-0.54). Children 5-15 years and young adults 15-25 years had higher prevalence of asymptomatic malaria (range 2.7-52% and 3.7-13.7%, respectively) as compared to children <5 years and adults (range 0.5-24.5% and 1.7-8.6%). *P. falciparum* remained the predominant species (2.0-25.4%) followed by P. malariae (0.3-3.1%); no cases of P. ovale or *P. vivax* were identified. Although the asymptomatic malaria prevalence has declined since 2005, this study revealed a substantial remaining reservoir of low-density, sub-microscopy/RDT but PCR detectable parasitaemias among asymptomatic individuals in Zanzibar. The results highlight the need for sensitive molecular methods for identifying and targeting the residual parasite reservoir in malaria elimination programmes.

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CLONAL OUTBREAK OF *PLASMODIUM FALCIPARUM* IN EASTERN PANAMA

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Reemerging parasite populations threaten to undermine efforts of global malaria eradication. Identifying the source of resurgent parasites is therefore paramount to strategic and successful intervention for malaria elimination. Genetic tools such as the malaria barcode to "fingerprint" parasite linages are anticipated to help identify sources of parasite outbreaks and track their spread. Panama has been notably successful in reduction of *Plasmodium falciparum* with only one reported case in 2011. Although malaria incidence in Panama has remained low over the past two decades, a major outbreak from 2001 to 2005 resulted in more than 60 percent of all cases reported over the past 35 years. We hypothesized that parasites from this epidemic might be highly related genetically and exhibit a largely clonal population structure. We thus tested the relatedness of *Plasmodium falciparum* parasites from this outbreak using informative single nucleotide polymorphisms and also examined drug resistance loci from among these parasites. We found the parasites to be clustered into three clonal subpopulations (AMOVA, 96%, $p = 0.001$) among eastern Panamanian isolates and shared genetic relatedness with parasites sampled from Colombia. Structure analysis revealed there was most likely two populations ($\Delta K = 108$), however given the clone-corrected clusters and AMOVA results, we found the second most likely number clusters ($\Delta K = 61$) revealed additional sub-population structure. Two clusters of Panamanian parasites from the epidemic shared identical drug resistance haplotypes, and all clusters shared a chloroquine resistance genotype matching the pfcrt haplotype found among parasites of Colombian origin. Our findings suggest these resurgent parasite populations are highly clonal and likely resulted from epidemic expansion of imported or vestigial cases. Outbreak investigation using genetic tools can illuminate the relatedness and potential sources of epidemic malaria cases and guide strategies to prevent further resurgence in areas of malaria elimination.

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THE AGRICULTURE - MALARIA RELATIONSHIP AND THE CONTRIBUTIONS OF DELOS LEWIS VAN DINE TO THE MALARIA PROBLEM IN THE SOUTHERN U.S.

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Human malaria has a strong association with agricultural activity. Increased food production via cultivation and irrigation often increases malaria risk in malaria-endemic zones, creating a conflict between agricultural enterprises and health. Conversely, malaria stultifies agricultural productivity by causing illness in laborers at critical times. This presentation highlights the efforts of USDA entomologist Delos Lewis Van Dine to quantify the impact of malaria on cotton production in Louisiana in 1914 and argues that it is was the first effort to connect malaria and agriculture fiscally. The study, conducted on the Hecla Plantation in the Mississippi delta, showed high malaria prevalence in black tenant farmers beholden to white plantation owners and that lost profits of $3.98 per acre could be attributed to malaria. It further demonstrated that impoundment of bayou waters and

water level manipulation eliminated *Anopheles* breeding near tenant farmers' shacks. Overall, this poorly known but seminal project provides an early, effective demonstration of intersectoral malaria control well before drugs and insecticides were available and that investment from the agricultural sector was key to malaria reduction.

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SUBMICROSCOPIC PARASITEMIA IN RURAL AND URBAN SOUTHWESTERN NIGERIA

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Epidemiology of submicroscopic parasitemia will become more relevant in determining malaria control measures in the era of elimination. This study evaluates the performance of microscopy and qPCR in detecting and quantitating malaria parasites in an urban and a rural sites in South West Nigeria.Cross-sectional surveys were conducted in hospital laboratories in rural Ijede and urban Lekki, spanning the period 2008-2010. Malaria parasite detection and quantitation using microscopy and qPCR were compared for these two sites. Prevalence at the urban site was 29% (330/1158) by PCR and 11%(111/997) by microscopy while at rural Ijede corresponding values were 30.2% (197/652) and 25%(168/658) respectively. The differences between the prevalence values at both sites were not significant by PCR (χ2=0.52, P=0.47) but very significant by microscopy (χ2=57.6, P<0.0001). Overall composite sensitivity for microscopy(41%) was lower compared to PCR (95%) while their respective specificities were 98% and 95%. At individual study sites, sensitivity of microscopy was better at the rural site with 98% than at the urban site 30% and specificities at both sites were 99% and 98% respectively. Sub-microscopic parasitaemia at the rural site was less, 74/197(37.5%), compared with the urban 211/336(62.2%), (χ2=30.34, P<0.0001). This was reflected in variance in their geometric mean parasite density(GMPD) observed-6999p/µL at the rural, and 2701p/µL at the urban sites by microscopy and corresponding values of 4693 p/µL and 1100 p/µL by qPCR (Cohen's Kappa agreement 0.48, Spearmans rank's correlation coefficient was 0.5, CI 0.37-0.59, mean difference of log units in the Bland Altman plot of 0.87; and 95% limits of agreement(mean ± 2SDs) between the two methods, 1.5 to 3.1.In spite of apparent lower prevalence of malaria parasites by microscopy in rural Ijede compared to urban Lekki, the actual prevalence when PCR technique is employed shows that parasite prevalence are similar in both study sites. Result from this study highlights a possible role for more sensitive diagnostic method in urban and other apparent regions of diminished parasitemia load and prevalence, over rural settings.

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ON THE ESTIMATION OF MALARIA TRANSMISSION INTENSITY USING SEROLOGICAL DATA: POWER CALCULATIONS AND ESTIMATION BIAS

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Parasite prevalence and entomological infection rate are two popular measures of malaria burden in a given population. An alternative measure is the so-called sero-conversion rate that can be estimated from sero-prevalence data of specific anti-parasite antibodies in the serum using a series of reverse catalytic models. In theory, sero-conversion rate is the frequency by which individuals convert from a sero-negative state into a sero-positive one upon parasite challenge. In practice, sero-conversion rate has proven to be strongly correlated with the underlying force of infection, suggesting its routine use in malaria epidemiology. Statistically speaking, little is known about the expected behavior of the corresponding estimates under different estimation methods, sampling schemes, and disease dynamics. This knowledge is particularly instrumental in helping the design of future field studies. In this work we perform a comprehensive theoretical study using simulated data from typical cross-sectional, health-facility and school surveys. Specifically, we generate sero-prevalence data assuming either a constant sero-conversion rate over time or assuming a change in it after a field intervention. We assess the underlying estimation bias and statistical power in order to calculate the minimum sample size required to (1) estimate sero-conversion rate with a given precision, (2) or to detect any change in disease transmission after an intervention with a given probability. We also suggest different statistical strategies to reduce estimation bias introduced by using convenience sampling or the maximum likelihood estimation method. Finally, we present a new R package for sero-prevalence data analysis that can be easily used by malaria epidemiologists.

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THE POTENTIAL TO TEST AND TREAT MALARIA IN NIGERIA: RESULTS FROM NATIONAL OUTLET SURVEYS (2009-2013)

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The WHO T3 initiative: Test, Treat and Track promotes effective management of suspected malaria cases through diagnosis, effective treatment and accurate surveillance. In Nigeria, children with fever are taken to private (46%) or public sector providers (18%) for treatment. We assessed readiness of these outlets to provide malaria testing and treatment and the cost to the consumer. Nationally-representative antimalarial medicine outlet surveys were conducted in 2009, 2011 and 2013 as part of the ACTwatch project. A complete census of all outlets with the potential to stock antimalarials within selected clusters was conducted. Outlet types were classified as either public/not-for-profit or private for profit. The primary monitoring indicators were the weighted proportion of outlets offering/stocking any malaria diagnostics or Quality Assured Artemisinin-based Combination Therapy (QAACT). Data on the cost QAACTs to the consumer were also collected. Among outlets that had any antimalarial medicines in the public sector, 22% (2009) and 31% (2011) reported having malaria blood testing available on the day of interview. This compares with 2% (2009) and 4% (2011) of outlets in the private sector. Availability of QAACTs in the public sector remained stable at 44% (2009) and 48% (2011). In the private sector availability remained low at 7% (2009) and 10 % (2011). In the private sector, QAACT prices decreased from $3.94 (2009) to $1.40 (2011). 2013 survey findings will be reported at the conference. Across survey rounds, availability of malaria blood testing and QAACTs is lower in the private sector compared to the public sector. While readiness for malaria case management is improving in the public sector, nearly half of all children with suspected malaria in Nigeria are taken to the private sector where most of these providers are not equipped to appropriately test and treat cases. Improving readiness of the private sector for malaria case management in Nigeria may be an important strategy to improve effective coverage.

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FORECASTING THE BURDEN OF MALARIA IN UGANDA USING CLINICAL AND ENVIRONMENTAL PREDICTORS

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Malaria thrives in poor tropical and subtropical countries where local resources are limited. Accurate disease forecasts can provide public health and clinical organizations with the information needed to implement targeted approaches for malaria control that make effective use of limited resources. Previous malaria forecasting work has not considered clinical predictors, such as antimalarial treatment, which is an important predictor of malaria burden in a population. The objective of the research was to identify the environmental and clinical predictors that produce the most accurate forecasts of malaria at six different health facilities in Uganda. Malaria forecasting models were developed using health facility data collected by the Uganda Malaria Surveillance Project and satellite-derived rainfall, temperature, and vegetation estimates. Short-term (4-week) and long-term (52-week) weekly forecasts of confirmed malaria from June 1, 2012 to May 31, 2013 were developed for each health facility using multivariate transfer function models. The model with the most accurate forecasts varied by site. Clinical predictors were retained in the most accurate models across all facility sites with the exception of one model. The average short-term error ranged from 20% to 96% over the forecasting period. The long-term models performed best for predicting the cumulative cases with error ranging from 2% to 22%. Study limitations included not knowing if the confirmed malaria cases were incident or recrudescent as well as measurement errors associated with the remote sensing and clinical data sources. Incorporating clinical predictors such as type of antimalarial treatment, improved the forecasting accuracy of several of the models. These results demonstrate the utility of using clinical predictors in conjunction with environmental predicators to forecast malaria. With the mounting cost of the global fight against malaria and the drive towards elimination in many countries, accurate forecasts of malaria remain essential.

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FETAL GROWTH RESTRICTION IS A MAJOR FACTOR INVOLVED IN GESTATIONAL MALARIA IN BENIN

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Malaria in pregnancy (MiP) leads to low birth weight, which is mainly due to intra-uterine growth restriction in Africa. However, when and how malaria impacts on fetal growth is still unknown. We investigated this issue using data on 774 Beninese pregnant women from the STOPPAM cohort. They were screened for malaria (using blood smears) every month during pregnancy until delivery and had 4 ultrasound scans at 20, 26, 30 and 36 weeks of gestation, allowing repeated measurements of growth within the same pregnancy. For the analysis, birth weights (BW) and estimated fetal weights for gestational age were converted into Z-scores using Tanzanian sex-specific charts as reference. First, BW Z-scores were compared between women infected and uninfected with malaria during pregnancy. Then, the effect of malaria on fetal growth, measured as a change in Z-scores between two consecutive measurements, was assessed. Both analyses were adjusted for potential confounding factors such as maternal undernutrition, maternal anemia and gravidity. More than 40% of women had at least one malarial infection during pregnancy. We showed that women infected in the first and at least one subsequent trimester of pregnancy had significantly lower BW Z-scores (-0.40 [-0.78; -0.01]) than uninfected women during pregnancy. We did not find that malaria limited to either the 1st trimester or the 2nd/3rd trimester was associated with BW Z-scores, suggesting a cumulative effect of malaria on fetal growth. A decrease in Z-scores during pregnancy was significantly associated with malaria infections that occurred several weeks before the decrease (Z-scores decrease in women infected with malaria compared to non infected women: -0.23 [-0.40; -0.05]), but not with recent malaria infections. In conclusion, we confirmed the effect of malaria on fetal growth in Africa. Our results suggest both a long-term and cumulative effect, starting from the 1st trimester onwards.

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USING GENOME-WIDE SINGLE NUCLEOTIDE POLYMORPHISMS (SNPS) TO TRACK MALARIA SPREAD AMONG LOCAL COMMUNITIES IN THE MYANMAR-CHINA BORDER AREAS

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Border areas are reservoirs of malaria because of frequent and recurrent parasite introductions via human movements. Areas along the international border of Myanmar and China have reported high malaria incidence in recent years. In Myanmar, civil unrest and establishment of internally displaced persons (IDP) camps along the Myanmar-China border have impacted much of the malaria transmission in the region. The growing IDP populations raise deep concerns about health impact on local communities. Moreover, in remote countryside of poor accessibility and general negligence, and where the ethnic minorities reside, over 60% of the populations are potentially at high risk of malaria. This study used genome-wide Single Nucleotide Polymorphisms (SNPs) and microsatellite markers to examine the source and spreading patterns of malaria parasites (*Plasmodium falciparum* and *P. vivax*) between IDP camps and surrounding villages in Myanmar, as well as villages/towns in the border areas of Myanmar-China. We compared genotypic composition of *Plasmodium* samples collected in 2011, 2012, and 2013 from the same area and examined demographic history with the goal to determine whether recent infections were caused by the same or different parasite genotypes. In addition, we tested if IDP camps are the source of malaria infections particularly concerning border-malaria cases based on genetic diversity differences among localities. Broadly, we tested the hypothesis of whether *P. vivax* is genetically more diverse than *P. falciparum* under the same environmental and temporal settings at a local scale. In-depth knowledge and information on the extent of malaria spread are keys to target disease control efforts in high-risk areas such as those near international borders and remote countryside. This is of particular relevance when most other parts of Southeast Asia are entering the malaria elimination phase.

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USE OF GPS DATA LOGGERS TO DESCRIBE SPATIO-TEMPORAL HUMAN MOVEMENT PATTERNS IN AN AREA OF EFFECTIVE MALARIA CONTROL IN SOUTHERN ZAMBIA

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The role of human movement in malaria transmission dynamics, particularly in pre-elimination settings, has not been fully elucidated. GPS data loggers allow for micro-scale estimates of human movement in rural areas of sub-Saharan Africa, which can aid in explaining the micro-epidemiology of malaria importation and transmission in these areas. Participants currently enrolled in a longitudinal cohort study of the impact of malaria control measures in a region of declining malaria transmission in southern, Zambia (one of three Southern Africa International Centers of Excellence for Malaria Research sites) were invited to participate in a population movement study using GPS data loggers. Approximately 10 participants at a time were asked to carry the loggers for a one-month period. Data will be collected over 12 months to account for seasonality in movement patterns. Enrollment began in October 2013 and is ongoing. Serial numbers of GPS data loggers were matched to participant IDs and geographic position logged every two minutes. Movement data from the GPS loggers were imported into ArcGIS for pre-processing and analysis. The movement tracts were used to determine the cumulative amount of time spent in different areas, derived from the frequency of visits and amount of time spent during each visit to different areas. An intensity map was created to display the cumulative amount of movement in different areas. An analysis of time spent in and around the residence was conducted. The distribution of time spent in and around the household and time spent at varying distances from the household was analyzed, and the cumulative amount of time spent in areas of high and low malaria risk was calculated. An analysis to determine whether time spent inside an area of high malaria risk is due to residence in a hotspot or routine travel to a location of high malaria risk was conducted.

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PLASMODIUM VIVAX MALARIA IS NOT A MAJOR THREAT IN MADAGASCAR

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Published reports on malaria in Madagascar in the last seven decades showed that *Plasmodium vivax*, *P. ovale* and *P. malariae* are present; however, *P. falciparum* remains the predominant species. Effort has been achieved with the introduction of artemisinin-based combination therapy (ACT) and the scaling-up of the vector control since 2006. The last two malaria index surveys among children in Madagascar from 6 to 59 months of age (n = 6154 in 2011 and n = 5504 in 2013) showed that malaria transmission is highly variable in different areas. Global malaria prevalence ranged from 1% in the highlands to 15% on the rainy eastern coast. *P. falciparum* infection was responsible for >99% of the cases. In those two surveys, only one *P. vivax* infection (1/863) and two *P. malariae* infections (2/863) were detected. It was shown recently that *P. vivax* is capable of breaking through the Duffy-negative barrier in Madagascar, leading some policy makers to consider introducing *P. vivax* specific treatments. Available data, however, indicate that at this time, *P. vivax* is not a real threat in Madagascar. With its local population of both Asian and African origin, Madagascar is an ideal place to investigate the fundamental aspects of the *P. vivax* biology including the molecular mechanisms underlying red blood cell invasion. The key issues in malaria elimination in Madagascar are the recurrent shortage of stocks of ACT; the interruption of insecticide indoor spraying and the irregularity in insecticide treated bed net distribution. Nonetheless, the impact of the intervention against malaria in Madagascar on *P. vivax* between 1940 and 2000 will be also discussed in our presentation.

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G6PD GENOTYPE AND THE IMPACT ON PREGNANCY OUTCOME IN WOMEN INFECTED WITH *PLASMODIUM FALCIPARUM* AND *P. VIVAX* MALARIA: DESCRIPTIVE STUDY OF 1749 WOMEN

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G6PD deficiency is the most common enzymatic disorder in human, and its distribution closely matches that of malaria. Nonetheless, a protection from malaria infection and parasitemia in G6PD deficient subjects does not seem to explain completely the rapid spread of this genetic trait in several populations. The associations between G6PD status, malaria and pregnancy outcome have not been well characterized although a protective effect might be hypothesized. A large cohort of pregnant women from malaria treatment and prevention studies carried out along the Thai-Myanmar border was characterized for G6PD genotype, and the impact of falciparum and vivax malaria infections on pregnancy outcomes was compared. The major local mutation, Mahidol, was detected through PCR-RFLP in DNA extracted from filter papers collected in the past 20 years in 1749 pregnant women. Malaria was detected by microscopy during active screening at ANC consultations. Demographic data, obstetric history, gestational age and clinical symptoms were recorded as well as pregnancy and neonatal outcomes. Significant differences in the proportion of women with symptomatic infections, onset of anaemia, miscarriage, stillbirth, birth weight and sex of the newborn were observed according to their G6PD*Mahidol genotypes. Protection from G6PD was not always observed for both falciparum and vivax infections.

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MALARIA IN BORDERLANDS: A CASE STUDY FROM THE THAILAND-MYANMAR BORDER

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The central plains of Thailand have been mostly malaria free now for several decades. However, malaria continues to persist in the borderlands connecting the Thai Kingdom with Laos, Cambodia, Malaysia, and Myanmar. The reasons for this persistence are complex. These are areas with high ecological, ethnic, and linguistic diversity. These borderlands have been exposed to political instability that has existed across the borders in Cambodia, Laos, and Myanmar. The common logic on the ground is that the malaria situation on the Thai side is a spill-over effect of the malaria situation on foreign soil. In this research, we take a micro, spatial-epidemiological approach to understanding border malaria, using a study site along the Thai-Myanmar border. The underlying goals of this work were to empirically test for demographic factors (including migration) as risks for malaria infection; in the potential for asymptomatic carriers to exist in a region that is considered to be a "low transmission" area; and in mapping space-time patterns in malaria cases in households. We find that a large proportion of cases are missed by microscopic diagnosis, that migration does not appear to be a significant risk factor for individuals or household members who live with migrants, and that individuals without

citizenship (either in Myanmar or Thailand) exhibit a significantly higher risk of malaria infection when compared to any other demographic group. Finally, the spatial distribution of malaria cases clusters tightly around year-round water sources within the village during the dry season, but expands throughout much of the village during the wet season. Taken together, these factors indicate that the border malaria situation is much more complex than generally considered. While this research does not rule out the potential of malaria importation from Myanmar, it does show that such importation isn't necessary for continued persistence of malaria on Thai soil.

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USING GEOSTATISTICAL METHODS TO ANALYZE SPATIO-TEMPORAL CHANGES IN CHILDHOOD MALARIA FOLLOWING SCALE-UP OF CONTROL EFFORTS IN CHIKWAWA DISTRICT, MALAWI

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Geostatistical methods are increasingly used to support disease control efforts and analyse spatial variation in disease prevalence. These methods are especially useful in settings where disease registries are non-existent or geographically incomplete, and data on disease prevalence must be obtained via cross-sectional surveys of the population of interest. In order to obtain timely, accurate and (sub-)district estimates of prevalence, however, continuous prevalence surveys could provide a more powerful approach for identifying local hotspots and guiding more targeted control efforts. Applying novel model-based geostatistical methods for malaria mapping, we estimated changes in the spatial distribution of malaria prevalence over time using data from a continuous Malaria Indicator Survey conducted within a 20 x 20 km area of Chikwawa District in Malawi, from May 2010 to June 2013, a period of district-wide scale-up of malaria control. We developed a statistical model that accounts for temporal and spatial variation in prevalence, and over-dispersion within households. This approach allows us to quantify the uncertainty in prevalence estimates more accurately than standard statistical methods that ignore the temporal and spatial correlation induced by unmeasured risk factors for malaria and, as a result, give an exaggerated impression of regression relationships. To model seasonality accurately we used a linear combination of sinusoidal curves at different frequencies. The resulting time-series of malaria prevalence maps show how the model can provide an evaluation of control progress. The method can also be used to predict changes in prevalence that would result from different control progress scenarios, by running the model with different values for the input variables. Finally, visualizations using animations of spatio-temporal prevalence allow for a more intuitive interpretation by end-users that can guide more targeted control efforts towards hotspots.

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DETERMINING THE CHANGE IN MALARIA ACROSS AFRICA FROM 2000 TO 2012

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Decreases in malarial prevalence over the past decade have been mainly associated with the expansion of vector control measures such as insecticide-treated nets (ITNs) and changes in environmental and socio-economic factors. Here we seek to formally evaluate the determinants and patterns of the change in malaria prevalence from 2000 to 2012 across all of Africa. We first build Bayesian spatial models of malarial prevalence using a large assembly of geopositioned parasite rate surveys, along with new dynamic environmental and socioeconomic covariates varying through time. Then, using a combination of Bayesian compartment and spatial modelling we build models of ITN usage through time. Using this rich data set of prevalence, environmental factors, socioeconomic factors and ITN usage, all modelled continuously at a 5km by 5km resolution across all of Africa, we characterise the change that has occurred in the last decade.

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AIR TEMPERATURE SUITABILITY FOR *PLASMODIUM FALCIPARUM* MALARIA TRANSMISSION IN AFRICA 2000-2012

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Air temperature affects multiple aspects of the malaria transmission cycle, including vector survival and sporozoite development. These factors have been combined within a biological model to produce a single metric (temperature suitability) that is useful as a predictor variable in modeling and mapping malaria endemicity. In this study we improve on previous research endeavors by creating a dynamic temperature suitability product for *Plasmodium falciparum* in Africa, from 2000-2012, with a 1km spatial resolution, and a monthly temporal resolution. The temperature suitability product is generated using land surface temperature data derived from satellite imagery, which is converted to air temperature using an approach we develop, and then ingested into an improved version of an established temperature suitability model. Results indicate that the dynamic temperature suitability product is an improvement over earlier synoptic products, particularly with respect to its ability to characterize spatio-temporal patterns in areas with seasonally variable infection rates.

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CONSENSUS FORECASTS OF CLINICAL DISEASE BURDEN FROM *PLASMODIUM FALCIPARUM*: A STOCHASTIC MODELING APPROACH

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Mechanistic transmission models for "micro-simulation"---the stochastic, computational realization of disease dynamics at the individual level within a mock human population---represent a key tool for forecasting the burden of clinical disease from community prevalence estimates. These models reveal a complex, non-linear relationship and age-dependence to the parasite prevalence--clinical incidence relationship, owing to the effects of exposure-driven immunity, which in turn depends on a combination of the historical mean entomological innoculation rate and its seasonality profile. In this study we examine the predictions of multiple model variants and forge consensus forecasts of *Plasmodium falciparum* disease burden on national and continental scales.

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MALARIA TRANSMISSION, INFECTION AND DISEASE AT THREE SITES WITH VARIED MALARIA TRANSMISSION INTENSITY IN UGANDA: IMPLICATIONS FOR MALARIA CONTROL

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Intensification of control interventions has led to reductions in malaria burden in some, but not all settings. To describe the malaria epidemiology in Uganda, we conducted entomologic surveys and cohort studies at 3 sites with varying transmission intensity. All households were enumerated in 3 sub-counties: Walukuba (peri-urban), Kihihi (rural) and Nagongera (rural). In each sub-county, 100 houses were randomly selected for measures of malaria transmission, infection, and disease over 24 months. Annual entomological inoculation rate (AEIR) was estimated from monthly collections using CDC light traps. All children aged 0.5-11 years were provided a long lasting insecticide treated bed net (LLITN) and followed using active surveillance for measures of parasite prevalence and anemia every 3 months and passive surveillance to measure the incidence of malaria. Episodes of uncomplicated malaria were treated with artemether-lumefantrine and complicated malaria treated with quinine. Transmission was highly seasonal at all 3 sites, with 2 annual peaks. Estimates from Walukuba, Kihihi, and Nagongera, respectively were as follows: AEIRs of 3.3, 31.5, and 315 infectious bites per person year (PPY); parasite prevalence of 8.8%, 15.0%, and 41.5%; and malaria incidence of 0.48, 1.67 and 3.52 episodes PPY. Comparing the 1st and 2nd years, there was a significant decrease in the incidence of malaria in Walukuba (0.55 vs. 0.36 PPY, p=0.01) and significant increases in Kihihi (1.24 vs. 2.13 PPY, p<0.001) and Nagongera (3.06 vs. 3.98 PPY, p<0.001). Of 3140 episodes of malaria diagnosed, only 9 (0.3%) met criteria for severe disease with no cases of cerebral malaria or deaths. The prevalence of moderate (Hb<10) and severe (Hb<7) anemia were 8.5% and < 0.4%, respectively, and did not vary by transmission intensity. In the setting of LLITNs and prompt effective treatment, the risk of complicated malaria and anemia was very low. However, the burden of malaria remains high and increased at two rural sites, suggesting that additional malaria control interventions are needed in Uganda.

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STATISTICAL INFERENCE OF *PLASMODIUM FALCIPARUM* TRANSMISSION NETWORKS BASED JOINTLY ON EPIDEMIOLOGICAL AND GENETIC DATA

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Decisions about how to best allocate finite resources for malaria surveillance and control depend on a range of factors, including epidemiological quantities such as the proportion of local versus imported cases, the origin of imported cases, and variation in local transmission potential. Measurement of these quantities is difficult, however. For a variety of viral and bacterial pathogens, recent advances in genetic sequencing and statistical inference methodology have enabled the estimation of transmission linkages between small geographic areas or groups of infected hosts, and in some cases between individual hosts. Methods for making such estimates currently depend on the assumption that ample genetic variation is generated by neutral mutations on a timescale that is fast relative to that of transmission. Because this assumption is likely violated for *Plasmodium* parasites, which undergo a sexual reproductive phase and have a lower mutation rate than viruses, existing methods to build transmission networks from genetic and epidemiological data cannot be applied to malaria. To overcome these obstacles, we have developed bespoke statistical methodology for making inferences about transmission linkages between human malaria cases. This methodology makes use of the multi-locus genetic composition of an individual's parasites, the individual's home location, and the date when the infection was detected. We make explicit assumptions about the processes that generate and erode genetic variation, including superinfection, the importation of parasites from source populations, and the stochastic loss of alleles over the course of multiple transmission events. Using this mathematical framework for epidemiological dynamics and parasite evolution, together with Bayesian statistical techniques, we made inferences about transmission networks using simulated data and data sets from Zanzibar and Swaziland. These networks can be used to determine both population and individual-level parameters, such as R0 and the probability of a given infection being imported, respectively. We furthermore used simulated data to explore the robustness of this method to assumptions about the proportion of cases detected, mechanisms of inheritance, and epidemiological heterogeneities. Our results demonstrate the potential for this method to estimate key epidemiological parameters for malaria surveillance and elimination.

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THE RELATIONSHIP BETWEEN THE PREVALENCE OF MALARIA IN PREGNANT WOMEN AND THE PREVALENCE OF MALARIA IN CHILDREN AND NON-PREGNANT WOMEN IN SUB-SAHARAN AFRICA

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In malarious areas, malaria is more frequent in pregnant women than in age-matched non-pregnant women and the magnitude of the excess risk varies with age and exposure to malaria in previous pregnancies. Pregnant women attending for their first antenatal clinic visit are a potential pragmatic sentinel group to track malaria transmission intensity, however the relationship between malaria infection prevalence in asymptomatic pregnant women and other transmission indices, such as the prevalence of malaria infection in children obtained from population based cross-sectional surveys, a commonly used indicator for malaria transmission intensity, is not yet known. To determine if pregnant women can provide an alternative source of transmission intensity information, we evaluated the relationship between the prevalence of malaria among asymptomatic pregnant women and a) asymptomatic non-pregnant women and b) asymptomatic children (0-59 months) in the same area. Studies in sub-Saharan Africa were obtained using the malaria in pregnancy library (January 2014) and national surveys. We used random effects meta-analysis and meta-regression. The summary risk ratio (RR) of the malaria prevalence among pregnant vs non-pregnant women was 1.45 (95% CI 1.33-1.59), I-square 68% among all gravidae (51 studies in 32 records), and 2.10 (1.82-2.43), I-square 74% among primigravidae (19 locations). Information from 58 studies (18 records) was available for the comparison between pregnant women and children aged 0-5 years. The prevalence was highest in children: compared to all gravidae RR 1.45 (1.31-1.60), I-square 80%, and compared to primigravidae RR 1.13 (1.00-1.28), I-square 72% (5 studies). The malaria prevalence among primigravidae at the first antenatal booking visit may be a good approximation of the prevalence of malaria in children obtained from household surveys.

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VARIABLE MALARIA PREVALENCE ON ISLANDS IN LAKE VICTORIA, WESTERN KENYA

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Kenya launched its second National Malaria Strategy with a notably ambitious vision for a "*malaria free Kenya*", but malaria remains a major health problem among communities in Lake Victoria, We conducted two malariometric surveys during the dry (January) and wet (August) seasons in 2012 to determine the prevalence, geographical and seasonal variation of malaria among communities in the coast (Ungoye: population ~2,000) and on islands of Lake Victoria (Mfangano [large island]: population ~25,000; Takawiri, Kibuogi, and Ngodhe [small islands]: population ~700 each). Overall, parasite rates (PRs) as determined by microscopy (18.9% vs 14.5%), rapid diagnostic test (36.9% vs 31.9%) and PCR (31.1% vs 25.8%) were higher in the dry season than in the wet season ($P<0.01$) with characteristic age distribution. The highest prevalence by RDT was observed in Ungoye (dry season): 54.4% in age group 0-5 years old, 68.4% in 6-10, 55.3% in 11-15, 13.2% in 16-30 and 11.3% in >30, while the lowest was in Takawiri: 13.1%, 21.5%, 11.5%, 7%, and 1%, respectively. Species-specific prevalence by PCR was 29.3% for *Plasmodium falciparum*, 8.5% for *P. malariae*, and 2.1% for *P. ovale* in the dry season, and 24.5%, 6.0%, and 2.1%, respectively, in the wet season. No *P. vivax* was detected. Prevalence of mixed infections was 8.3% and 5.8% in the dry and wet seasons, respectively. In both seasons, PRs were highest in the coast, followed by large island and lowest in the small islands, with significant fluctuations in islands but not the coast. Fluctuations in PRs among island settings were significant only in children and young adolescents but not in adults. PRs were correlated with prevalence of fever in both seasons ($P<0.05$), however they were correlated with enlarged spleen in the dry season only ($P< 0.01$). Paradoxically, PRs were either not correlated (wet) or negatively correlated (dry) with rates of anaemia. Overall prevalence of G6PD deficiency was 12% in male and 2% in female. No significant correlation between the deficiency rates and PRs was observed among islands and the coast. Variation in malaria prevalence reflected the different dynamic of malaria transmission between the islands and the coast of Lake Victoria. Our results provide baseline data for the planned feasibility study of malaria elimination on islands in Lake Victoria.

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MALARIA AND ANEMIA PREVALENCE TWO YEARS FOLLOWING MASS NET DISTRIBUTION IN PLATEAU STATE, NIGERIA

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Nigeria suffers the world's largest malaria burden and in 2009 it launched a plan to provide two long-lasting insecticidal nets (LLINs) to every household via state-level mass distribution campaigns. In Plateau State (pop. 3.6 million) in central Nigeria, a baseline survey was conducted in September 2010 prior to mass net distribution of 1.4 million LLINs in December 2010. Two years later (October 2012), a modified malaria indicator survey was conducted to compare changes in net ownership, utilization, IRS coverage, *Plasmodium* prevalence and childhood anemia. In each survey >1,300 households in at least 58 clusters were sampled. Household ownership of at least one net increased from 35.1% in 2010 to 74.0% in 2012 ($P<0.001$), while ownership of two or more nets increased from 14.5% to 50.1% ($P<0.001$). In 2012, households had a mean of 1.6 nets per household, 0.62 nets per sleeping space and 0.31 nets per person. Overall reported net use the night before the survey among all individuals, children <5 years, and pregnant women was 49.0%, 59.0% and 60.5%, respectively in 2012 among all households (all $P<0.001$ versus 2010) and 64.5%, 77.6% and 79.1%, respectively, among households owning at least one net (all $P<0.001$ versus 2010). IRS coverage remained low (<1% in both surveys). Between 2010 and 2012, crude *Plasmodium* prevalence by microscopy decreased by 58% from 47.7% (n=4,209) to 19.9% (n=3,911; $P<0.001$). However, parasite prevalence by rapid diagnostic test (RDT) in 2012 (36.7%) was not significantly lower compared to 2010 RDT prevalence (40.5%, $P=0.16$) using the same test (CareStart Pf/PAN). Prevalence was highest among children 5-9 years old. *Plasmodium malariae* accounted for 3.7% of infections diagnosed by microscopy. Anemia in children ≤10 years was equally prevalent in 2012 (57.8%) and 2010 (57.1%). These results, believed to be the first state-level report of the impact of mass net distribution in Nigeria, document significant increases in net coverage and usage that correspond with a decrease in parasite prevalence as diagnosed by microscopy, but not RDT.

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DETERMINANTS OF SOCIO-ECONOMIC STATUS AND RISK OF MALARIA INFECTION IN PANAMA (2009-2012)

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In the Americas malaria remains a major problem in areas with low socioeconomic-status (SES). In order to achieve elimination, current strategies must have a multi-sectorial approach that addresses social and environmental determinants of malaria infection. The current focal low incidence of malaria in Panama presents a unique opportunity to start an elimination phase. In this study we examine the epidemiology of malaria in Panama with special reference to social and environmental determinants of malaria infection at the corregimiento level (smallest political division), analyzing census demographic and epidemiological data for 2,295 malaria cases between 2009-2012 in 621 corregimientos. Our analysis indicated that the burden of *P. vivax* infections by health region was higher among Amerindian reservations (where the incidence of *Plasmodium falciparum* and vivax infections was estimated between < 1 % to 8.4 % respectively), and female individuals less than 15 years old. In order to test the hypothesis that those corregimientos with the highest proportion of type 2 households (build with deciduous construction materials) were more likely to be infected, we performed a multivariate logistic regression analysis to evaluate the association between risk of malaria infection and type 2 houses, controlling for other predictors of low SES such as dirt floor, lack of potable water, lack of electricity, lack of sanitary facilities, unemployment and illiteracy. Results indicated that those corregimientos with the highest proportion of type 2 households were more likely to be infected with malaria (OR = 43.24 (2009), 821.20 (2010), 1359.23 (2011) 729.52 (2012) ($p < 0.05$), all other predictors held constant. Pairwise correlations indicated a protective effect of type 1 households ($p < 0.05$), while risk of malaria infection was positively correlated with determinants of low SES such as type 2 houses, dirt floors, illiteracy and lack of electricity but was not with lack of potable water, sanitary facilities or unemployment. In conclusion, risk of malaria infection was associated with corregimientos having the highest proportion of type 2 households. We expect that this data will help implement a multi-sectorial approach for the elimination of malaria based on determinants of SES in Panama.

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DETERMINANTS OF EFFECTIVE DELIVERY OF INTERMITTENT PREVENTIVE TREATMENT IN PREGNANCY (IPTP) IN SUB-SAHARAN AFRICA: A MULTI-COUNTRY ANALYSIS

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Malaria infection during pregnancy leads to adverse health outcomes for both mothers and infants. IPTp of at least two doses of sulphadoxine-pyrimethamine (SP), administered at antenatal care (ANC) visits, is an effective malaria prevention intervention. Despite increasing investment in IPTp programs over the past decade, and despite high rates of attendance at ANC visits, use of IPTp remains low. To identify bottlenecks in IPTp delivery, service effectiveness analyses were performed on data from 16 Demographic and Health Surveys (DHS) and Malaria Indictor Surveys (MIS) conducted between 2007 and 2011 in malaria-endemic countries in SSA. Multi-country, pooled, multivariate logistic regressions were used to identify determinants of IPTp delivery. Distributions of key determinants were compared for lower IPTp coverage countries (<20% IPTp use) and higher IPTp coverage countries (≥20% IPTp use). IPTp was effectively delivered for only 18% of targeted women. Access to ANC services was not identified as a major reason for this low rate. In fact, 83% attended ANC at least once and 97% of those receiving one dose of SP attended ANC twice. However, levels of SP delivery to those attending ANC was low: 42% of those attending one ANC visit received one SP dose, and 57% of those attending two ANC visits received two SP doses. Determinants of IPTp use included number of ANC visits, receipt of other maternal health interventions, and malaria transmission level. Effectiveness of IPTp delivery systems varied substantially between higher and lower IPTp coverage countries. Women in higher coverage countries made fewer ANC visits, attended ANC for the first time earlier in gestation, and were more likely to use ANC services at public or religious facilities than were women in lower coverage countries. Results show that most pregnant women are obtaining ANC services at sufficient frequency and appropriate timing to permit IPTp delivery, but the intervention is not being effectively delivered in these settings.

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ACCESS TO MALARIA CONTROL INTERVENTIONS AMONG SCHOOL-AGE CHILDREN IN MALAWI

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In Malawi, school-age children have the highest rates of malaria illness at health facilities and asymptomatic infections in the community. Whether this is due to age-related biological susceptibility or difference in access to malaria control interventions is unclear. We used community-based surveillance data to assess interventions, including bednet use and prompt diagnosis and treatment, in school-age children (6-15years) compared to younger children (0-5years) and pregnant women, groups that are typically the target of anti-malaria interventions. During cross-sectional surveys in September 2012 and May 2013, we enrolled 7653 participants. School-age children were less likely to report using bednets (645/1130, 57%) than younger children (600/790, 76%, p<0.001) and pregnant women (57/74, 77%, p<0.001). Among school-age children, the likelihood of bednet use was not affected by gender or rural vs. urban setting, but they were more likely to sleep under a net in the rainy season and when the ratio of household members to nets was lower (2.6 vs 4.5 people per net, p<0.001). Effects of bednets in this age group will be determined by comparing rates of anemia, microscopic and submicroscopic parasitemia. Fever in the last two weeks was reported in fewer school-age children (163/1130, 14%) than younger children (200/790, 25%); p<0.001). Among those with fever, there was no difference between the groups in seeking treatment or duration of fever prior to seeking treatment. School-age children were most commonly taken to a shop for treatment compared to younger children, who were most commonly taken to a government health facility. By parent report, the two groups were equally likely to be tested for malaria and to receive antimalarial drugs. In Malawi, school-age children have less access to antimalarial interventions than younger children and pregnant women. This may partially explain their high rates of disease and infection. New interventions targeting this group or strategies to increase access to existing programs may decrease both disease burden and transmission.

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MALARIA IN CHILDREN UNDER-TWO IN RURAL SINDH, PAKISTAN - RESULTS OF A COMMUNITY BASED ACTIVE SURVEILLANCE STUDY

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Burden of malaria is not clearly defined in Pakistan, and is believed to be sporadic and limited to certain high risk areas. These high risk zones have not been clearly defined. With the MDGs and Roll Back Malaria's targets approaching in 2015, it is important to characterize this malaria burden better. We aimed to determine the incidence of malaria in children less than two years old in a rural community typically considered at low risk of malaria. Prospective surveillance was carried out in children between 0 - 24 months over a period of two years from October 2011 - November 2013 in the district of Matiari, in Sindh, Pakistan. Children of parents and / or guardians able to give informed consent were recruited as newborns, and underwent routine fortnightly active surveillance for two years. Children meeting the World Health Organization's Integrated Management of Childhood Illness' criteria for febrile illness and severe pneumonia were tested for malaria using Malaria Pf/Pan rapid immunochromatographic tests. Positive malaria cases were confirmed by light microscopy. Febrile children had weekly follow-ups and were treated or referred. 817 children were followed and total child-years of follow-up were 1374 after adjusting for children lost to follow-up or missed on surveillance visits. 409 (50.1%) were male. Malaria incidence rate was 9.5 cases per 1000 child years (95% CI 5.0 - 16.1). No cases of neonatal malaria were detected. Clinical presentation was nonspecific and overlapped with pneumonia. There were no mortalities due to appropriate treatment and follow-up. Malaria in rural Sindh in Pakistan is a more common occurrence than previously recognized. This has implications for diagnostic and management algorithms used for young febrile children in the community.

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THE TRIPLET METHOD: A QUICK AND RELIABLE METHOD FOR DELIVERING PARASITE INFECTION CLEARANCE AND DETECTION SENSTIVITY ESTIMATES

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Malaria infection and clearance rates can be estimation by analysis of longitudinal molecular typing data. In general, infections appear and disappear over short time intervals, but these highly dynamic patterns

are mainly the result of imperfect detection owing to samples that fall below the detection limit of PCR. Several different statistical techniques, such as hidden Markov models, have been used to estimate infection and clearance rates allowing for imperfect detection, but these can be difficult to implement. We present improvements on a simple method for estimating force of infection for *Plasmodium falciparum* from longitudinal typing data. The longitudinal sequence of observations for any one genetic type of parasite is evaluated as overlapping triplets observations, at time steps zero, one and two in order to estimate the true clearance rate between times steps zero and one. Counts from the set of triplets beginning with a positive measurement at time step zero are evaluated as realizations of a multinomial distribution conditional on the true clearance and detection sensitivity. We amend the original method to estimate the force of infection, clearance, and detection rates, as part of the same Bayesian model. The triplet method is straightforward to calculate, does not require extensive statistical modelling, and produces estimates comparable to more involved approaches for longitudinal data. Missing data are naturally accommodated as a category of the multinomial. We provide a program that calculates the carriage prevalence for each genotype, estimates of infection incidence, and the clearance rate, all corrected for imperfect detection. The method applied to the Albinama cohort (ages five to nine) of the TransEpi study in Papua New Guinea produces overall *Plasmodium falciparum* parasite mean daily clearance rate of 0.142 [0.005, 0.743] and annual incidence of infection 4.011[3.837, 4.093] (median[95%CI]); both the recovery rate and the infection rate are higher than those found for younger children in Tanzania and Ghana using similar methodology.

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INDIVIDUAL AND HOUSEHOLD LEVEL FACTORS ASSOCIATED WITH RAPID DIAGNOSTIC TEST (RDT)-POSITIVITY IN A HIGH MALARIA TRANSMISSION SETTING OF NORTHERN ZAMBIA, 2012- 2013

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While malaria transmission has declined substantially throughout parts of Zambia, some areas continue to experience high transmission levels despite deployment of malaria control efforts. Understanding factors associated with continued malaria transmission in these areas may inform control efforts. Household malaria surveys were conducted in Nchelenge District, Luapula Province, Zambia. Households were enumerated based on satellite imagery, 5 x 5 km grid cells were overlaid, and households were randomly chosen within selected grid cells. Households were enrolled into cross-sectional (one visit) or longitudinal (visits every other month) cohorts; analyses were restricted to cross-sectional and the first visit to longitudinal households. During study visits, adults and caretakers of children were administered a questionnaire and a blood sample was collected for a malaria rapid diagnostic test (RDT). Individual and household level factors associated with RDT positivity were analyzed using logistic regression models. A total of 1,201 individuals from 339 households were enrolled. Over the study period, 43% of participants were RDT positive. Over half of RDT positive individuals were between the ages of 5 and 17 years, and half of RDT positive individuals had visited a health center or health post for malaria in the past 6 months. In the multi-variable logistic regression analysis, RDT positive individuals were over twice as likely to be between the ages of 5 and 17 years as compared to children younger than 5 years (OR=2.06; 95% CI: 1.23, 3.44), over half as likely to report a fever within the past two weeks (OR=1.57; 95% CI: 1.04, 2.37), and 73% more likely to live in a household using an open well as the main water source (OR=1.73; 95% CI: 1.15, 2.6). RDT positivity was highest among children and adolescents between the ages of 5 and 17 years. RDT positives were likely to experience symptoms and have sought care. Open wells may be a breeding site for mosquito vectors, potentially contributing to malaria transmission.

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SPATIAL PREDICTION OF MALARIA RISK IN A HIGH TRANSMISSION AREA OF NCHELENGE DISTRICT IN NORTHERN ZAMBIA, 2012-2013

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Despite gains in malaria control in much of Zambia, burden of malaria remains high in some areas. Transmission may be explained by proximity to environmental features associated with mosquito-breeding sites. Household malaria surveys were conducted in Nchelenge District, Luapula Province in northern Zambia from February 2012 through December 2013. Households were enumerated based on satellite imagery and randomly selected for study enrollment. At each visit, adults and caretakers of children were administered questionnaires and a malaria rapid diagnostic test (RDT). Data on the spatial distribution of malaria cases were used to generate a risk map based on environmental features, including proximity to category 1, 2, and 3 streams, slope (range 0 to 90 degrees), distance from lake Mweru, distance to health facilities, distance to roads, population density and vegetation. Streams were categorized using hydrological models based on a digital elevation model (DEM) derived from the Shuttle Radar Topography Mission version 3 and correspond to the size of the stream. Logistic regression modeling and spatial risk maps were built using programs from the R statistical software and ArcGIS. A total of 300 households were visited, comprising 1,171 participants, of whom 43% were RDT positive. Households within 500 meters of any stream, specifically located closer to a category 2 stream (per 50 m), and households located closer to the lake (per 50 m) were more likely to have RDT-positive residents. The odds of an RDT-positive resident also increased 14% per unit increase in the degree of slope where the household is located. These environmental features were used in the logistic regression model to predict and map malaria risk, along with a measure of risk uncertainty. Malaria transmission is heterogeneous in a high transmission area. Proximity to any streams within 500 meters, specifically distance to category 2 streams, higher degree of slope, and being closer to the lake increased the risk of transmission. Prediction maps may be useful in targeting control interventions.

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PLASMODIUM VIVAX POPULATION STRUCTURE AND TRANSMISSION DYNAMICS IN CENTRAL CHINA

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In Central China the declining incidence of *P. vivax* has been interrupted by reemergence and outbreak since 2000. In this study the impact of these changes on the local parasite population, and concurrent risks of future resurgence, was assessed. *P. vivax* Isolates collected from Anhui (n=94) and Jiangsu (n=25) provinces, Central China by passive case detection between 2007 and 2010 were genotyped using capillary electrophoresis at 7 polymorphic short tandem repeat markers. Spatial and temporal analyses of within-host and population diversity, population structure, and relatedness were conducted on these isolates. Polyclonal infections were infrequent in the 94 isolates from Anhui (4%) and 25 from Jiangsu (12%), with a trend for increasing frequency from 2008 to 2010 (2 to 19%) when combined. Population diversity was high in both provinces and across the

years tested (HE = 0.8 - 0.85). Differentiation between Anhui and Jiangsu was modest (F'ST = 0.1). Several clusters of isolates with identical multi-locus haplotypes were observed across both Anhui and Jiangsu. Linkage disequilibrium was strong in both populations and in each year tested (IAS = 0.2 - 0.4) but declined two- to four-fold when identical haplotypes were accounted for, indicative of occasional epidemic transmission dynamics. None of 5 imported isolates shared identical haplotypes to any of the Central Chinese isolates. The population genetic structure of *P. vivax* in Central China highlights unstable transmission, with limited barriers to gene flow between the central provinces. The challenge of imported cases and risks of resurgence emphasise the need for continued surveillance to detect early warning signals. Although parasite genotyping has potential to inform the management of outbreaks, further studies are required to identify suitable marker panels for resolving local from imported *P. vivax* isolates.

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PLASMODIUM VIVAX MORBIDITY AFTER RADICAL CURE: A TWO-YEAR COHORT STUDY IN THE PERUVIAN AMAZON

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The current national treatment guidelines for vivax malaria in Peru include a 7-day primaquine (0.50mg/kg/day) course together with chloroquine (25mg/kg, 3days). In order to evaluate the efficacy of this protocol a cohort of *P. vivax* infected patients was treated and monitored for 2-years. The study was conducted between 2008 and 2011 in 29 communities around Iquitos city. *Plasmodium vivax* infected individuals were enrolled in the cohort after individual informed consent. Treatment was directly observed and participants were monitored actively to assess treatment efficacy at Day28, then visited monthly for clinical examination and blood sampling (microscopy and PCR). A total of 303 *P. vivax* infected individuals were recruited and treated, and 270 (89%) of them completed follow-up. At baseline, males and females were equally represented and the median age was 20 years [IQR: 11-38].Two late parasitological failures, both at day 28, were detected, though at the same time point 16 participants (5%) had a PCR detectable *P. vivax* infection. Almost half (144) of the participants had *P. vivax* recurrent infections most of them (70%) repeatedly (median=3, range [2-11]. The incidence of *P.vivax* recurrent infections by microscopy and by PCR was analyzed using negative binomial regression; and time to event was analysed by survival analysis and cox regression. Results will be compared to a sister cohort study carried out in Vietnam. In conclusion, after radical treatment, a high number of *P. vivax* recurrent infections were observed, most of them asymptomatic and sub-microscopic. Our results show that a substantial amount of the *P. vivax* transmission occurs silently in the Peruvian Amazon, a finding that calls for improved diagnosis and treatment if elimination is to be achieved.

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SEIZURE OCCURRENCE DURING TWO-YEAR FOLLOW-UP OF PEDIATRIC MALARIA PATIENTS: PRELIMINARY OBSERVATIONS

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Seizures are common following cerebral malaria (CM). Previous studies have demonstrated that epilepsy develops in 5-10% of survivors of CM. Less is known regarding the incidence of provoked and unprovoked seizures in other forms of severe malaria. At Mulago Hospital, Kampala, Uganda we conducted a prospective cohort study of pediatric malaria between 2008 and 2013. Children with cerebral and severe malarial anemia (SMA) were enrolled, with community children (CC) without a history of seizures or neurodisability enrolled as controls. The children's caretakers were asked whether the child had seizures during follow-up at 6, 12 and 24-month visits. 218 children with CM, 180 children with SMA and 182 CC were enrolled. Seizures occurred before admission in 205 children with CM (94%) and 3 children with SMA (1.7%), and during admission in 125 children with CM (57%) and 2 children with SMA (1.1%). 182 children with CM, 164 children with SMA, and 171 CC completed 2-year follow-up. Among children who completed follow-up, 5 children with CM (2.7%), 11 children with SMA (6.7%) and 2 CC (1.2%) had febrile seizures during the follow-up period, while 4 children with CM (2.2%), 1 child with SMA (0.6%) and 0 CC (%) had unprovoked seizures during follow-up. 3 children with CM (1.6%), 1 child with SMA (0.6%) and no CC met the definition of epilepsy (two separate incidents of unprovoked seizures). In this cohort of children with CM or SMA, febrile seizures were reported, but epilepsy was infrequent, occurring in <2% of the cohort.

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NEXT GENERATION DURABLE WALL LINERS, A NEW STRATEGY FOR MALARIA CONTROL: BASELINE RESULTS FROM A CLUSTER RANDOMIZED TRIAL

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Despite widespread adoption of long lasting insecticide nets (LLINs) and indoor residual spraying for vector control, malaria remains a major source of morbidity and mortality. Pyrethroid insecticide treated wall liners (ITWL) have been efficacious in reducing malaria prevalence in small-scale studies, however the increasing prevalence and distribution of pyrethroid resistance poses a threat to many pyrethroid-based vector control strategies. A new generation ITWL made of polymer based fabric impregnated with a mixture of non–pyrethroid insecticides has been developed. ITWLs act as a physical barrier to vectors and they release insecticide gradually, killing vectors that come into contact with them. They are expected to last several years before needing to be replaced. Like IRS the efficacy of ITWLs does not depend as heavily on human behaviour as LLINs do. A three arm cluster randomized controlled trial is underway in Muheza district, north-eastern Tanzania to measure and compare the efficacy and cost effectiveness of (1) LLIN (reference group), (2) IRS + LLIN, and (3) ITWL + LLIN. We present baseline findings collected prior to the randomisation of clusters into study arms. The study area was mapped and a census was performed. Sixty clusters were created, and 15 to 20 houses were randomly selected from each. All consenting household members were

asked demographic and behaviour questionnaires. Blood samples were drawn for malaria diagnosis, anemia testing, and immunochromographic testing (ICT) for *Wuchereria bancrofti*. Entomologic evaluations by WHO cylinder tests were performed. A total of 92,538 individuals from 24,198 households in the study area were enumerated, and 3208 people from 954 houses were sampled in the baseline epidemiological survey. Malaria parasitemia by mRDT was 21.6 % (95% CI: 20.2 -23.1%) and 15.7% (95% CI: 14.4 -17.2%) were positive for *W. bancrofti* by ICTs. Anemia prevalence for under fives (haemoglobin <8g/dL) was 4.3% (95% CI: 2.7 – 6.0%). Malaria infection was more common in 5-12 years olds 37.2% (95% CI 33.5- 40.9%) compared with < 5 children 17.8% (95% CI: 14.6-21.0%) (p = 0.001). WHO cylinder tests showed reduced susceptibility to pyrethroids of *An. gambiae s.l* 24 h post exposure ranging from 51 to 90%. When completed, this study will provide important information to National Malaria Control Programes and international agencies to guide future malaria control strategy and allocation of resources.

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FACTORS ASSOCIATED WITH DECREASED *PLASMODIUM FALCIPARUM* INFECTION RISK IN MALIAN CHILDREN

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Malaria control efforts remain suboptimal for many reasons including knowledge gaps in malaria epidemiology and immunology. To better understand the epidemiologic and immunologic factors associated with risk of *P. falciparum* infection and clinical malaria, we conducted a longitudinal cohort study of 695 individuals aged 3 months to 25 years in the rural village of Kalifabougou, Mali. We did bi-weekly active surveillance for *P. falciparum* infection by PCR and weekly active clinical surveillance for clinical malaria. Nearly all adults and children over four years of age became infected during the malaria season at a rate that was independent of age (log-rank test, p =.37), indicating that sterile immunity to *P. falciparum* infection is not acquired through natural exposure; and as expected, the risk of clinical malaria decreased with increasing age (log-rank test, p =.0038). Surprisingly, we observed that children under 4 years of age were less likely to be infected with *P. falciparum* compared to older subjects (p <0.0001), and indeed, 24% of children under 4 years of age remained PCR negative throughout the intense 6-month malaria season. Exposure was measured by antibody response to gSG6, an *Anopheles gambiae* specific salivary protein, and results indicate that uninfected children were less likely to have serologic evidence of exposure to the mosquito vector over the course of the malaria season. Self-reported bed net use was not different between infected and uninfected children. Because evidence of decreased exposure did not fully explain decreased infection risk in young children, we are taking several approaches to test the hypothesis that uninfected children have enhanced pre-erythrocytic immunity and/or that developmental differences render young children less permissive to *P. falciparum* infection. Findings from this study may help inform strategies to prevent *P. falciparum* infection in malaria endemic areas.

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INDIVIDUAL AND HOUSEHOLD LEVEL FACTORS ASSOCIATED MALARIA INFECTION AS DETERMINED BY PASSIVE AND REACTIVE CASE DETECTION OF MALARIA IN CHONGWE DISTRICT, ZAMBIA

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In moderate and high malaria transmission areas, current surveillance systems rely solely on passive detection methods. As malaria transmission in Zambia declines, it will become important to identify continued foci of infection via active case detection to bolster malaria control efforts. Individuals seeking care at the Chinyunyu clinic within Chongwe District, Zambia with a chief complaint of fever were administered a questionnaire, a rapid diagnostic test (RDT) and a blood sample was obtained for microscopy. Field teams were dispatched to the homes of RDT positive individuals where an additional survey and RDTs were administered to members within the household. GPS coordinates were recorded for each household. Descriptive statistics and multi-level modeling methods were implemented to compare index cases to RDT positive household contacts, and compare RDT positive to RDT negative household contacts. A total of 472 index cases were identified between June 2012 and June 2013, with 1,621 household contacts investigated and 731 (45%) testing positive for malaria. Index cases were significantly more likely to report symptoms (fever, headache) and be of younger age than RDT positive household contacts (p<0.05 for all comparisons). The mean age of passively detected cases was 12.35 (SD=15.82), the mean age of RDT positive household contacts was 14.74 (13.86), and for RDT negative household contacts was 22.16 (19.88). The proportion of index cases with fever and headache was 97% and 85%, the proportion for RDT positive household contacts was 2.7% and 54.5%, and RDT negative household contacts was 1.1% and 19.3%, respectively. In conclusion, almost half of household contacts of index cases were identified as RDT positive for malaria during the 1-year study period, suggesting passive surveillance underestimates the malaria prevalence for the clinic catchment area. Age and symptoms were the most important factors in seeking care at the clinic. RDT positive household contacts were less likely to report symptoms and slightly older than those that sought care.

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MODELING LONG-TERM MALARIA TRANSMISSION CHANGES IN A TANZANIAN VILLAGE USING CROSS-SECTIONAL DATA ON AGE SPECIFIC PREVALENCE AND LEVELS OF ANTIBODIES

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Robust estimates of *Plasmodium falciparum* transmission intensity are imperative for planning, implementation and evaluation of malaria control interventions. Seroconversion rates (SCR) to asexual blood-stage antigens can provide estimates of transmission intensity that correlate with entomological inoculation rates. We study past transmission trends in a rural village and evaluate new models to improve SCR based methods by analysis of antibody levels from multiple cross-sectional serological surveys. The study was conducted in Nyamisati, Rufiji, Tanzania, where parasite

prevalence decreased from 65 to 18% from 1999 and 2010. A single intervention with ITNs was performed in 1999. IgG levels to recombinant *P. falciparum* antigens (MSP-1_{19}, MSP-2, MSP-3, AMA-1) and *An. gambiae* salivary protein gSG6 were measured in children (1-16y) sampled in cross-sectional surveys in 1999 and 2010. SCR and rates of antibody decay were estimated by fitting mathematical models to data from the two cross-sections, assuming three profiles of exposure: (i) stable; (ii) stepwise decrease; or (iii) continuous decrease. Results suggest an average 66% decrease in malaria transmission intensity and an 89% reduction in *Anopheles* exposure. Transmission trends were best described by a stepwise decrease model with a reduction predicted to occur shortly after distribution of ITNs. The new models provide estimates of the duration of antibody responses under this transmission decline. MSP-1_{19} seropositive individuals were estimated to convert to seronegative with a half-life of 12 (95% CI 7-20) years due to antibody decline with a half-life of 3 (95% CI 2-6) years. The reduction in transmission may in part be attributed to reduced anopheles exposure following the introduction of ITNs, but is not likely to be explained by ITNs alone. Despite reduced parasite prevalence many children remained seropositive to blood-stage antigens. The new sensitive models using antibody levels enabled detection of reduced exposure among seropositive children and provided estimates of both antibody and transmission dynamics.

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MALARIA AT NATIONAL UNIVERSITY HOSPITAL, SINGAPORE

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Globally, malaria affects 300 to 500 million people each year. It is endemic to several countries in South East Asia. Singapore has maintained its malaria free status since 1982 despite occasional clusters due to local transmission. However, travel related malaria is seen in hospitals here. We describe the current epidemiology of malaria at our institution. The National University Hospital (NUH) is a 1000 bed multi-specialty, tertiary teaching hospital in Singapore. Laboratory surveillance for malaria is part of the infectious disease surveillance at NUH since 2004. A retrospective study, describing the epidemiology of malaria cases presenting between January 2009 and mid- April 2014, was conducted. A total of 44 cases were analyzed. 39 (88.6%) of them were male, 25 (6.8%) were Indian, 6 (13.6%) Chinese, 1 (2.27%) Malay and 1 (2.27%) Caucasian. 28 (63.6%) of the 44 patients had travelled to a malaria endemic area. All 9 Singaporeans with malaria had preceding travel history. The most common species of malaria was *Plasmodium vivax* (n=31, 70.5%). Of the other species, 7 (15.9%) were *P. falciparum*, 4 (9.09%) were *P. knowlesi* and 1 (2.27%) had a mixed *falciparum* and *vivax* infection. 17 of the 44 patients had traveled to India. 15 of the 17 patients who traveled to India were infected with *vivax*. Amongst the 4 patients with *P. knowlesi*, 3 had traveled to Malaysia. In particular, they had visited forested areas for recreation or training. Other travel destinations included Indonesia, Thailand, Hong Kong and Ghana. 39 of 44 patients were admitted and their mean length of stay was 4 days. 4 (9.09%) patients required ICU care and all 39 were discharged well. The global disease burden, modern travel dynamics and emergence of *P. knowlesi*, a zoonotic malarial species, contribute to the continued presence of cases of malaria in Singapore. The existence of *Anopheles* mosquito vectors on the island warrant ongoing vigilance to limit the risk of local transmission.

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EARLY DETECTION OF MALARIA RESURGENCE IN THE PERUVIAN AMAZON REGION USING SEROLOGICAL MARKERS

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In the past decade, increased support from international donors, e.g. the Global Fund-PAMAFRO Project (2005-2010), allowed for the scale-up of comprehensive malaria control strategies in the Amazon Region. During this period, malaria declined drastically in Peru from 87,805 reported clinical cases in 2005 to 29,355 and 23,075 cases in 2010 and 2011, respectively. Since 2011, malaria control activities are mainly supported by the MoH budget, prioritizing passive and reactive case detection and treatment of confirmed infections. Since 2012, several malaria outbreaks have been detected in diverse Amazonian areas, a phenomenon that had not been observed since 2007. In order to assess recent changes the malaria transmission intensity (MTI), a cross-sectional survey was conducted during November 2012 in eight peri-Iquitos villages using molecular and serological tools. After a full census of the study villages, each household was visited and all available children<7 years plus one randomly selected individual>7 years were enrolled. A total of 651 survey participants were interviewed, clinically examined and a blood sample taken for the detection of malaria parasites (microscopy and PCR) and antibodies to *Plasmodium vivax* (PvMSP119, PvAMA1) and *P. falciparum* (PfGLURP, PfAMA1) antigens by ELISA. Age-specific seroprevalence was analyzed using a previously published catalytic conversion model based on maximum likelihood for generating seroconversion rates (SCR). Overall parasite prevalence by microscopy and PCR were low, i.e. 1.8 and 3.9%, respectively for *P. vivax*, and 1.5 and 6.7%, respectively for *P. falciparum*, while seroprevalence was much higher, 23.3% for PvMSP119 and 18.0% for PfGLURP. Most of infections were asymptomatic (79.2%) and sub-patent (71.6%). Likelihood ratio tests supported age seroprevalence curves with two SCR for both *P. vivax* and *P. falciparum* indicating a significant increase in MTI since 2011. Additional data including antibody responses to two antigens for each species and a risk factor analysis for malaria infection and exposure will be presented. In conclusion, this sero-epidemiological analysis allowed for an in-depth characterization of the current malaria transmission pattern as well as for the identification of a recent increase in MTI in the peri-Iquitos area of the Peruvian Amazon following a reduction of control efforts since 2011.

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ROLE OF AUTOPHAGY AND POLYMORPHIC VARIATION IN AUTOPHAGY GENES IN CONDITIONING CLINICAL OUTCOMES IN CHILDREN WITH MALARIAL ANEMIA

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Plasmodium falciparum polymerizes free heme into hemozoin (*Pf*Hz) as a byproduct of hemoglobin (Hb) digestion. Phagocytosis of *Pf*Hz by leukocytes promotes dysregulation in the immune response and enhanced pathogenesis. Autophagy is a process that eliminates intracellular components to maintain homeostasis. Although no studies have investigated autophagy in malaria, inactivation of autophagy is associated

with enhanced susceptibility to infectious and inflammatory disorders. To investigate the role of autophagy in malaria, we genotyped autophagy genes [*ATG9* (i.e. -2896G/C, -4970C/T, -6561A/G) and *ATG10* (-2442G/C, -4322T/A, -7723G/T)] in parasitemic children (n=1220; 3-36 mos.) and determined the association between variation and severe malarial anemia (SMA, Hb < 5.0 g/dL). Regression analyses, controlling for covariates of anemia, revealed that carriage of CCA (-2896C/-4970C/-6561A) and GTA (-2896G/-4970T/-6561A) haplotypes (*ATG9*) were associated with reduced risk of developing SMA (Avg: OR, 0.26; 95% CI, 0.08-0.66; *P*<0.001). Similarly, carriers of CTG (-2442C/-4322T/-7723G) and GTT (-2442G/-4322T/-7723T) in *ATG10* had decreased susceptibility to SMA (Avg: OR, 0.52; 95% CI, 0.18-1.05; *P*=0.070). Analysis of intragenic haplotypes revealed that carriage of CCAGTT, CCGGTT, GCACTG, GTAGTT, and GTGGTT were correlated with a reduced risk of developing SMA (Avg: OR, 0.27; 95% CI, 0.05-0.94; *P*<0.001). Analysis of gene expression profiles showed increased levels of autophagy genes in children with SMA (*ATG9,* 1.57 fold, *P*=0.022; *ATG10,* 2.29 fold, *P*=0.001). In addition, treatment of cultured PBMCs with *Pf*Hz enhanced autophagy, as illustrated by elevated LC3-II (*P*<0.05). Consistent with previous studies showing the importance of inflammatory mediators in autophagy, analysis of malaria-associated cytokines/chemokines revealed that haplotypes associated with disease susceptibility had dysregulation of IL-1Ra, IL-1β, IL-8, and IFN-γ (*P*<0.05). Collectively, these studies suggest that autophagy plays an important role in conditioning clinical outcomes in children with malaria.

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ASSOCIATION BETWEEN INDUCIBLE HEME OXYGENASE (*HMOX*)-1 GENE VARIANTS AND SEVERE MALARIAL ANEMIA AMONG CHILDREN RESIDENT IN A *PLASMODIUM FALCIPARUM* HOLOENDEMIC REGION OF WESTERN KENYA

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Inducible heme oxygenase (HO)-1, the rate-limiting enzyme in the catabolism of heme into biliverdin, releasing free ferrous iron and carbon monoxide, is a protective factor with potent anti-inflammatory, anti-oxidant, and anti-proliferative effects, and is upregulated by multiple stimuli. Polymorphisms in the *HMOX-1* gene have been associated with various disease entities, including pulmonary, cardiovascular, neurological and infectious diseases, renal impairment and transplantation, as well as hematological and serological disorders. The current study investigated the association between *HMOX-1* intronic 917A>G (rs5755720) and promoter -495A>T (rs2071746) variants and susceptibility to severe malarial anemia (SMA; hemoglobin, Hb<5.0g/dL) among parasitemic children (age: 3-36 months; n=1,224) with acute malaria presenting at Siaya County Hospital, western Kenya. Demographic, clinical and laboratory measures were determined and children were stratified based on Hb levels into non-SMA (Hb≥5.0g/dL; n=1,014] and SMA (Hb<5.0g/dL; n=210). Genotyping was performed using the TaqMan 5' allele discrimination assay. Proportions of *HMOX-1* 917 and -495 genotypes were comparable between the groups (*P*=0.479 and *P*=0.275, respectively). Similarly, frequencies of haplotype constructs failed to show differences between the groups [917A/-495A (AA; *P*=0.805), AT (*P*=0.133), GA (*P*=0.171) and GT (*P*=0.448), respectively]. Bivariate logistic regression analyses, controlling for covariates of anemia, revealed that carriers of *HMOX-1* 917 GG mutant genotype protected children against SMA (OR=0.569, 95% CI-0.328-0.988; *P*=0.045). Additionally, carriers of a mutant 917G/-495T (GT) haplotype had >41% reduced risk of developing SMA (OR=0.585, 95% CI-0.349-0.981; *P*=0.042). By contrast, carriage of the AT haplotype increased the risk of developing SMA (OR=1.867, 95% CI-1.063-3.279; *P*=0.030). Collectively, these results suggest that variation at 917 and -495 in the *HMOX-1* loci play an important role in conditioning the development of SMA in children resident in *Plasmodium falciparum* endemic areas.

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INFERRING MOSQUITO POPULATION BIOLOGY FROM GENETIC DATA

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Anopheline mosquitoes are malaria vectors, many questions concerning the vectors' demographics remain unanswered. Our study attempts to infer the seasonal dynamics of the effective population size, and estimate the average generation time of the vector species. We are also interested in what happens during the dry season - especially for the female mosquitoes. A dataset consisting of the full genome of 200 mosquitoes from Africa were collected and analysed. Such data contain information about demographic history of the species. Studying the genetic signals not only sheds light on the above problems, but also plays a critical role in designing and evaluating vector control technologies.

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EVOLUTION OF THE MEROZOITE SURFACE PROTEIN 7 (MSP7) IN *PLASMODIUM* SPP., WITH EMPHASIS ON SPECIES CLOSELY RELATED TO *P. VIVAX*

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Multigene families are considered to be one of the main sources of genome innovation and diversification; particularly, in the case of *Plasmodium* spp. numerous multigene families are involved on processes such as immune evasion and host cell recognition. We have studied the evolution of the merozoite surface 7 (MSP7) multigene family, which it thought to participate on the initial steps of parasitic recognition and attachment to the host's erythrocyte surface. MSP7 paralog sequences for a total of twelve *Plasmodium* species were obtained by sequencing laboratory isolates and/or publicly available genomic data. An analysis of each paralog showed that similar amino acidic composition is shared among all members of the MSP7 multigene family. We observe numerous gene gain and loss events among *Plasmodium* species particularly within the clade that includes *Plasmodium vivax* (further referred as *P. vivax* clade) with a marked increment in the gene family size in this human parasite and its closely related macaque parasite *P. cynomolgi*. Specifically, there are several duplication events leading to MSP7 paralogs within the *P. vivax* clade and some of them increased the gene family size specifically in *P. vivax* and *P. cynomolgi*. Whether these events have been driven by natural selection is a matter that remains unclear. However, others have described five high activity binding peptides (HABP) to the host's erythrocyte in *P. falciparum* (gene PF3D7_1335100). We observe that three of them are frequently found in other PfMSP7 paralogs and other *Plasmodium* species. One, HABP_26114, shows 67 to 43% similarity (including conserved and same type amino acids) in all *P. falciparum* paralogs as well as in MSP7 paralogs found on species of the *P. vivax* clade (62-38%). This finding indicates that at least certain erythrocyte binding activity is to be expected in all members of the MSP7 multigene family as a mean to assure host infection. However, how the number of paralogs affects this putative binding activity across *Plasmodium* species is a matter that deserves further investigation.

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ASCERTAINING THE DIVERSITY AND RATE OF EVOLUTION OF THE COMPLETE MITOCHONDRIAL GENOME IN HAEMOSPORIDIAN PARASITES

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Our understanding about the origin of human malarias has been invigorating by the discovery of many new species in non-human primates. Many of those species, however, have been described solely using molecular data. Furthermore, zoonotic malarias have been misidentified due to the poor resolution of some morphological characteristics. Thus, the traditional view that relies solely on morphology to define malaria parasite species has been challenged. There are few systems that allow contrasting morphological and molecular data, one of those are the avian haemosporidians. In this study, we compare the rate of evolution of mitochondrial genes using standard molecular clock methods in both mammalian and avian parasites. We include newly described species belonging to three Haemesporidian genera: Leucocytozoon, Haemoproteus and *Plasmodium*. We contrast the results of single mitochondrial gene approaches with those from complete mitochondrial genomes and test both the potential and limitations of mitochondrial genes, including complete Cytb sequences, as ways to delimit species in malarial parasites that have been identified using morphology. Overall, we found that Cytb allows the correct differentiation of morphologically distinct species. However, the Cytb cannot, by itself, reliably uncover many recent phylogenetic relationships of species that radiated at a scale of 2-7 million years ago. Our conclusion is that single gene approaches do not provide enough information to properly differentiate or estimate molecular phylogenies on species that have recently diverged. Whether having morphological information is valuable in the description of species, molecular differences using multiple mitochondrial genes are sufficient to discover species.

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ULTRASENSITIVE DETECTION OF *PLASMODIUM FALCIPARUM* BY AMPLIFICATION OF MULTI-COPY SUBTELOMERIC TARGETS USING QUANTITATIVE PCR

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To inform up-scaled malaria control efforts, populations in endemic areas must be actively screened by molecular tools to identify the transmissive human reservoir including asymptomatic *Plasmodium* carriers. A major challenge is the large number of samples and the associated cost, which can be minimized by pre-screening of sample pools. Current molecular methods lack sensitivity and cannot reliably detect low-density infections, especially after dilution in sample pools. Novel and ultra-sensitive malaria detection assays are thus urgently needed. We therefore selected high-copy subtelomeric sequences to design two ultra-sensitive qPCR assays for detection of *P. falciparum* (Pf) infections. Amplification targeted the telomere-associated-repeat-element 2 (TARE-2, ca. 350 copies/genome) and the var-gene acidic terminal sequence (varATS, 60 copies/genome). In sensitivity tests using parasite culture, both assays reliably detected 0.034 parasites/µl blood, and could amplify from samples containing as little as 0.00034 parasites/µl blood. The sensitivity of TARE-2 and varATS assays relative to a 18S rDNA assay was assessed on 503 Tanzanian field samples covering all ages. The highest gain in Pf prevalence was observed in infants (0-1y) by TARE-2 qPCR (22.2%; varATS: 5.6%), and thus TARE-2 emerged as the most sensitive assay. From the age of four, TARE-2 and varATS qPCRs perfomed similarly with an average gain in Pf prevalence of 11.2% and 10.1%. To evaluate the applicability of our assays in pooling strategies, we produced 5-sample pools containing one low-density field sample (1-5 parasites/µl) plus four negatives and co-extracted DNA. Both TARE-2 and varATS assays reliably identified all pools containing a Pf sample. Our results demonstrate that a large proportion of asymptomatic Pf carriers is missed using 18S qPCR, leading to underestimation of the transmissive reservoir. Due to their enhanced sensitivity, TARE-2 and ATS qPCRs can be used to screen sample pools for high-throughput and cost-effective detection of Pf infections.

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RHOPTRY PROTEINS ARE INVOLVED IN SPOROZOITE INVASION OF THE SALIVARY GLAND

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It has been shown that rhoptry proteins are secreted and localized to the tight junction, during *Plasmodium* merozoite invasion of erythrocytes. One of rhoptry proteins, Rhoptry Neck Protein 2 (RON2) is known to form complex with RON4 and RON5. The interaction between RON complex and Apical Membrane Protein 1 (AMA1) at the tight junction confers the invasive motility on merozoites. Previously, we reported that RON2 is also localized to the rhoptries in sporozoites and that RON2 is involved in sporozoite invasion of the salivary glands by generation of sporozoite stage-specific ron2 silencing transgenic parasites. Here, we investigated whether RON complex is also formed in oocyst-derived sporozoites or not, by co-immunoprecipitation assay. Sporozoites were collected from infected *Anopheles* mosquito midguts at day 19th post-feeding and lysed in 1% CHAPS buffer. Solubilized proteins were then precipitated with anti-RON4 antibodies with protein G Sepharose beads. Western blotting showed that RON2 and RON5 were co-precipitated with RON4, indicating that RON complex is formed in oocyst-derived sporozoites. Next, the functions of RON4 and RON5 were examined by sporozoite-stage specific gene silencing system. The efficiency of salivary gland invasion was significantly decreased in ron4 or ron5 gene repressed sporozoites. These data suggested that sporozoite rhoptry proteins, RON2, RON4 and RON5, are involved in invasion of the salivary glands in a coordinated manner.

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EFFECT OF DECREASING MALARIA TRANSMISSION ON THE GENETIC DIVERSITY OF THE *PLASMODIUM FALCIPARUM* MEROZOITE SURFACE PROTEIN 2 IN A TANZANIAN VILLAGE

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Plasmodium falciparum is a genetically highly diverse organism. The genes coding for blood stage antigens are particularly polymorphic being under immune selection. Understanding this diversity is important when considering potential vaccine candidate antigens. Merozoite surface protein-2 (MSP2) is one of the most polymorphic antigens and has been thoroughly studies as vaccine candidate. MSP2 is coded by the single copy gene, *msp2,* which is widely used as a marker to define *Plasmodium falciparum* diversity. This study assessed the diversity of *msp2* in relation to transmission intensity in a village in Tanzania, where malaria transmission has dramatically declined over the last decades. Asymptomatic individuals, aged 0-84 years, sampled in annual cross-sectional surveys 1994, 1999 and 2010 (n=577, 370 and 758, respectively), were included in the study. Parasite prevalence decreased significantly after 1999 (57.2%, 58.6% and 16.1%). Similarly, the mean number of concurrent clones declined significantly in 2010 (2.72, 2.15 and 1.66, respectively, $P<0.0001$). The proportion of infections that were multiclonal was 61.2% in 1994, 54.4% in 1999 and 36.1% in 2010 ($P<0.0001$). The number of different alleles (defined as 3 base pare size bins) of FC27 type were 21, 23 and 13; and of IC/3D7 was 82, 66 and 53 at the three respective surveys. These data

suggest that the genetic diversity of *P. falciparum* populations is affected by transmission. More in depth analyses including sequencing are in process to clarify more in detail the dynamics of the genetic diversity of the *msp2* gene in this setting.

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FUNCTIONAL CHARACTERIZATION OF RNA REGULATORS IN MALARIA TRANSMISSION

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The malaria parasite has a complex life cycle with two bottlenecks that occur during its transmission between mosquitoes and mammals. These transmission events require translational repression to ensure that only the proper proteins are being expressed, while allowing the parasite to prepare the mRNAs it will need for the next developmental stage. Puf2 is an RNA-binding protein and translational regulator, which when knocked out, causes the parasites in the salivary gland to become less infectious over time. In these *puf2*$^{-}$ parasites, the mRNA abundance of two putative deadenylases (CCR4, CCR4L) increases substantially.CCR4 is a deadenylase with the same functional domain as CCR4L. CCR4 is well characterized in yeast and mammals as a major translational regulator, and initiates the degradation of ribonucleic acids as part of the CCR4-Not complex. These proteins have yet to be well characterized in the malaria parasite, but the lack of a leucine-rich region in CCR4L may indicate that it does not interact with the CCR4-Not complexes suite of proteins and thus may have a specialized function. We have produced transgenic parasites that either knock-out the CCR4 or CCR4L genes, or express epitope-tagged versions of these proteins in order to compare their functions, localizations, binding partners and essentiality. Localization data in blood stage suggests that there is expression of CCR4L in schizonts but little to no expression in ring and trophozoite stages. Characterization of these proteins in the remainder of the life cycle may show that they are potential drug targets and by disrupting their function one might prevent parasite transmission, halt the infection, or reduce the infectivity of the parasite.

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ENHANCING LONGEVITY OF *PLASMODIUM VIVAX* AND *P. FALCIPARUM* SPOROZOITES DISSECTED FROM MOSQUITO SALIVARY GLANDS

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Many unknowns still exist regarding the liver stage forms of *Plasmodium* species that cause malaria. To investigate the liver stage invasion and development, malaria parasites need to be preserved in the infectious sporozoite form by preventing maturation during mosquito dissection. Mimicking salivary gland conditions with a dissection buffer comprised of insect media may aid in the preservation of sporozoites, by creating a more stable transition out of the mosquito. Our goal is to determine which buffer variation prolongs life and viability of sporozoites over time as measured by gliding motility. Variations of Hank's Balanced Salts and Grace's Insect Media were compared to RPMI 1640, the current standard dissection buffer. *Plasmodium vivax* and *P. falciparum* sporozoites were harvested from *Anopheles* mosquitoes into each test buffer, and gliding assays were performed at time 0, 4, 8, and 24 hours post dissection. Gliding percentages were compared for statistical difference between buffers and time points within three experimental groups. *P. vivax* experiments were split into two groups based on whether or not sporozoites were harvested from mosquitoes that were internationally shipped after blood feeding, and *P. falciparum* experiments composed the third group. At time 0 hours, RPMI and Grace's both showed strong gliding percentages in all groups (RPMI: 31-57%, Grace's: 33-57%), but by time 4 hours, RPMI consistently had the lowest gliding percentage (0-35%). Grace's had statistically higher ($p<0.001$) or equivalent gliding percentages compared to all other buffers at time 4, 8, and 24 hours (4 hours: 12-56%, 8 hours: 9-56%, 24 hours: 5-22%). Based on gliding percentages, our variation of Grace's preserved sporozoites over time better than both Hank's variations and the standard dissection buffer RPMI. Using a buffer variation such as Grace's that is more similar to the salivary gland environment, increases access to sporozoites for essential liver and pre-erythrocytic stage studies. *Rapatbhorn Patrapuvich and Alison Roth contributed equally

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ANALYSIS OF THE GENETIC PROFILE OF *PLASMODIUM FALCIPARUM* ISOLATES FROM URBAN AND RURAL AREAS FROM GABON

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Plasmodium falciparum malaria is a major public health problem in Africa. Indeed, in many African countries, new malaria control strategies recommended by the World Health Organization (WHO) have been adopted during the last decade. In Gabon, the deployment of these was followed by a decrease and a rebound in malaria prevalence, a change in the age of populations at risk, suggesting a changing epidemiology. It is therefore important to assess the impact of these strategies on the genetic diversity of circulating parasites. The aim of the study was to analyze and to compare the allelic diversity of *Plasmodium falciparum* isolates in Gabon. Febrile children and adults were recruited in 2011 at Oyem, Port-Gentil and Libreville. All patients had a malaria diagnosis based on microscopy. Peripheral blood samples were collected from and msp1 and msp2 were analysed by nested- PCR in malaria positive samples. The allelic family Ro33 was the most frequent (> 50%) in isolates from all sites. The highest diversity was found in the K1 allelic family with a total of 14 alleles while 10 and one Mad20 and Ro33 alleles was identified, respectively. The majority of 3D7 alleles was detected in Libreville and Oyem whereas most of the FC27 alleles was found at Oyem. Among isolates, 42 msp2-type alleles were detected, with nearly half belonging to each allelic family. The complexity of infections was the highest with msp1 gene: 1.95 in Port-Gentil, 1.91 and 1.66 in Oyem and Libreville. With the msp2 gene it was 1.33 in Port-Gentil, Libreville 1.24 and 2.15 Oyem. A significant allelic diversity was found in all isolates. The complexity of infections and the different genetic profile of the detected parasite strains varying according to the site suggest a heterogeneous transmission in the different sites. However, strategies for malaria control appear to have a limited impact on the diversity of *Plasmodium* strains.

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GENETIC DIVERSITY OF *PLASMODIUM FALCIPARUM* IN HAITI: INSIGHTS FROM MICROSATELLITE ANALYSIS

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Hispaniola is the only Caribbean island with endemic malaria, and the majority of malaria cases, all due to *Plasmodium falciparum*, are reported in Haiti. Recently, there have been renewed discussions on malaria elimination for Hispaniola, with the focus on interventions in Haiti. Effective antimalarial treatment policies are crucial to successful malaria elimination programs. Presently, Haiti employs chloroquine + primaquine regimen for patients diagnosed with malaria. Although antimalarial resistant *P. falciparum* is not present on a wide scale in Haiti, the emergence of chloroquine resistance remains a threat in Haiti.

Previous studies investigating *P. falciparum* evolution in Haiti in response to widespread antimalarial use have focused on specific genes associated with antimalarial resistance. The present study takes a broader approach to understanding *P. falciparum* evolution by measuring genetic diversity using microsatellite loci located on multiple chromosomes. Eighty-nine samples were collected on blood spot cards from Terre Noire, Leogane, Jacmel, Chabin, Nippes, North Cap Haitien, and Hinche between 2010 and 2013. DNA was extracted and amplified for 12 putatively neutral microsatellite loci. Based on analysis of five loci (*TA1*, *TA60*, *POLY*α, *ARA2*, and *Pfg377*), we identified multiple infections in 6.3% of our samples. Expected heterozygosities for the five loci ranged from 0.55 to 0.72, suggesting a highly diverse *P. falciparum* population in Haiti. Future analysis of all 12 loci will compare the level of diversity across multiple geographic sites and assess population structure within Haiti. Overall genetic diversity and geographic distribution of parasite diversity can aid in understanding present malaria transmission and impact of antimalarial drug use in Haiti.

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DISTINCT ESSENTIAL FUNCTIONS FOR POLYAMINES BIOSYNTHESIS ENZYMES IN MALARIA PARASITE BLOOD AND MOSQUITO STAGES DEVELOPMENT

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Polyamines are positively charged organic molecules that play important roles in cell cycle regulation, cell proliferation, senescence and death of eukaryotic cells. Polyamine analogues have been considered and applied in cancer therapy. Moreover, DFMO (α-Difluoromethylornithine), one of the main drugs targeting African Trypanosomiasis, is an inhibitor of Ornithine Decarboxylase, an essential enzyme in the biosynthesis of polyamines. Despite of the importance of this pathway as possible target for multi-stage malaria intervention, little is known about the cellular functions of polyamine biosynthesis enzymes for *Plasmodium* development. We applied gene-targeting techniques for *Plasmodium yoelii* to target enzymes of this pathway for deletion. Our results indicate that the bifunctional Ornithine Decarboxylase/ S-Adenosylmethionine Decarboxylase (ODC/ SAMDC) enzyme is not essential for sexual and asexual blood stage (BS) development. However, BS growth was reduced in odc/samdc(-) and male gamete exflagellation was completely abolished with no development of mosquito stages. Spermidine Synthase, the downstream enzyme of ODC/ SAMDC, was shown to be essential for BS development by knock-out/ knock-in approach. These results indicate alternative essential roles for the polyamine biosynthesis enzymes during BS and early mosquito stages development. This validates the enzymes of this pathway as multi-stage drug targeting candidates.

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START1 IS A MITOCHONDRIAL PROTEIN INVOLVED IN UBIQUINONE METABOLISM IN *PLASMODIUM FALCIPARUM*

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The mitochondrial electron transport chain (mtETC), which utilizes ubiquinone (Coenzyme Q or CoQ) as an electron carrier, is a validated target for antimalarial drugs such as atovaquone. CoQ is also involved in the pyrimidine biosynthesis pathway, which is essential for parasite survival. Genes encoding several of the CoQ biosynthesis enzymes have been identified in *Plasmodium falciparum*; however, much remains unknown about CoQ synthesis and interactions in the mtETC. We have identified START1, a mitochondrial protein that complements *Saccharomyces cerevisiae* Coq10p, despite minimal homology. In *S. cerevisiae*, disruption of *COQ10* results in inhibition of mitochondrial respiration and impaired – but not inhibited – CoQ synthesis. It has been suggested that Coq10p binds to CoQ, and is likely involved in the transport of CoQ within the mitochondria. This is supported by overexpression of *COQ10*, which leads to deficient respiration, presumably through sequestering CoQ and preventing its use in the mtETC. Given *COQ10*'s essential role in yeast, and the pressing need for new antimalarial drug targets, our aim is to characterize the role of START1 in *P. falciparum*. To support the hypothesis that START1 is essential, we are attempting to disrupt the endogenous gene in wild type parasites and in parasites expressing an exogenous copy of the gene. We are also generating parasites in which endogenous *START1* is tagged with the GlmS ribozyme sequence for a regulatable mRNA knockdown. We are ascertaining whether overexpression of START1 in parasites results in a similar phenotype to that of *S. cerevisiae*. Finally, we are identifying whether START1 can be found in complex with other proteins in parasites by immunoprecipitation and blue native gels. This research will characterize a new and important element of the mtETC and ubiquinone pathways in *P. falciparum*.

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EUPATHDB: AN ONLINE GENOMICS RESOURCE FOR EUKARYOTIC PATHOGENS

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The Eukaryotic Pathogen Database Resource (EuPathDB) is a family of free online databases that integrate genomic data with functional genomics and clinical/field isolate data for numerous eukaryotic pathogens within Amoebazoa, Apicomplexa, Diplomonadida, Microsporidia, Trichomonadida, Kinetoplastida. EuPathDB also integrates data informing upon host-parasite interactions from datasets that analyze mixed parasite-host samples such as human cells infected with parasites. An interactive data exploration platform, EuPathDB provides data mining and visualization tools for discovering meaningful relationships between

genomic features to support hypothesis-driven research. The databases are updated and expanded bimonthly with data ranging from genome sequence and annotation to expression data, to parasite field isolates, to host data in response to infection. Despite the breadth of data (140 genomes, 150 functional datasets), it is easy to mine, visualize, download and browse different data types. Data is mined using the Strategy System to search within and between datasets, developing in silico experiments that identify features with similar biological characteristics. Search strategies and results can be downloaded, saved and shared with a colleague. Data may be visualized in the context of the genome sequence and annotation using an interactive and configurable browser. Individual record pages that compile all available data for a feature (e.g. gene, isolate, genomic sequence) provide a comprehensive view of the feature. Our extensive user-support system includes video tutorials, a rapid-reply email question hotline, and hands-on workshops at locations worldwide. Attend our poster or exhibit booth for an overview of this NIH/NIAID-funded resource. Or visit one of our sites: AmoebaDB.org, CryptoDB.org, EuPathDB.org, GiardiaDB.org, MicrosporidiaDB.org, PiroplasmaDB.org, PlasmoDB.org, ToxoDB.org, TriTrypDB.org, TrichDB.org, OrthoMCL.org, and HostDB.org.

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DETECTION OF *PLASMODIUM FALCIPARUM* IN THE BLOODMEAL OF *ANOPHELES GAMBIAE* USING QUANTITATIVE NUCLEIC ACID SEQUENCE BASED AMPLIFICATION (QT-NASBA)

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Control of the human-to-mosquito transmission of *Plasmodium* is being increasingly explored through drugs against sexual parasite forms in the human and mosquito, transmission blocking vaccines, and mass drug administrations. Successful implementation of these measures will require a greater understanding and quantitation of stage-specific infections of mosquitoes from humans. Past work on the human to mosquito portion of the *Plasmodium* life cycle has primarily focused on dissection and staining of wild blood fed mosquito midguts for early sexual forms and later stage oocysts, or examination of human infectiousness by direct skin feeds and standardized membrane feeding assays. These approaches, while important, tend to be laborious and may lack sensitivity for early infection events. As an alternative, we present a method of detecting the acquisition of *P. falciparum* in the bloodmeals of field caught *Anopheles gambiae* utilizing the modern molecular technique of Quantitative Nucleic Acid Sequence Based Amplification. QT-NASBA was performed on individual and pooled RNA extracted from fresh bloodmeals preserved on FTA cards for detection of Pfs25 transcript. We were able to detect *P. falciparum* in 5 of 6 RNA pools and 5 of 6 individual mosquitoes. Negative controls of RNA extracted from bloodmeals taken from uninfected individuals were all negative. Future experiments will focus on discrimination of this technique and parasite quantification from laboratory infected and naturally-infected mosquitoes. We see a variety of uses for this approach, including the correlation of human gametocytemia detected by QT-NASBA on human blood spots to the presence of early sexual forms in mosquitoes that bite upon these same individuals, performing sensitive spatial and temporal microepidemiology, and investigating biting tendencies of wild mosquitoes as they relate to human gametocytemia in a natural setting. These measures should lead to a more complete understanding of *Plasmodium* transmission, and may become an important measure to validate transmission-blocking interventions.

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DEVELOPMENT OF A SINGLE-CELL GENOMICS PLATFORM FOR MALARIA INFECTIONS

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Mixed- or multiple-clone malaria infections are commonplace in areas of high endemicity, are a major outcome in drug and vaccine efficacy trials, and can confound most parasite genetic studies. Though, surprisingly, their composition is still poorly understood. A major roadblock in exploring multiple-clone infections is that current genome sequencing tools only allow us to examine infections in bulk, providing little information on individual parasite genomes within an infection. To address this we have developed a single-cell genomics approach to dissect multiple-clone infections. By combining cell sorting and whole genome amplification we are able to capture single malaria parasite-infected red blood cells and generate sufficient high quality material for genomic analysis. We optimized our approach by assaying >260 single cells across fourteen experimental conditions. To quantify accuracy, we created artificial mixtures of *Plasmodium falciparum* laboratory lines (Hb3/Dd2/3D7) and obtained highly accurate (>99.9%) single cell genotypes. We genome sequenced 4 single cell amplifications obtained from these mixtures (Hb3 (n=2), 3D7 (n=2)), confirming 99.29% of the 196,332 SNP calls made across these 4 sequencing reactions. We saw no contamination from other genomes present in the mixtures in single cell genome sequences. This single cell genomics platform can be extended to malaria parasite species where long-term culture is not possible, precluding the direct dissection of infections through dilution cloning. We performed single cell genotyping and single cell sequencing of *P. vivax* infections obtained directly from patients, and obtained comparable accuracy and coverage to *P. falciparum* assays. These methods open the door for large scale analysis of within-host variation of malaria infections at single cell resolution.

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POPULATION GENETICS AND NATURAL SELECTION OF CANDIDATE MALARIA VACCINE ANTIGENS IN MALIAN *PLASMODIUM FALCIPARUM* ISOLATES

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Vaccination, in combination with other control measures, is an essential strategy for malaria eradication. However, the highly polymorphic nature of many candidate vaccine antigens can result in allele-specific immune responses that limit vaccine efficacy against diverse strains found in nature, as has been observed with vaccines based on apical membrane 1 antigen and merozoite surface protein 1. Little is known about the genetic diversity in field isolates of the next generation of vaccine antigens even as vaccines based on these antigens are entering Phase 1 clinical testing. Some of these candidates include the liver and blood stage antigen merozoite surface antigen 5 (MSP5), the blood stage antigens glycosylphosphatidylinositol-anchored micronemal antigen (GAMA), and the RH5-interacting protein (PfRipr). We estimated the haplotype prevalences and genetic diversity within vaccine antigen-encoding genes in 91 specimens collected from asymptomatic infections and clinical malaria

episodes experienced by children in Bandiagara, Mali. We hypothesized that field isolates of at least some of these candidate antigens would exhibit low genetic diversity that would predict cross-protective efficacy against heterologous strains found in endemic areas. We estimated heterozygosity of the full-length malaria vaccine antigen sequence by the parameter π, estimate D* and F* to detect significant departures from the neutral model with *Plasmodium* reichenowi, the chimpanzee malaria parasite as the out-group, and test for balancing selection using Tajima's D test. Preliminary results indicate that MSP5 and PfRipr are well-conserved while GAMA is highly polymorphic. The results from this study will be used to down select conserved candidate antigens and antigenic variants for possible inclusion in a broadly cross-protective, multivalent malaria vaccine.

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A NEW MALARIA VACCINE CANDIDATE, GPI PROTEIN TRANSAMIDASE RELATED PROTEIN SHOWED GOOD PROTECTIVE IMMUNE RESPONSE IN MOUSE MODEL

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Following a genome-wide search for a blood stage malaria candidate with GPI anchor motif using bioinformatics, and DNA-based vaccine screening and mouse malaria model, our study identifies PyGPI8p-transamidase related protein (PyTAM) as hypothetical protein with a protective immune response (Shuaibu et al., 2010). The homology search of PYTAM molecule using Conserved Domain Architecture Retrieval Tool (CDART) reveled that it's belonging to Cysteine proteases C13 family. Cysteine peptidases of parasitic protozoa have been implicated in a variety of biological events including invasion of host cells, immune evasion, pathogenesis and virulence and proteolytic degradation of hemoglobin and therefore some have also been validated as drug targets. Interestingly, we have found by immunofluorescence staining and immune-electron microscopy that the molecule is cytosolic at ring stage, and then located finally onto the parasitophorous vacuole at the late schizonte. Also, we successfully demonstrated the vaccination efficacy of PYTAM candidate as DNA vaccine formulated with Nanoparticle delivery system in controlling malaria in mouse model, as previously reported. Although the protection enhancing mechanism is not clearly understood. It is clear that antibody levels, was not significantly increased but Th1-mediated antigen-specific immune responses (INF-γ producing CD4 and CD8 T cell) was significant. Currently, we are working on molecular characterization of PYTAM and its mechanism of protective immune responses. These data indicate that PyTAM could a promising malaria vaccine candidate for further development.

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IMMUNOGENICITY AND EFFICACY OF CHAD63-MVA ME-TRAP PRIME-BOOST VACCINATION AGAINST *PLASMODIUM FALCIPARUM* INFECTION IN HEALTHY ADULTS IN SENEGAL

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Malaria transmission is in decline in some parts of Africa, which is partly due to the scaling up of control measures. Previous attempts at malaria elimination ended with mixed success. It is currently agreed that additional control measures including vaccination will be required. Recent studies using viral vectors with prime-boost approach to deliver ME-TRAP (multiple epitopes thrombospodin related adhesion proteins) showed promising safety, immunogenicity and significant efficacy in sporozoite challenge studies. Our study reports on the efficacy and immunogenicity of the prime-boost vaccines in peri-urban area of Dakar West Africa. We conducted a single-blind, randomised controlled phase IIb efficacy trial of 120 healthy men aged 18-50 years, living in a malaria endemic area of Senegal.Eligible study participants were randomised to receive either the active vaccine ChAd63 encoding the pre-erythrocytic antigen ME-TRAP as prime vaccination, followed eight weeks later by Modified Vaccine Ankara also encoding ME-TRAP as booster or two doses of anti-rabies vaccine as comparator . They were all followed up for eight months. The immunogenicity was determined by ELISPOT to quantify T cells responses to TRAP, and ELISA to identify the specific antibodies. We used finger-prick thick blood film to evaluate parasitemia throughout the study follow-ups. We determined time to first *P. falciparum* infection and re-infection by Real time PCR. Prior to intensive PCR survey, all study participants received a 3-day malaria treatment with atovaquone-proguanil and artesunate at the end of the vaccinations to clear all traces of parasitaemia. We also assessed the reactogenicity by recording all adverse and serious adverse events which occurred during follow-ups. This pre-erythrocytic malaria vaccine is safe and induces high immunogenicity with a mean T-cell response at 1266 SFU/106 PBMCs compared to 84SFU/106 PBMCS for the control group. qPCR Data analysis is ongoing and efficacy results will be presented at the meeting Vaccine efficacy against infection in adults may be rapidly assessed in peri-urban areas using this efficient trial design

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HIGH PARASITE DENSITY IS ASSOCIATED WITH DIMORPHISM VARIATION IN THE ID1 DOMAIN OF VAR2CSA DURING PREGNANCY-ASSOCIATED MALARIA

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Sequestration of *Plasmodium falciparum* infected erythrocytes (EI) in the placenta is the key phenomenon that characterizes the placental malaria. This feature is conferred to EI by their ability to bind Chondroitin Sulphate A (CSA) molecules expressed on the syncytiotrophoblastes. Immunity acquired by multigravidae allows controlling parasite density which limits the impact on the pregnancy outcomes. VAR2CSA has been identified as the main *P. falciparum* erythrocytes membrane protein involved in this interaction and is therefore considered as the major target of the acquired protective antibodies. But, the sequences polymorphism observed in VAR2CSA is one of the biggest challenges to be overcome in order to achieve development of an effective VAR2CSA-based vaccine. The N-terminal part of VAR2CSA has been indicated to 1°) contains the minimal CSA-binding site and; 2°) induce adhesion blocking antibodies with cross-reactive properties. In this study, we analyzed the sequence polymorphism in the N-terminal part of VAR2CSA expressed by field isolates and investigated the relationship between a particular genotype and the ability to bind CSA, the parasite density and other mother-related factors and pregnancy outcomes. A total of 398 NTS-ID2a sequences were generated from transcripts of 45 isolates collected from Beninese pregnant women resulting in 92 distinct sequences at protein level. The analysis demonstrated the existence of a dimorphic region within the structurally critical ID1 domain that revealed a very interesting association with the occurrence of infections with very high parasite density. Primers specific for this polymorphism were designed and this association was further validated on a second study population. Sequence analyses have helped define a distinct cluster of parasites, without any geographical bias and representing 20% of all analyzed clones. These observations are of

relevance to understand the molecular mechanisms mediating the severity of malaria infection in pregnant women and indicate interesting ways for potential optimization in the ongoing effort to develop a VAR2CSA based vaccine.

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INTRODUCTION OF MALARIA VACCINE IN NIGERIA: STATUS AND PROGRESS UPDATE

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Malaria is endemic in Nigeria. About 97% of the population is at risk and it accounts for 25% under-5 mortality. Artemisinin-based Combination Therapy and Intermittent Preventive Treatment are current curative and preventive interventions. Clinical trials using malaria vaccine were conducted in Enugu and Jos, Nigeria. The RTS,S/AS01 coadministered with Expanded Programme on Immunization (EPI) vaccines provided modest protection against both clinical and severe malaria in young infants. Meetings were held in-country to discuss the pros and cons, in preparation for the adoption of malaria vaccine in Nigeria. Nigeria officials held Stakeholder and advocacy meetings with facilitators from MVI PATH in 2011 and 2012. Key informants were interviewed about national policy decision making processes on adopting new malaria control interventions and new vaccines in Nigeria and the readiness of the health system in adopting the candidate vaccine. The results were analyzed by content analysis. Progress is being made with adoption of malaria vaccine in Nigeria. There was no National Immunization Technical Advisory Group/Committee for all vaccine preventable diseases in Nigeria, and no standard documented guideline for decision making process for the adoption of new vaccines. The existing public-private partnership for the adoption of Human Papilloma Virus vaccine and Expanded Programme on Immunization in Nigeria was proposed for Malaria vaccine. Challenges foreseen with the decision-making process include large population size, weak health system, resistance to change by health staff, inadequate trained staff, high cost of implementation, budgetary deficits, inadequate cold chain and storage supply. A Ministerial memo has been sent to the National Council of Health and the Federal Executive Council. A proposal presentation would be made to the Technical Working Group on Malaria and relevant stakeholders. Lessons will be learnt from neighbouring countries with functional health system. Funding will be harnessed from World Health Organization and Global Alliance for Vaccine and Immunization. A proper cost benefit analysis would be done to ascertain cost-effectiveness. High level advocacy will be carried out at all levels.

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EVIDENCE OF SELECTION IN POLYMORPHIC *PLASMODIUM FALCIPARUM* MEROZOITE ANTIGENS DURING THE RECOVERY OF CHILDREN FROM MALARIA

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Several *Plasmodium falciparum* merozoite invasion genes are highly polymorphic, potentially allowing the parasite to evade the host's immune responses and utilise alternative invasion pathways. The extent to which immune evasion shapes the parasite's population dynamics in natural infections is unknown. The Reticulocyte binding homologue (Rh), erythrocyte binding antigen (EBA), merozoite surface protein (MSP) 3-like gene families, apical membrane antigen (AMA) 1 and MSP1 have been shown to be involved in invasion as antibodies to these proteins inhibit invasion *in vitro*. Fifteen of these merozoite genes were sequenced from parasite DNA isolated from children, under 5, with uncomplicated malaria recruited into a drug trial between 2007and 2008, which consisted of pre (samples at recruitment)- and post (malaria slide positive sample during 84 days of weekly follow up) -treatment samples. The AMA1, MSP142, EBA175, MSPDBL1, MSPDBL2 and Rh2b loci were 100% heterologous genotypes in pre- and post-treatment parasite pairs. In contrast, 70% of the pre- and post-treatment parasites were homologous genotypes at the Rh5 locus. An analysis of the proportion of individual alleles revealed that the proportion of certain alleles in MSP142, EBA175, EBA181, Rh5 and AMA1 loci were significantly different ($p<0.05$) pre- and post-treatment. These changes in allele frequencies reflect the highly polymorphic nature of these antigens and suggest there may be a selective mechanism potentially allowing the parasite to evade immune responses, by allele-specific immunity. The allele proportions of MSP3, MSP6, MSPDBL2 and Rh1 remained similar pre- and post-treatment, suggesting that they may generate cross-reactive immune responses and thus there is no distinction between the multiple allelic types. In summary, the antigens which showed allele frequency changes may require a multi-allelic approach for vaccine development. Also, the antigens that showed 100% heterologous parasite pairs are good candidates for discriminating between recrudescent and new infections in antimalarial drug trials.

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IDENTIFICATION OF THE NOVEL TRANSMISSION-BLOCKING VACCINE TARGET EXPRESSING ON THE SURFACE OF MALE GAMETES

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Plasmodium transmission from mosquitoes to mammalians requires sexual stage parasite development and fertilization in mosquito midguts. After ingestion of gametocytes by mosquitoes, fertilization occurs to form zygotes, which develop into invasive forms, ookinetes. These stages could be promising targets for transmission blocking strategy, which intends to break malaria life cycle inside the mosquito vector. Despite large research effort on screening for parasite molecules expressing on the surface of gamete, zygote or ookinete, the number of the candidate antigens of transmission-blocking vaccine is still limited. Previously, we reported that a novel male specific protein, designated PyGM75, is localized to the osmiophilic bodies of male gametocytes then transported to the surface of microgametes in *Plasmodium yoelii*. Furthermore, we demonstrated that pygm75 disrupted parasites impaired the exflagellation ability. In this study, we investigated whether PyGM75 can be a novel transmission-blocking vaccine target. First, to examine if anti-PyGM75 antibodies can prevent parasite transmission to mosquitoes, we mixed the specific antibodies and parasitized RBCs, then let them feed on *Anopheles* mosquitoes using membrane-feeding system. As the result, the numbers of oocysts formed on the mosquito midgut were greatly reduced by adding anti-PyGM75 antibodies, as a dose dependent manner. In addition, the antibodies strikingly impaired the motility of microgametes *in vitro*. Taken together, our data demonstrates that anti-PyGM75 antibodies have potential to prevent the malaria transmission, presumably by interfering fertilization occurred in midguts. Since PyGM75 is conserved among *Plasmodium* species, including *P. falciparum* and *P. vivax*, this study strongly suggests that GM75 is a novel candidate target of transmission-blocking vaccine.

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INVESTIGATING THE ROLE OF GILT ON IMMUNE RESPONSES TO A MALARIA VACCINE ANTIGEN

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Malaria ranks fifth as leading cause of death from infectious diseases. Our overall research goal is focused on the development of a malaria transmission-blocking vaccine (TBV). One such vaccine target (Pfs48/45 protein) is based on antigens expressed on male and female gametocytes, which establish infection in the mosquito vector. The targets of blocking antibodies are disulfide bond constrained conformational epitopes. As Pfs48/45 has not been crystallized, precise location of the disulfide bonds

and the topology of epitopes are unknown. The disulfide bonds have been shown to greatly influence the ability of an APC to process and present epitopes, and elicitation of an appropriate immune response. GILT, a thiol reductase constitutively expressed in APCs, mediates endocytic reduction of antigens and display of peptides on MHC class I and II. Using recombinant Pfs48/45 as the model antigen, this project seeks to identify mechanisms involved in presentation of relevant epitopes to T and B cells leading to effective antibody responses and generation of the memory B cell pool. We hypothesize that reduction of disulfide bonds in Pfs48/45 will dramatically impact the generation of T cell epitopes, and thus influence downstream B cell stimulation and protective antibody responses. We have conducted immunogenicity studies in wildtype and GILT-/- mice using both non-reduced and reduced forms of Pfs48/45 and analyzed the responses using full length and five overlapping (~100aa long, spanning full-length Pfs48/45) sub-fragments and 39 peptides by ELISA, western blotting, ELISpot and T cell proliferation assays. Results from these studies have indeed revealed significantly different patterns of recognition of putative B and T cell epitopes. These ongoing studies have revealed the presence of immunodominant B cell and T cell epitopes in the sub-fragment 2 (aa 108-200) and sub-fragment 5 (aa 341-420), respectively. We are now initiating studies to investigate the influence of immune epitopes in functional responses and transmission-blocking protection. Results of the study will impact vaccine considerations.

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IDENTIFICATION OF CORRELATES OF DISEASE AND PROTECTION IN A CONTROLLED HUMAN MALARIA INFECTION MODEL

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Long-lasting protection against homologous *Plasmodium falciparum* infection can be induced in malaria naive subjects taking chloroquine prophylaxis during immunizations with bites of infected mosquitoes followed by a Controlled Human Malaria infection (CHMI). However, identification of correlates of disease and protection, which could be prospectively explored as biomarkers for host protective immunity to malaria, is still critically limited. Here, we monitored the kinetics of the human immune response at the transcriptomic and cellular level during immunizations and controlled infection. Direct ex-vivo whole blood gene expression profiling and cell subset analysis was performed using dual-color Reverse-Transcriptase Multiplex Ligation-dependent Probe Amplification (dcRT-MLPA) and polychromatic (16-color) flow cytometry, respectively. Volunteers from three CHMI trials were included in this study: (1) a mosquito/sporozoite dose titration CHMI using chloroquine prophylaxis during immunizations, (2) a non-inferiority CHMI comparing the efficacy of chloroquine to mefloquine prophylaxis during immunizations, and (3) a heterologous challenge of previously (under chloroquine prophylaxis) immunized and homologously protected volunteers. Genes differentially expressed between protected and unprotected volunteers were identified by the Global test at each time point during vaccination and challenge. Biomarker signatures for disease and protection were generated using Lasso and Ridge regression analysis. Critical signalling networks that discriminate between protective and non-protective immune responses will now be generated by pathway analysis. In addition, key cellular changes, both quantitative and qualitative, have been analyzed by multi-parameter flow cytometry. This will be followed by integration of the molecular and cellular datasets to correlate data to protective immunity and/or disease.

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PROFILING THE HUMORAL IMMUNE RESPONSES TO *PLASMODIUM VIVAX* INFECTION AND IDENTIFICATION OF CANDIDATE IMMUNOGENIC RHOPTRY-ASSOCIATED MEMBRANE ANTIGEN (RAMA)

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Completion of sequencing of the *Plasmodium vivax* genome and transcriptome offers the chance to identify antigens among >5000 candidate proteins. To identify those *P. vivax* proteins that are immunogenic, a total of 152 candidate proteins (160 fragments) were expressed using a wheat germ cell-free system. The results of Western blot analysis showed that 92.5% (148/160) of the targets were expressed, and 96.6% (143/148) were in a soluble form with 67.7% of solubility rate. The proteins were screened by protein arrays with sera from 22 vivax malaria patients and 10 healthy individuals to confirm their immune profile, and 44 (27.5%, 44/160) highly reactive *P. vivax* antigens were identified. Overall, 5 candidates (rhoptry-associated membrane antigen [RAMA], Pv-fam-a and -b, EXP-1 and hypothetical protein PVX_084775) showed a positive reaction with >80% of patient sera, and 21 candidates with 50% to 80%. More than 23% of the highly immunoreactive proteins were hypothetical proteins, described for the first time in this study. One of the top immunogenic proteins, RAMA, was characterized and confirmed to be a serological marker of recent exposure to *P. vivax* infection. These novel immunoproteomes should greatly facilitate the identification of promising novel malaria antigens and may warrant further study.

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IMMUNOGENICITY OF A SYNTHETIC VACCINE BASED ON THE *PLASMODIUM VIVAX* DUFFY BINDING PROTEIN REGION II

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Molecules that play a role in *Plasmodium* merozoite invasion of host red blood cells represent attractive targets for blood-stage vaccine development against malaria. In *Plasmodium vivax*, merozoite invasion of reticulocytes is mediated by the Duffy binding protein (DBP), which interacts with its cognate receptor, the Duffy antigen receptor for chemokines (DARC) on the surface of reticulocytes. The DBP ligand domain, known as region II (DBPII), contains the critical residues for receptor recognition making it a prime target for vaccine development against blood-stage vivax malaria. In natural infections DBP is weakly immunogenic and DBPII allelic variation is associated with strain-specific immunity, which may compromise vaccine efficacy. In a previous study, a synthetic vaccine termed DEKnull that lacked an immunodominant variant epitope in DBPII induced functional antibodies to shared neutralizing epitopes on the native Sal1 allele. Anti-DEKnull antibody titers were lower than anti-Sal1 titers but produced more consistent, strain-transcending anti-DBPII inhibitory responses. In this study, we further characterized the immunogenicity of DEKnull, finding immunization with rDEKnull produced an immune response comparable to native recombinant DBP alleles. Further investigation of DEKnull is necessary to enhance its immunogenicity and broaden its specificity.

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TARGETING MALARIA PARASITE INVASION OF RED BLOOD CELLS: HOST-CELL ENGAGEMENT AND ANTIBODY NEUTRALIZATION

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Host-cell invasion is a critical step in the pathogenesis of malaria. Erythrocyte Binding Like (EBL) ligands mediate critical interactions during RBC invasion by *Plasmodium falciparum* and *P. vivax*, and are targets of antibodies that play a role in naturally acquired immunity. However, the structural basis and mechanisms of host-cell engagement, as well as the neutralization mechanisms of antibodies that prevent parasite growth have not been defined. Lack of this knowledge has hampered the effective rational design of agents to disrupt invasion. We will present structural, mechanistic and functional studies of EBL ligands from both *P. falciparum* and *P. vivax* in association with receptors and antibodies. These studies demonstrate that multimeric assembly of receptor-ligand interactions is crucial for host-cell invasion, and highlight critical functional regions that can be exploited for targeted disruption, including receptor-binding pockets and multimeric assembly interfaces. We found that potently neutralizing antibodies target the assembly interfaces and receptor-binding residues, while non-neutralizing antibodies target decoy epitopes far removed from functional regions of ligands. These results explain why only a subset of antibodies that recognize EBL ligands are neutralizing. The results reveal the complex nature of EBL-RBC interactions and highlight new approaches to target the molecular mechanism of invasion. Vaccine efficacy may be improved by targeting critical functional regions and protective epitopes of EBL ligands, while avoiding decoy-epitopes identified by these studies.

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A NEXT GENERATION GENETICALLY ATTENUATED *PLASMODIUM FALCIPARUM* PARASITE CREATED BY TRIPLE GENE DELETION

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A malaria vaccine could dramatically reduce the tremendous morbidity and mortality due to *Plasmodium* parasites. Immunizations with live-attenuated sporozoites in animal models and humans have shown the feasibility of a pre-erythrocytic malaria vaccine that confers complete and protracted protection against infection. Genetic engineering offers a versatile platform for controlled and consistent design of pre-erythrocytic genetically attenuated parasites (GAPs) as vaccine candidates. We previously generated a GAP by deleting the *P. falciparum* P52 and P36 genes (Pf p52-/p36- GAP) preclinical assessment of which indicated an early liver stage growth defect. However, human exposure to >200 Pf p52-/p36- GAP-infected mosquito bites caused peripheral parasitemia in 1 of 6 safety trial volunteers, revealing that this GAP was severely but incompletely attenuated. We modeled this phenotype with rodent malaria *P. yoelii* p52-/p36- GAP in highly susceptible Balb/cByJ mice. Encouragingly, Pf p52-/p36- GAP induced substantial human immune responses including antibodies that blocked *in vitro* hepatocyte infection by sporozoites. We have now created a triple gene deleted GAP by additionally removing SAP1 (Pf p52-/p36-/sap1- GAP). SAP1 deletion alone was sufficient to completely attenuate *P. yoelii* in Balb/cByJ mice. Deletion of genes whose encoded proteins perform distinct biological functions (as do P52, P36 and SAP1) should improve the robustness of attenuation and greatly reduce the possibility of compensatory changes. Pf p52-/p36-/sap1- GAP and wildtype parasites were indistinguishable in blood and mosquito stages of development. Using an improved humanized mouse model transplanted with human hepatocytes and erythrocytes, we demonstrate that despite a high dose sporozoite challenge, Pf p52-/p36-/sap1- GAP did not transition to blood stage infection and appeared completely attenuated. We also used FLP/FRT recombination to remove all drug selectable markers from Pf p52-/p36-/sap1- GAP. Preparations are underway to test this next generation GAP in a human phase I clinical trial.

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IMMUNOSCREENING OF THE TARGET PROTEINS CONTRIBUTING TO GROWTH INHIBITORY ACTIVITY OF HUMAN IGG AGAINST *PLASMODIUM FALCIPARUM*

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Residents in malaria endemic area acquire the protective antibodies against the disease by continuous infection with *Plasmodium falciparum*. It has been shown that a part of the antibodies have growth inhibitory activity (GIA) against *in vitro* cultured *P. falciparum*. We then hypothesized that identification of responsible antigens to GIA may support the discovery of novel blood-stage vaccine candidates. The GIA of individual IgG purified from a Malian immune adult was measured (n=51). To identify the antibody responses correlating with GIA, we established a modified AlphaScreen system for high-throughput detection of antigen-antibody reaction. Genome-widely expressed 1,848 parasite proteins by a wheat germ cell-free system were screened with the Malian adult IgGs. Known malaria vaccine candidates were also included in the 1,848 proteins. First, we selected approximately 900 immunoreactive proteins with the Malian adult IgGs (giving the AlphaScreen count more than negative control). Of those, we selected antigens which showed significant positive correlations between their AlphaScreen counts and GIA. As a result, the ratio of the known malaria vaccine candidates among the finally selected antigens was significantly higher than that in the original 1,848 proteins. The result suggests that this screening system would be effective to discover novel malaria blood-stage vaccine candidates. In addition to the analysis of the individual antigens, we are now analyzing which combination of antigens will better explain the GIA of total IgG. The results of this analysis will be discussed in this presentation.

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CHARACTERIZATION OF *PLASMODIUM FALCIPARUM* MAS170 AS NOVEL MALARIA BLOOD-STAGE VACCINE CANDIDATE

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A novel 170-kDa merozoite apical surface protein of *Plasmodium falciparum* (PfMAS170) was characterized as a novel malaria blood-stage vaccine candidate in this study. The PfMAS170 is conserved among *Plasmodium spp.* and is predicted as 170-kDa protein with a signal peptide at the N-terminus. We expressed recombinant protein corresponding to the C-terminal region of PfMAS170 using a wheat germ cell-free system to obtain anti- PfMAS170 sera. Western blot analysis detected approximately 170-kDa signal corresponding to the full-length of PfMAS170 in late blood-stage parasites, and PfMAS170 fragments from approximately 120-kDa to 30-kDa were released in the culture medium. Immunofluorescence assay of free-merozoite without Triton X-100 permeabilization revealed that PfMAS170 localizes on the surface of apical end of merozoite. Erythrocyte binding assay of the conditioned culture medium showed that the secreted 30-kDa fragment of PfMAS170 binds to erythrocyte

surface. In order to test whether antibodies to PfMAS170 could block merozoite invasion, growth of *P. falciparum* 3D7 parasite in the presence of anti-PfMAS170 antibody was tested. The anti-PfMAS170 antibody significantly inhibits the merozoite invasion to erythrocytes. Since anti-PfMAS170 antibodies inhibited merozoite invasion *in vitro*, we decided to investigate whether PfMAS170 is exposed to the human immune system in *P. falciparum*-infected individuals. The sera from *P. falciparum* infected individuals in Thailand reacted with the recombinant PfMAS170, indicating PfMAS170 is immunogenic in humans. Taken together, these results suggested that PfMAS170 plays important role in the merozoite invasion process. The C-terminal erythrocyte-binding domain is of interest for the development of blood-stage malaria vaccine.

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EVALUATING THE POTENTIAL IMPACT OF TRANSMISSION BLOCKING VACCINES AGAINST *PLASMODIUM FALCIPARUM* INFECTION ALONGSIDE EXISTING INTERVENTIONS

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Transmission-blocking vaccines are under development with the aim that they will reduce or interrupt transmission in malaria endemic settings when used alongside existing interventions. However, their utility will depend not only on their efficacy and durability, but also on characteristics of the transmission setting including the intensity and seasonality of transmission. We extended a published mathematical model to identify settings in Sub-Saharan Africa in which a TBV could interrupt transmission if implemented alongside existing interventions, and the characteristics required of the TBV and of the vaccination programme. In all settings repeated annual rounds of vaccination will be required, with more frequent rounds required if the duration of protection is shorter or if the initial transmission intensity is high. The protective efficacy of a typical TBV vaccine with a 1-year half-life is predicted to be substantially higher in seasonal settings compared to perennial settings with the same average transmission intensity, with greater efficacy achieved if the vaccination programme is aligned with the start of the transmission season. Overall the protective efficacy is predicted to be greatest in areas of low transmission but the number of cases averted greatest in areas of moderate transmission. Thus the optimal location to undertake Phase III trials for candidate vaccines would be in existing low to moderate transmission areas.

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IMMUNOGENICITY OF DNA VACCINES ENCODING PFS48/45, A *PLASMODIUM FALCIPARUM* TRANSMISSION-BLOCKING VACCINE ANTIGEN IN RHESUS MONKEYS BY *IN VIVO* ELECTROPORATION INCLUDING EVALUATION OF CODON OPTIMIZATION AND N-LINKED GLYCOSYLATION

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Antigens expressed on various sexual stages of *Plasmodium falciparum* are being pursued as targets of transmission blocking vaccines (TBV). These include gamete surface proteins (Pfs230 and Pfs48/45) and zygote/ookinete surface protein (Pfs25) expressed after fertilization in the mosquito midgut. Ingestion of antibodies against these antigens effectively blocks parasite development in the midgut. Efforts are underway to develop vaccines based on either recombinant proteins-adjuvant formulations or as DNA vaccines. While our laboratory has previously expressed Pfs48/45 as a functionally effective recombinant molecule in E. coli, structural complexity has continued to be a challenge for further development of an effective vaccine. The goal of this study was to investigate Pfs48/45 using DNA vaccine platform. The rationale is to develop a TBV that is: (i) technically less challenging in comparison to recombinant protein production, (ii) cost-effective, (iii) stable, and (iv) easy to manufacture. In addition, DNA vaccine platform allows a multivalent approach by combining several antigens. Our vaccine design included codon optimization of DNA sequence for optimum expression, in-vivo electroporation for enhanced immunogenicity, mutations to block any N-linked glycosylation in the expressed protein, and a heterologous prime-boost approach. Additionally, we evaluated a combination of DNA vaccines encoding Pfs48/45 and Pfs25. All 7 putative N-linked glycosylation sites in Pfs48/45 were mutated (N to D or K) based on available sequences of P48/45 orthlogs in various *Plasmodium* species. Rhesus macaques (N=4) were assigned to each of three groups and immunized (IM) with 3 DNA vaccine doses (2.5mg), using *in vivo* electroporation at 4 week intervals followed by a recombinant protein boost (50ug protein in Alum). Antibody titers were determined by ELISA and functional activity in mosquito membrane feeding assays. Results on various test parameters as well as outcome of combining two different TBV target antigens will be discussed. (Funded by AI47089, AI101427 and AI 103466).

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DISTRIBUTIONAL IMPACT OF RTS,S VACCINATION IN SUB-SAHARAN AFRICA: IMPLICATIONS FOR POLICY IMPLEMENTATION

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A growing body of evidence has demonstrated that many public health interventions developed to aid the poor are not reaching their intended target. These concerns are relevant for the introduction of a malaria vaccine, the most advanced of which is RTS,S, currently in Phase III clinical trials. Initial results indicate that RTS,S can provide modest protection against both clinical and severe malaria in young infants. We examine the potential distributional impact of the RTS,S vaccine in 6 African countries by evaluating differences in the relative risk of malaria against projected vaccine coverage and health benefits across beneficiaries grouped by socio-economic characteristics using an asset index. To accomplish this we first link country Demographic and Health Surveys with the distributions of entomological inoculation rate derived from the prevalence data assembled by the Malaria Atlas Program. We then combine information on transmission, access to health services and baseline vector control interventions coverage to assess the impact of vaccine deployment on disease burden and its distribution across different population groups using a stochastic simulator of malaria epidemiology and control. We estimate the extent of forgone health due to disparities in access to immunization services by simulating a scenario assuming immunization coverage of the group in the highest socio-economic quintiles for the whole vaccination cohort. We highlight the importance of malaria case management in sustaining the health gains achieved with the RTS,S by simulating vaccine impact at levels of highest wealth quintile for both immunization and case management coverage. Our findings suggest that substantial additional reductions in burden could be realized with RTS,S by targeting the underserved population with either extensive outreach or through innovative distribution channels. We further illustrate the gains in program effectiveness if vaccine deployment in combined with systems strengthening to improve access to malaria case management.