the greatest difference seen at 11-13 yr (mean 5912 ng/ml). Increased serum IL-6 correlated with parasitic infection, anemia, and acute and chronic malnutrition. IL-10 levels peaked at 9-11 yr in the α-SWAP positive group (mean 430 ng/ml) and were inversely correlated with IL-6 levels. Children in the α-SWAP positive group and infected with hookworms and *P. falciparum* had significantly increased serum levels of IL-10 (*P*=0.045 and *P*= 0.015). Elevation of TNF-α in the α-SWAP group was also associated with malaria infection in 7-9 yr olds (*P*=0.009*).* Our results show a marked difference in the cytokine profile among α-SWAP positive *vs.* α-SWAP negative children, with an early inflammatory response in α-SWAP positive young children (5-7 yr old), measurable by increased IL-6 and low IL-10, before eggs are detected in urine. Schistosomiasis-malaria co-infection strongly correlated with higher pro-inflammatory cytokines in serum, suggesting an important morbidity-related interaction between these parasite species in children.

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SPATIAL ANALYSIS OF DETERMINANTS OF DENGUE TRANSMISSION WITHIN A PROSPECTIVE COHORT STUDY IN VENEZUELA

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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. Mapping technology and spatial analysis of epidemiological data will be used to draw risk-maps and identify key factors that determine clusters of high dengue transmission and the spatial spread of dengue within a prospective cohort in Maracay, an endemic city of Venezuela. 2000 individuals aged 5-30 years have been enrolled between August-December 2010 into a cohort study. Geolocation of households, water bodies and other environmental factors as well as epidemiological data comprising demographic, socioeconomic, clinical, serological and hematological data were collected at baseline. Annual cross-sectional surveys will determine seroconversion and collect further epidemiological information. Active and passive surveillance is performed to identify dengue cases. Collected data will be imported into geographic information systems software for spatial statistical analysis (regression models) at household level. Risk maps of dengue occurrence measured as confirmed cases by RT-PCR and/or serology both overall and stratified by serotype will be presented. The effect of serotype-specific transmission will be explored. Preliminary results and implications for dengue control will be discussed.

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VARIATION IN DENGUE VIRUS PLAQUE REDUCTION NEUTRALIZATION TESTING: SYSTEMATIC REVIEW AND POOLED ANALYSIS

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The plaque reduction neutralization test (PRNT) is the gold standard for quantifying antibody responses against dengue virus (DENV). Despite the importance of comparable results in diagnostics and vaccine development, the effects of differing laboratory techniques, particularly the use of different viral strains within a dengue serotype, have not been well-characterized. This systematic review and pooled analysis aims to characterize differences in laboratory methods between articles reporting PRNT titers and quantify the effect of these differences on measured PRNT titers. We identified 32 articles reporting 4,411 titers from 605 individuals enrolled in vaccine trials (8 articles), serological surveys (3 articles) or observational studies (23 articles). These articles reported the use of 4 different neutralization end points, 3 different cell lines, 12 different virus concentrations and 51 different virus strains (9 for DENV1, 17 for DENV2, 17 for DENV3 and 8 for DENV4). Pooled analysis showed that the strain used in PRNT assays had a substantial effect on the measured titer, and accounted for 5% (90% credible interval: 1%, 11%) of inter-observation variation after adjusting for other factors. Differences between articles (in part a proxy for inter-laboratory differences) accounted for 37% (90% credible interval: 27%, 61%) of inter-observation variance after adjusting for other factors. These results call into question the comparability of dengue PRNT titers reported from different laboratories. These results highlight the importance of standardizing PRNT methods in order to permit inter-laboratory comparisons and reduce variability between study results.

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DENGUE VIRUS E PROTEIN DOMAIN III-REACTIVE ANTIBODIES IN POLYCLONAL IMMUNE SERA AND THEIR ROLE IN PROTECTION OR ENHANCEMENT *IN VIVO*

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The four dengue virus serotypes (DENV1-4) are responsible for the most prevalent arboviral disease in humans. The DENV virion surface is composed of 180 copies of envelope (E) protein, which is comprised of three domains (EDI, II and III). E is the main target of neutralizing antibody, and studies with mouse monoclonal antibodies indicate that antibodies that bind to EDIII can strongly neutralize DENV. Few studies have explored the properties of antibody subpopulations in polyclonal immune sera responsible for DENV neutralization and enhancement. Recent studies with human sera indicate that anti-EDIII antibodies contribute little to binding or neutralizing potency of human immune sera *in vitro.* The goal of this study was to assess the role of anti-EDIII antibodies in immune sera in neutralizing or enhancing DENV *in vivo*. We show that mouse dengue-immune sera have more EDIII-reactive, neutralizing antibodies than human immune serum. Using a depletion strategy to remove EDIII-specific antibodies from polyvalent serum, we demonstrate that anti-EDIII antibodies in DENV-immune human serum are not required for reduction of viral load after infection with a homologous serotype in our AG129 dengue mouse model, consistent with *in vitro* neutralization data. Depletion of anti-EDIII antibodies in mouse sera led to a significant reduction in neutralization potency. However, mice were protected by increasing the quantity of EDIII antibody-depleted mouse serum, indicating that the mice develop both EDIII-reactive and -non-reactive neutralizing antibodies. We also used our mouse model to evaluate whether EDIII-specific antibodies contribute to virus and disease enhancement *in vivo*. Depletion of anti-EDIII antibodies from mouse serum led to an increase in disease enhancement, indicating that anti-EDIII antibodies suppressed the ability of other antibodies in polyvalent immune sera to enhance infection. However, administration of larger amounts (greater neutralizing titers) of EDIII-depleted mouse serum was not enhancing. Finally, we conclude that neutralizing titer, measured in a flow cytometry-based assay with human U937-DC-SIGN cells, is a strong predictor of viral load and disease outcome *in vivo*, and serves as a better indicator than peak enhancement titer, as measured in K562 cells. This data supports the hypothesis that neutralization titer can serve as an important immune correlate of dengue vaccine-derived protection.

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THE IMPORTANCE OF PLATELET-DERIVED MICROVESICLES IN DENGUE PATHOGENESIS

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Platelets, the second most common cell-type in the circulation, are important for homeostasis of the body's physiology. They possess a unique feature- the ability to respond instantaneously to subtle changes in the surroundings and become activated. Platelet activation results not only in secreting tons of releasates but also unleashing platelet-derived microvesicles (PMVs) by shedding subsections of its membrane, a process similar to that of apoptosis. PMVs are the most frequently detected form of microparticle found in the circulation of human subjects. Cumulating evidence suggests that PMVs have significant pathophysiologic effects including the orchestration of inflammatory conditions. In addition to its role as a marker for cell damage, circulating cell-derived microvesicles are being recognized for their roles as signaling elements in cell-cell communication and as transport vesicles for proteins, nucleic acids and receptors in certain diseases and infections. However, the status of PMPs in acute dengue virus infected patients remains largely unexplored. PMVs were isolated from plasma of dengue patients by differential centrifugation. Viral RNA was quantified by qRT-PCR, viral isolation was done by co-culture with Vero cells, and proteomic profiling was performed in the isolated PMVs. In addition, some of the plasma proteins observed in proteomic were verified by ELISA. Results revealed that i) the levels of dengue viral RNA were significantly higher in the PMVs than in the platelet and serum fractions; ii) infectious virus could be recovered from the isolated PMVs, iii) although proteins with extracellular functions, chaperone and transport, and blood coagulation were dominant in isolated PMVs, several unique proteins were noticed, such as lactadherin and vitamin-D-binding protein; and iv) significantly lower lactadherin was observed in the plasma of dengue patients. These results indicated that dengue virus could be disseminated via PMVs in dengue patients and that the proteins associated with PMVs may have a protective role since lactadherin has been shown to have an enhancement activity in the clearance of PMVs by phagocytic cells. The information could be a critical step to further understand the complicated pathogenesis of dengue disease.

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RISK FACTORS FOR DENGUE SHOCK SYNDROME: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Several risk factors are reportedly associated with dengue shock syndrome (DSS), but the results from these reports are highly inconclusive. In order to estimate overall association of risk factors and DSS over dengue hemorrhagic fever (DHF), we systematically reviewed and performed a meta-analysis of relevant studies in both DSS and DHF patients. PubMed, EMBASE, Scopus, Google Scholar, Dengue Bulletin, Cochrane Library, Virtual Health Library, Cochrane Library, and manual search of reference lists of articles published before September 2010 were used to retrieve relevant studies. Two reviewers independently selected articles and extracted data on study characteristics and data regarding the association between factors and DSS over DHF in the form of 2x2 tables. A meta-analysis using fixed-effects or random-effects models to pooled odds ratios (OR) or difference in mean with corresponding 95% confidence intervals were calculated only if more than one study had investigated particular factor. We found 173 articles that met our eligibility criteria. Our meta-analysis showed that younger age, female, vomiting, jaundice, abdominal pain, hepatomegaly, gastrointestinal bleeding, hematemesis, ascites, pleural effusion, gallbladder wall swelling, DEN-2, thrombocytopenia, leukopenia, hematocrit increase over 20%, elevated ALT, prolonged APTT, prolonged PT were risk factors for DSS, whereas normal nutrition, DEN-1, DEN-4 were protective factors against the disease.

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CROSS-NEUTRALIZING ANTIBODY RESPONSES AGAINST CIRCULATING DENV FIELD ISOLATES AFTER HUMAN VACCINATION WITH A TETRAVALENT DENGUE VACCINE

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The potential of a vaccine to prevent natural infections may depend on the capacity of vaccine-induced antibodies to neutralize currently circulating strains. A tetravalent dengue vaccine (TDV) based on 4 recombinant, live, attenuated viruses (CYD1-4) has been developed at Sanofi Pasteur and is in clinical phase III evaluation. Serum neutralizing activity after vaccination is routinely evaluated against the vaccine parental, wild-type DENV strains: DENV-1/PUO-359, DENV-2/PUO-218, DENV-3/PaH881/88, and DENV-4/1228, all isolated between 1978 and 1988. We previously demonstrated that a pool of sera generated during preclinical evaluation of the vaccine in rhesus monkeys broadly neutralized contemporary DENV lineages of diverse geographical origin and genotype. This work presents data obtained in a similar evaluation, conducted with sera from volunteers currently enrolled in a phase II immunogenicity and safety clinical study in Singapore and vaccinated with TDV. Pools of serum were assembled from subjects according to the patient age group (adult / adolescent / children), the individual's flavivirus immune status before vaccination (positive / negative), and the post vaccination level of the neutralizing antibody response against the vaccine parental strains. For each serotype, 6 DENV strains were tested: two prototype strains (parent vaccine strains and WHO strains DENV-1/West Pac 74, DENV-2/S16803, DENV-3/CH53489 and DENV-4/TVP360), and 4 field isolates of different genotype and geographic origin, including 2 Latin American strains and 2 Asian strains. This data will be discussed with regards to their implications for human vaccination.

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TRAVEL-ASSOCIATED CASES OF DENGUE REPORTED TO THE CENTERS FOR DISEASE CONTROL AND PREVENTION 2006 THROUGH 2010

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Dengue is the leading cause of systemic febrile illness in travelers returning from dengue endemic areas of the Caribbean, Latin America, and Asia. We describe Travel-associated dengue cases occurring among persons residing in the 50 US states and the District of Columbia (DC) and illness onset during 2006 through 2010. Dengue case data reported to the Centers for Disease Control and Prevention's (CDC) national arbovirus surveillance system (ArboNET) and case data from patients with specimens submitted to the CDC Dengue Branch (DB) but not reported to ArboNET were analyzed. For ArboNET cases, laboratory result interpretations and travel classifications were determined by the reporting jurisdiction. For DB cases, laboratory-confirmed cases were patients with DENV RNA detected by real-time PCR. Laboratory-probable cases were patients with anti-DENV IgM antibodies detected by ELISA in a single convalescent specimen. Travel-associated illness was defined as dengue-like illness in a resident of the 50 United States and DC traveling abroad within 14 days of illness

onset. Between 2006 and 2010, 1,315 laboratory-confirmed or probable Travel-associated cases were reported (annual average = 263; p value for trend = 0.3); mean age was 40 years and 50% were male. Of those with clinical information, 89% (1,023), 5% (53), 3% (30), and 4% (44) were classified as dengue fever (DF), DF with hemorrhage, dengue hemorrhagic fever or shock syndrome or not classified, respectively. The average annual number of hospitalizations was 110 (p value for trend = 0.8) and deaths was 1 (p value for trend = 0.3). Over 54% of cases were reported from Florida, New York, Texas and Minnesota; > 45% of cases were attributed to travel to the Dominican Republic, Puerto Rico, India, Mexico, and Haiti. In conclusion, travel to dengue endemic areas continues to pose a risk to US travelers. Persons traveling to these areas should seek pre-travel consultation, minimize mosquito exposure while traveling, and seek medical attention if fever develops during travel or after return.

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THE ROLE OF ANTIBODIES IN DENGUE VIRUS PATHOGENESIS: UNDERSTANDING PROTECTION VERSUS ENHANCEMENT

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Primary infection with one Dengue virus (DENV) serotype generally confers lifelong homotypic immunity but only short-term heterotypic immunity to the other three serotypes. Secondary infection with a heterologous serotype typically results in severe Dengue Hemorrhagic Fever (DHF). Studies support a role for pre-existing antibody (Ab) to DENV in DHF pathogenesis through Antibody Dependent Enhancement (ADE), in which Abs induced by the initial infection enhance virus infectivity rather than neutralize leading to increased viral uptake into cells. Evidence supports a role for Fc-FcR interactions on non-neutralizing anti-DENV Abs in DENV pathogenesis. Elucidating the role of DENV-specific ADE in increased DENV infectivity, virus replication and viremia is the basis for this research. We hypothesize that non-neutralizing DENV Abs are responsible for increasing infectivity of host cells with heterologous DENV, and the increased viremia results in severe pathology. Memory B cells from convalescent DENV-infected patients were used to generate libraries of anti-DENV human monoclonal antibodies (HMAb) by molecular cloning. HMAbs were characterized based on DENV serotype specificity, cross-reactivity, antigenic binding sites, and neutralizing and/or enhancing ability *in vitro*. HMAbs with potent neutralizing activity against DENV-1 demonstrated decreased enhancement activity at higher concentrations, consistent with their presumed ability to block viral entry at full Ab site occupancy, whereas non-neutralizing Abs showed a positive correlation between enhancement and concentration. Thus both neutralizing and non-neutralizing HMAbs were able to enhance DENV infection *in vitro*; however, the degree of enhancement appears to be dependent on the concentration of the individual HMAbs. These HMAbs provide insight into the human immune response to DENV, which can be used to assess the role of Abs in DENV pathogenesis, specifically their ability to regulate the immune response to DENV, and may help determine Ab characteristics associated with protection versus enhancement of disease.

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HUMAN MOVEMENT DETERMINES RISK OF INFECTION WITH DENGUE VIRUS

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Knowledge of human mobility and how it influences pathogen transmission remains limited, especially at fine scales. We studied the importance of individual human movements, measured in terms of exposure to pathogen, for predicting risk of infection with dengue virus (DENV) over two transmission seasons dominated by DENV-4 in Iquitos, Peru. We used a survey to identify locations visited recently by 48 febrile individuals (23 DENV+, 25 DENV- controls) and sampled for evidence of acute dengue infection (RT-PCR, IgM ELISA) among contacts residing in those locations. Overall, we identified 97 total acute (PCR+ or IgM seroconversion) infections and 77 recent (elevated acute IgM) infections in 166 households (mean 3 contact houses per index case), 31% of which occurred >100m from the home. These data and serotype-specific plaque reduction neutralization test results from a prospective longitudinal cohort were then used to parameterize risk and attack rate models. Based on a simple theoretical model, we estimated exposure as a composite index of the number of recently visited locations with concurrent acute DENV infections, and the number of acute infections and susceptible hosts per location. We show that risk of infection with DENV is overwhelmingly driven by variation in exposure ($P<0.001$) and herd immunity ($P<0.05$). Attack rates in the activity spaces of DENV+ cases were markedly higher than DENV- controls (17% [95% CI:12-21%] vs 6% [95% CI:3-8%]), with no difference in household incidence between the home and contact sites of DENV+ clusters (16.9%). We conclude that human mobility is central to the transmission of this virus and for predicting who and what locations are at greatest risk for infection with DENV during an outbreak. We discuss the implications of our results for the design of dengue control and surveillance programs and argue our findings and methods are not specific to DENV and are relevant to understanding other infectious diseases.

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ECONOMIC COST OF DENGUE IN MALAYSIA: MERGING MULTIPLE DATA SOURCES

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While incidence of most infectious diseases has been declining worldwide, dengue cases are increasing. In 2010, the mainland United States experienced dengue transmission for the first time in decades. Vaccines and new mosquito control technologies are being tested, but their implementation will require additional resources. Information about economic burden is needed for setting priorities, but accurate estimation is difficult due to incomplete data. We are overcoming this limitation by literature review, engaging experts and data from both the health and surveillance systems, and using a Delphi process. The annual economic cost of a disease (e.g. dengue in 2009) can be calculated as the number of cases per year times the cost per case. While dengue is a reportable illness in Malaysia (e.g., 41,454 cases reported in 2009), the surveillance system is passive. To address possible underreporting of cases, we first obtained estimates of expansion factors (the number by which reported

cases need to be multiplied to obtain the true number) from previous studies in the literature and Malaysia's work permit system, FOMEMA. Private hospital laboratories found about 25,000 dengue-positive cases. Finally, clinicians estimated that about 50%-60% of dengue cases were treated in the ambulatory sector. Altogether, our first round expansion factors from 10 experts were 1.2 for reported hospitalized cases, 34.7 for reported ambulatory cases, and 2.3 overall, corresponding to 96,000 dengue cases per year. To estimate unit costs of dengue cases, we combined a publication from the University of Malaya, data from special studies, national health accounts, and inflation adjustments. Altogether, the 2009 annual cost of dengue illnesses was about US$ 36 million (US $1.20/capita). Of this, 41% was direct costs and 59% indirect costs; 66% of costs occurred in public sector cases and 34% in private sector cases. This study suggests that implementing a technology which would control dengue efficiently would be economically valuable.

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INTEGRATING HUMAN AND VECTOR MOVEMENT DATA INTO DENGUE VIRUS TRANSMISSION NETWORKS

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Human movement patterns and social structure play an important role in modulating human-vector contact rates, affecting transmission dynamics, and the spread and persistence of vector-borne pathogens. For dengue virus (DENV), limited dispersal range of its day-biting vector, *Aedes aegypti*, points to movement of viremic humans as a plausible explanation for the rapid spread of infection across urban environments. We used field data from spatially-explicit semi-structured interviews (SSI) and GPS data-loggers to derive contact networks of individual humans for DENV transmission in Iquitos, Peru. We obtained movement data for 1,200 participants and expressed their contact network as an undirected bipartite graph representing the locations participants had in common as a consequence of their routine movements. Vector dispersal was explicitly accounted for by linking locations within the hypothesized home range of the vector. Different measures of network topology were estimated for the full contact network and "key sites" network containing only those locations where exposure to *Ae. aegypti* was most likely (houses and schools). Places where participant's spent the most time outside their home during daytime were other residential locations (71% of total time); markets and stores (18%); parks, cemeteries, and recreational areas (3%); and hospitals and health posts (2%). Average degree of a participant (number of locations visited) increased with age from an average (SD) of 2.8 (1.1) for 3-8 yr-olds to 7.1 (4.3) for 45-69 yr-olds. By plotting the in-degree distribution of locations we identified places highly visited by infected individuals (key transmission sites). Our quantitative empiric contact networks indicate that residential exposure can occur beyond 100 m of a person's home and are consistent with the notion that movement of viremic people is a prime driver of rapid DENV propagation in urban environments.

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DENGUE VIRUS TRANSMISSION: THE APPLICATION OF MATHEMATICAL MODELS TO DEVELOP A FRAMEWORK FOR RISK ASSESSMENT

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Development of tools to predict dengue disease, and therefore enable timely intervention, is a topic of intense debate. Risk prediction algorithms should stem from understanding the interaction between different sources of virus transmission regulation that span the environment, human populations, vector populations, and biology of the virus. Location and timing of outbreaks are difficult to predict due to the nature of random events and mobility of humans at large spatial scales. In contrast, probabilities associated with a small outbreak locally escalating into a large epidemic, the rate of reproduction within the local human population, and the spatial extent of transmission related to an outbreak event are aspects of risk assessment that can be quantified statistically. We characterize risk as a space-time probability map that depicts the likelihood of specific events. Thus it is important to (1) establish a set of predictive factors that have a theoretically-measureable role in regulating virus transmission, (2) quantify the relationship between variation among different factors and risk of infection, and (3) develop probability profiles that map space-time risk in relation to changing conditions of transmission. We used a 4-serotype stochastic hybrid SEIR dengue virus transmission model to examine sensitivity of different factors that regulate transmission (environmental, human, vector, and virus strain) for predicting risk. Across simulations, we quantified changes in timing and magnitude of recurrent epidemics under varying conditions of transmission. We examined effects of heterogeneity in relation to dynamics of risk. Heterogeneity has an important cost-benefit relationship in model driven risk assessments. Such models are complex and costly, yet heterogeneity in ecological and epidemiological processes is strongly linked with risk and informs risk prediction. In this presentation, we characterize fundamental relationships between conditions of transmission and assessment of risk for endemic transmission settings with models of varying complexity.

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ENVIRONMENTAL DETERMINANTS OF WEST NILE VIRUS EPIDEMIC IN SOUTH DAKOTA THROUGHOUT 2003 TO 2007

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West Nile virus (WNV) first invaded the Northern Great Plains (NGP) in 2002 and caused a tremendous outbreak in South Dakota in 2003. This study summarized the spatial patterns of human WNV cases in Aberdeen, Sioux Falls, and Rapid City in South Dakota from 2003 to 2007 and investigated the influences of land cover types, hydrological factors, soil conditions, and elevation. We estimated the percentage of urban, open developed space, cropland, grass/hay, and wetland within the neighborhood of geo-coded cases and random controls. We also measured distances from irrigation draw points, hydrological features, and soils susceptible to ponding. The best fitting model was selected according to Akaike's Information Criterion (AIC). Aberdeen has the highest 5-year WNV cumulative infection rate (697.5 per 100,000), following by Rapid City (327.1 per100,000) and Sioux Falls (91.9 per100,000). The statistical models demonstrated the distinctive effects of the environment drivers in the different study areas. Grass/hay (OR=2.8, $p<0.01$) and emergence wetland (OR=1.7, $p<0.05$) predicted the higher risk in Aberdeen. Proximity to soils with high ponding frequency were associated with higher risk in Sioux Falls (OR=1.9, $p<0.01$), however, urban land cover type showed protection protective effect (OR=0.2, $p<0.01$). In Rapid City, risk was associated with both soil conditions (OR=1.5, $p<0.05$) and forest land

cover (OR=5.1, p<0.05) after adjusting for elevation. Our finding indicated that the spatial pattern of human WNV risk can be determined by the environmental variables which represented the unique of topological and hydrological features, soil conditions, and human activity at the local regions. These results suggest that fine-scale spatial models of WNV can be enhanced by adapting them to specific regions.

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GENETIC ANALYSIS OF WEST NILE VIRUS IN THE U.S. SHOWS INCREASING VARIABILITY, 2002-2010

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West Nile virus (WNV) is endemic in the U.S., where it was recognized in 1999, and caused annual outbreaks for 12 consecutive years. By 2010, WNV had caused over 30,000 serious illnesses, including 12,676 neuroinvasive cases and 1,200 deaths reported to the CDC. Viral adaptation to domestic mosquitoes and birds played a major role in the spread of WNV in North America. Reoccurring outbreaks suggest viral adaptation through genetic mutations which have the potential to: alter viral phenotype and virulence; degrade the performance of assays; and affect efficacy of vaccines and potential therapeutic agents. We studied genetic sequences of 140 WNV isolates produced from human plasma, mosquito and bird specimens, obtained from different geographical locations of the U.S. between 2002 and 2010. Genetic sequences were compared with existing sequences in GenBank using Vector NTI. Analyses of phylogenetic relationships were based on parsimony algorithms using MEGA software. In order to expedite surveillance of genetic changes we have developed a microarray-based assay composed of 5 slides containing 1274 overlapping oligoprobes covering the entire WNV genome. Microarray assay validation was performed with 10 previously sequenced WNV isolates. We detected unambiguously all mutations identified in each one of the isolates by traditional sequencing analysis. When compared to the NY99 sequence, results showed increasing genetic variability over the years including deletions and insertion in the 3'UTR. Most mutations were silent transitions (U ~~C~~, A ~~G~~); the number of nucleotide mutations ranged from 20 to 76 resulting in 3 to 17 amino acid substitutions. The 2D RNA analysis of the 3'UTR regions using *mfold* showed that the deletions and insertions identified affect the conformation of some regulatory elements critical for viral replication. Preliminary results of ongoing studies suggest that fixed mutations impact viral phenotype. Further studies are needed to confirm and investigate more phenotypic differences using *in vivo* and *in vitro* models.

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PROCESS-BASED ESTIMATES OF WEST NILE VIRUS TRANSMISSION RISK

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Control programs for West Nile virus (WNV) rely on a variety of surveillance methods focused on mosquitoes and avian hosts to evaluate the risk for pathogen transmission. Intervention strategies are often guided by considering surveillance components individually or by threshold-based risk assessments that provide an overall estimate of human infection risk. These approaches work well for sampled areas, but due to the focal nature of transmission, they are not easily extended to predict risk for unobserved places or times. Here, we evaluated two process-based risk metrics derived from a new dynamic transmission model: the basic reproduction number (R_0) and a novel temperature-driven estimate of the number of vector bites required for transmission (T). The model accounted for several important features of WNV transmission, including the effects of temperature on the virus and mosquito vectors and variation in host competence. For the period since the 2003 invasion of WNV in California, R_0 and T closely tracked the spatial and temporal dynamics of WNV transmission to avian hosts. 5.7% of chickens were seropositive when R_0 was above 1, implying amplification, compared with 1.7% when R_0 was lower. Most (59%) of all seroconversions occurred when transmission was expected to occur within 2-3 mosquito bloodmeals. Mechanistic risk metrics provided earlier warning of the potential for transmission, especially in areas where surveillance was sparse, and were useful for projections to future temperature scenarios that may result from climate change.

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CHARACTERIZATION OF A NOVEL FLAVIVIRUS ISOLATED FROM *CULEX (MELANOCONION) OCCOSSA* FROM IQUITOS, PERU

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In recent years, a number of flaviviruses that replicate only in arthropods have been discovered and characterized. Herein, we describe the isolation and molecular characterization of a novel mosquito-only flavivirus. The novel flavivirus was isolated from *Culex (melanoconion) occossa* mosquitoes collected in 2009 from an urban area of Iquitos, Peru, located in the Amazon basin in the northeastern region of the country. Evidence for a flavivirus was detected by indirect immunofluorescent assay (IFA) in cell culture supernatant of infected C6/36 cells using polyclonal flavivirus group antibodies and confirmed by RT-PCR. In pairwise comparison of the ENV region sequences, the highest nucleotide (47.4%) and amino acid (39.8%) identity was observed with Nounané virus (NOUV). In pairwise comparison of the NS5 region, the highest nucleotide identity was observed with Spondweni virus (65.9%), Iguape virus (IGUV; 65.7%) and Kedougou virus (65.6%); however, at the amino acid level, the highest pairwise identity was observed with IGUV (69.8%), Naranjal virus (69.6%) and Bussuquara virus (69.3%). Phylogenetic analysis using partial ENV and NS5 amino acid sequences revealed this flavivirus forms a clade with NOUV. To investigate the host range of the novel flavivirus, we inoculated a variety of mammalian cells (Vero 76, Vero E6, BHK, LLCMK, and MDCK) with pools of third passage C6/36 isolates and monitored for cytopathic effect (CPE). No CPE was detected, and all mammalian cells lines were negative for flavivirus antigen by IFA and flavivirus RNA by RT-PCR following fourteen days of incubation. We propose that this genetically distinct flavivirus be named Nanay Virus, after the zone of Iquitos, Peru, where it was first detected.

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HETEROLOGOUS NEUTRALIZING ACTIVITY OF JAPANESE ENCEPHALITIS VIRUS GENOTYPE III FORMALIN-INACTIVATED NAKAYAMA VACCINE AGAINST EMERGING GENOTYPE I VIRUS IN TAIWAN

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The circulating Japanese encephalitis virus (JEV) was shifted from genotype III (GIII) to I (GI) virus in Taiwan recently. All commercial JEV vaccines were made from GIII virus and formalin-inactivated Nakayama JEV vaccine was used in Taiwan. To evaluate the homologous and heterologous neutralizing activity of Nakayama vaccine, the vaccinated-children serums were collected in Taiwan, and neutralizing antibodies were measured against JEV GIII vaccine strain (Nakayama), GIII field isolate (CJN-2K) and GI field isolate (TC2009-1). After 4 doses of JEV vaccination, the positive rates of neutralizing antibodies against Nakayama, CJN-2K, and TC2009-1 were 80, 75, and 35%, respectively. However, the neutralizing antibodies were weaning rapidly, because the geometric mean titer (GMT) of neutralizing antibodies persisted 6, 6, and 0 years for against Nakayama, CJN-2K, and TC2009-1, respectively. Immunized with the formalin-inactivated Nakayama vaccine was offered heterologous protection for circulating GIII and GI viruses, when the homologous neutralizing titer (against Nakayama virus) reached above 1:10 and 1:80, respectively. But, among some of low- or non-heterologous neutralization samples, the antibody-dependent enhancement of JEV infection has been observed using undiluted serum samples. This was the first study to evaluate the heterologous neutralizing activity of JEV GIII inactivated vaccine using vaccinated serum samples. Taken together, our study was shown that JEV GIII vaccine offered less neutralizing activity against circulating GI virus, and then might be increased the risk of enhancement of JEV infection.

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TEMPORAL DYNAMICS OF TICKS AND TICK-BORNE ENCEPHALITIS VIRUSES IN A NATURAL FOCUS IN SOUTHERN GERMANY DURING A PERIOD OF TWO YEARS

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Tick-borne encephalitis virus (TBEV) is a member of the genus Flavivirus in the family Flaviviridae. It is transmitted in nature by ticks. So far, the dynamics of TBE natural transmission foci is only partially understood. Different models are used to predict TBEV occurance and risk of infection for humans. In the current study we present data on the abundance of ticks and of TBEV in a single TBE focus duirng a period of two years. Ixodes ricinus ticks were sampled monthly in a standardized way from May 2009 until October 2010. Ticks were sorted according to developmental stage and tested for presence of TBEV using a real time-RT-PCR. Positive tick samples were cultivated in cell culture. The E genes from positive TBEV ticks and positive cell cultures were sequenced and compared to the available sequences. In both years the highest total numbers of ticks were detected in May and June. The total numbers of ticks decreased in June and remained on a stable number for the rest of the year. In 2010 the decrease of tick numbers during the summer was more prominent than in 2009. TBEV infection rates in ticks differed significantly during the two years. While in 2009 eight TBEV positive ticks were from adult stages and one of nine positives came from a nymphal tick, in 2010 two of eight positive ticks were in adults and six positive ticks were nymphs. Although while in 2010 the highest number of ticks was sampled in April, the first positive ticks were only found in May. Ticks as well as TBEV in ticks show significant seasonal abundance. The actual data will help to better understand the dynamics of TBEV in ticks and to predict the risk of infection for humans.

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NON-HOMOLOGOUS INTRA-GENIC RECOMBINATION OF CHIKUNGUNYA VIRUS BUT NOT YELLOW FEVER VIRUS 17D

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Recent phylogenetic analyses of naturally occurring recombinant flaviviruses have raised concerns regarding the potential for the emergence of virulent recombinants either post-vaccination or following co-infection with two distinct wild-type viruses. To characterize the conditions and sequences that facilitate RNA arthropod-borne virus recombination, experiments were performed using yellow fever virus (YFV) 17D and chikungunya virus (CHIKV). Recombinant YFV 17D virus was not detected under any of the experimental conditions examined, despite achieving estimated YFV replicon co-infection levels of ~$2.4x10^6$ in vertebrate and ~$1.05x10^5$ in arthropod cells respectively. Furthermore, YFV 17D specific superinfection resistance was observed in cells harboring a primary infection with wild-type YFV Asibi. Non-homologous recombination was observed for CHIKV within the structural gene coding sequence resulting in an in-frame duplication of capsid and E3 gene. Since this observation demonstrated that the experimental approaches and methods employed were valid and sensitive for recombination detection, we conclude that the generation of viable flavivirus recombinants is extremely unlikely, even in the improbable event of a high level acute co-infection with two distinct YFV genomes.

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MALNUTRITION IS ASSOCIATED WITH ORAL POLIO VACCINE FAILURE IN INFANTS IN BANGLADESH

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Since the 1988 World Health Assembly commitment to global eradication of poliomyelitis, all but 4 countries have interrupted the transmission of wild type 1 and 3 poliovirus. With the subsequent development of bivalent oral poliovirus vaccine, the number of annual cases of poliomyelitis is decreasing. Children with numerous doses of trivalent OPV (tOPV) are still being diagnosed with poliomyelitis in endemic areas. Diarrheal disease has been associated with decreased oral vaccine response, such as tOPV. It is not known if malnutrition is a risk factor for poor oral vaccine response in children from Bangladesh. Our aim is to determine if decreased oral poliovirus vaccine response is associated with covariates of malnutrition. A cohort of 200 infants was followed for 1 year for diarrheal episodes, enteric infections, and malnutrition. Blood samples at 6 and 12 months were tested for neutralization antibodies to poliovirus (PV) types 1,2 and 3. Monthly measurements of height and weight were used to calculate Height and Weight for Age Z-scores. Malnutrition was defined as a Z-score of <-2. Breastfeeding history was obtained from mothers. Sera was tested for C-reactive protein(CRP) and anti-endotoxin, as marker of inflammation, and increased gut permeability, respectively. Results: Average birth weight was 2.67kg (+0.38SD) with 35.5% weighing under 2.5kg; Average Birth HAZ -0.92 and WAZ -1.39. At 1 year of age, the overall sera-response rate was 98.2%, 98.2% and 91% for serotype PV1, PV2, and PV3, respectively. Infants with HAZ <-2 were less likely to seroconvert to PV serotype 2 (P =0.0014) and 3 (P=0.0127). Infants with WAZ< -2 were

more likely to be sero-negative against PV serotype 3 (P =0.0044). Infants who were exclusively breastfed for longer period of time had better seroconversion rates (p-value PV1 0.0597, PV2 0.0506; PV3 0.0048). Anti-endotoxin was not associated with diarrheal events, but there was a negative association with serotype PV2(P=0.0289). Anti-endotoxin at 6 months did predict infants with HAZ<-2 at 12 months of age. No difference found in CRP and decrease vaccine response. In conclusion, by 1 year of age, tOPV underperforms in malnourished infants who received at least 3 doses of oral vaccine. There is a trend for improved vaccine response in exclusively breastfed infants.

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SEROLOGIC CROSS-REACTIVITY OF HUMAN IGM AND IGG ANTIBODIES TO FIVE SPECIES OF EBOLA VIRUS

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Five species of Ebola virus (EBOV) have been identified, with nucleotide differences of 30-45% between species. Four of these species have been shown to cause Ebola hemorrhagic fever (EHF) in humans and a fifth species (*Reston ebolavirus*) is capable of causing a similar disease in non-human primates. While examining potential serologic cross-reactivity between EBOV species is important for diagnostic assays, the nature of cross-reactive antibodies following EBOV infection has not been thoroughly characterized. In order to examine cross-reactivity of human serologic responses to EBOV, we developed antigen preparations for all five EBOV species, and compared serologic responses by IgM capture and IgG enzyme-linked immunosorbent assay (ELISA) in groups of convalescent diagnostic sera from outbreaks in Kikwit, Democratic Republic of Congo (n=24), Gulu, Uganda (n=20), Bundibugyo, Uganda (n=33), and the Philippines (n=18), which represent outbreaks due to four different EBOV species. For groups of samples from Kikwit, Gulu, and Bundibugyo, some limited IgM cross-reactivity was noted between heterologous sera-antigen pairs, however, IgM responses were largely stronger against autologous antigen. In some instances IgG responses were higher to autologous antigen than heterologous antigen, however, we observed strong cross-reactive IgG antibody responses to heterologous antigens among all sets of samples. Finally, we examined autologous IgM and IgG antibody levels, relative to time following EHF onset, and observed early peaking and declining IgM antibody levels (by 80 days) and early development and persistence of IgG antibodies among all samples, implying a consistent pattern of antibody kinetics, regardless of EBOV species. Our findings demonstrate limited cross-reactivity of IgM antibodies to EBOV, however, the stronger tendency for cross-reactive IgG antibody responses can largely circumvent limitations in the utility of heterologous antigen for diagnostic assays and may assist in the development of antibody-mediated vaccines to EBOV.

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BURDEN AND CLINICAL CHARACTERISTICS OF NOROVIRUS DISEASE IN GUATEMALA

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Noroviruses are recognized as a leading cause of diarrheal disease in developed countries. However, limited availability of diagnostics has hindered understanding of their role in developing countries, where most severe diarrheal disease and deaths occur. We therefore sought to determine the disease burden, seasonality, and clinical characteristics of norovirus disease in Guatemala. Centralized local capacity for norovirus diagnostics was established and applied to a population-based surveillance system at multiple government health care facilities. Patients of all ages presenting with acute diarrhea to participating hospitals and ambulatory clinics in two departments, Santa Rosa (October 2007-August 2010) and Quetzaltenango (August 2009-August 2010), were recruited. Demographic and clinical data were collected along with stool specimens for norovirus detection by real-time reverse transcription-polymerase chain reaction. Clinical severity was evaluated using a modified Vesikari score for gastroenteritis on a 21-point scale. Incidence rates were calculated using the catchment area population and adjusted for healthcare utilization rates developed from household surveys. We enrolled 2403 patients with diarrhea in the study, including 528 (22%) hospitalized and 1875 (78%) ambulatory patients; 1460 (61%) were children aged <5 years. Norovirus was detected in 114 (22%) hospitalized patients and 227 (12%) ambulatory patients, with seasonal increases during November-January. Patients infected with norovirus had a median clinical severity score of 6, slightly less severe than that of rotavirus-infected patients (median=8) but more severe than patients with bacterial or parasitic infections (median=4). Overall, we estimate norovirus was associated with 21 hospitalizations, 358 ambulatory visits, and 2261 community illnesses annually per 100,000 population. This study demonstrates that norovirus is a common cause of both moderate and severe diarrheal disease in Guatemala and should inform appropriate clinical management and public interventions for diarrheal disease.

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PRELIMINARY EVIDENCE OF CACHE VALLEY VIRUS INFECTIONS AND ASSOCIATED HUMAN ILLNESS IN WESTERN CANADA IN 2009

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Cache Valley virus (CVV) is a mosquito-borne virus belonging to the family Bunyaviridae, genus Orthobunyavirus that is widespread throughout North America. The virus has been documented to cause congenital defects in livestock but two cases of CVV-associated neurological disease in humans have been reported in the United States. For this study aliquots of sera from Manitoba (MB) and Saskatchewan (SK) residents previously suspected to be WNV cases (febrile and neurological symptoms) but testing negative for WNV during the 2009 mosquito season were tested for CVV antibody. Testing of human sera for CVV antibody was carried out by plaque reduction neutralization tests (PRNT). CVV was propagated in Vero E6 cells and a viral plaque titration was carried out to determine the viral titre per ml. A constant viral dose of 100 plaque forming units was added to serially diluted sera in a PRNT assay to identify CVV specific antibodies. 216 WNV suspect-case sera from SK were initially screened by PRNT at a titre of 1:20 with 9 (5%) of the sera giving CVV specific neutralizing titres of ≥1:20. Sera were further end point titrated with several samples exhibiting significant titres of 40-80 to CVV. An initial testing of 55 sera from WNV suspect cases in MB indentified 9 (16%) patients with CVV antibodies indicating a significant level of virus exposure in this province as well. The application of serological procedures to identify probable CVV infections in symptomatic patients provides preliminary evidence that this pathogen may be contributing to a certain level of exposures and possible illness among patients in MB and SK. Additional surveillance and diagnostic testing is warranted to verify if CVV is associated with disease not only in western Canada but other regions within the country.

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EXPERIMENTAL NIPAH VIRUS TRANSMISSION STUDIES

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Nipah virus first emerged in humans in Malaysia in 1998-1999, during a large outbreak of respiratory disease and encephalitis in humans, causing 276 cases of encephalitis, with 106 fatalities. Nipah virus outbreaks continued to occur in India in 2001 and in Bangladesh 2001 - 2011. Within the outbreaks of Nipah virus occurring in Bangladesh 2001 - 2007 it was estimated that ~50% of Nipah virus cases were due to human-to-human transmission. Nipah virus has been isolated from human urine, saliva, nasal and oropharyngeal secretions suggesting that direct contact with these secretions could result in human-to-human transmission. Epidemiological data suggest that for certain outbreaks a large proportion of Nipah virus patients were exposed to Nipah virus within a hospital setting. Given the potential for nosocomial transmission it is important to understand the mode of transmission of Nipah virus and implement measures to prevent human-to-human transmission in future outbreaks. Three potential modes of human-to-human transmission of Nipah virus could be implicated in human-to-human transmission: transmission via fomites, transmission via direct contact or transmission via aerosols. In this study we assessed of Nipah virus to transmit between humans experimentally through systematic transmission studies using the hamster model.

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CHARACTERIZATION OF A *BRUGIA MALAYI*-ENCODED HUMAN IL-5 RECEPTOR ANTAGONIST (BMIL-5RA) BY RNA INTERFERENCE AND IMMUNOFLUORESCENCE MICROSCOPY

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Understanding the strategies used by helminth parasites to evade the human immune system is of paramount importance if intervention programs are to be successful. We have previously identified a *Brugia malayi*-encoded human IL-5R antagonist (BmIL-5Ra) that not only binds to the human IL-5R, but inhibits human IL-5's ability to signal through its receptor on eosinophils. To further characterize the BmIL-5Ra and to begin to understand its role in both the parasite and mammalian host, antibodies were raised to recombinant BmIL5Ra and used to visualize the expression of the molecule on L3 and other lifecycle stages. To this end, the BmIL-5Ra was localized to the tegument of *Brugia malayi* using immunofluorescence confocal microscopy and immunoelectron microscopy. Constructs were then developed to perform RNAi in *B. malayi* L3 and methods optimized for performing RNAi in these organisms. Using soaking to deliver the RNAi constructs to L3s *in vitro*, we were not only able to show internalization of the double stranded RNA throughout the L3, but we were able to demonstrate inhibition of the BmIL5Ra mRNA (between 1.4 fold and 1.9 fold) in multiple experiments. More importantly, this inhibition caused a quantifiable decrease in the production of excreted/secreted BmIL-5Ra protein as well as a marked decrease in expression on the parasite surface. Thus, we have developed the tools to characterize the function of this protein in the Bm parasite and to assess the role of this protein (and its absence in using RNAi) in modulating IL-5 mediated events in *in vivo* infection models (immunodeficient mice and jird) that in turn should provide important new insights into the host/parasite relationship in human helminth infection.

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ENHANCING IMMUNITY TO *TRYPANOSOMA CRUZI* BY HETEROLOGOUS EXPRESSION OF TLR-LIGANDS

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Chagas disease, caused by the protozoan parasite *Trypanosoma cruzi* affects more than 8 million people worldwide. Infection by *T. cruzi* typically is very 'silent' immunologically and this may contribute to the ability of the infection to become established and persist indefinitely in most hosts. We hypothesize that the relative lack of accessible Pathogen Associated Molecular Patterns (PAMPs) in *T. cruzi* results in a weak activation of innate immune responses and thus the substantial delay in eliciting adaptive immunity. To test this hypothesis, we have heterologously expressed established exogenous PAMPs, namely *Salmonella typhimurium* flagellin (FliC), and *Neisseria meningitides* Porin (NmPorB) in *T. cruzi*. Parasite lines expressing either of these proteins elicited an enhanced innate immune response, as evidenced by their increased ability to activate NFkB/AP-1 reporter cell lines, higher IL-1 induced in macrophages, elevated IL-12 production in IL-12 reporter mice and the earlier generation of relevant serum cytokines in infected BL6 mice. These PAMP-expressing *T. cruzi* lines also elicited a stronger and more rapid *T. cruzi* specific $CD8^+$ T cell response in infected mice, as well as increased IFNγ producing $CD4^+$ and $CD8^+$ T cells. The enhanced immune response generated by PAMP-expressing *T. cruzi* may also have effected a better control of the parasite chronically, as suggested by the higher numbers of central memory$CD8^+$ T cells and reduced parasite load. The strategy of heterologous expression of exogenous PAMPs may be applicable to the generation of improved live-attenuated vaccines for *T. cruzi* and other pathogens.

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MACROPHAGES AND NEUTROPHILS FROM HUMANS AND MICE KILL LARVAL *STRONGYLOIDES STERCORALIS* DURING INNATE IMMUNITY

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The parasitic nematode *Strongyloides stercoralis* (*Ss*) infects 30-100 million people worldwide, yet little is known about the immune response in humans. Previous studies on innate immunity to *Ss* in mice have demonstrated a role for eosinophils, neutrophils (PMN) and complement activation in the protective immune response. The goal of this study was to determine the role of macrophages (MΦ) in innate immunity to *Ss* in humans and mice. Human MΦ were derived from CD34-negative monocytes from G-CSF primed donors and PMN were isolated from the blood of healthy donors. When cultured independently, MΦ and PMN did not kill the larvae; however, larval killing did occur when both human MΦ and PMN were combined *in vitro* in the presence of complement. To examine the role of mouse MΦ in the immune response against *Ss*, bone marrow-derived MΦ were either: 1) cocultured with PMN and larvae *in vitro* or 2) placed in diffusion chambers with larvae and implanted subcutaneously into naïve mice. Larval killing only occurred *in vitro* if both MΦ and PMN were present. In addition, MΦ implanted in naïve mice killed the larvae within 7 days. To determine the phenotype of MΦ during the immune response to *Ss,* mice were infected subcutaneously with larvae and peritoneal exudates cells (PEC) were analyzed by flow cytometry to quantify classically activated MΦ (CAMΦ) and alternatively activated MΦ (AAMΦ). Analysis of PEC from mice with primary infections revealed that both CAMΦ and AAMΦ were present in the peritoneal cavity at levels higher than in control mice. To determine if CAMΦ and/or AAMΦ functioned in killing the larvae, MΦ were stimulated *in vitro* with IL-4 to induce AAMΦ or IFN-γ/LPS to induce CAMΦ. AAMΦ, but not CAMΦ, killed the *Ss* larvae both *in vitro* and after 3 days within diffusion chambers *in vivo*. We conclude from these studies that both human and mouse MΦ,

in conjunction with PMN, kill the parasitic nematode *Ss.* Furthermore, infection of mice with *Ss* results in the induction of AAMΦ which kill the parasite both *in vitro* and *in vivo*.

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EFFECTS OF ANTENATAL MATERNAL PARASITIC TREATMENT ON INFANT ANTIBODY RESPONSE TO *HAEMOPHILUS INFLUENZAE* TYPE B (HIB) VACCINATION IN A MOTHER-CHILD COHORT IN COAST PROVINCE, KENYA

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Antenatal maternal parasitic infections are known to have an effect on fetal immunity. The mechanisms are not fully understood, but it is known that *in utero* exposure to parasite antigens can lead to fetal imbalance of Th1 versus Th2 development that persists into childhood. Studies have shown that schistosomiasis infection has a negative effect on tetanus and BCG vaccination, and malaria infection is associated with decreased response to tetanus, Hib and typhoid vaccination. Positive effects of deworming on vaccine response have been shown with BCG and oral cholera. We sought to determine the impact of treatment of maternal antenatal infection on infant antibody response to Hib vaccination, which is currently unknown. Mothers were tested prenatally and at delivery for parasitic infections, including filariasis, intestinal helminths and malaria. Children had blood drawn every 6 months until 36 months of age, and plasma tested for IgG antibodies against the protective epitope of Hib, poly-ribitol phosphate (PRP), via ELISA. Children were divided into groups by maternal infection status: Uninfected, Infected Treated (prenatal infection; no infection at delivery) and Infected Untreated (infection prenatally and at delivery). 260 mother-child pairs (N=144 Uninfected, N=110 Infected Treated, N=32 Infected Untreated) were analyzed for maternal infection status and infant anti-PRP titers. At 6 months, there was no difference in mean titers between the groups (7.44, 7.61, and 8.32 ug/mL, respectively). At 12 months, the Infected Untreated group had significantly lower mean titers than Uninfected and Infected Treated groups (3.82 v. 6.25 and 6.72 ug/mL, respectively; p=*0.04*). Treatment of maternal antenatal helminth infection is associated with normal infant anti-PRP antibody titers at 12 months, while infants of untreated mothers have markedly lower mean Hib titers. Research is ongoing to determine if this effect persists in older ages. These results suggest that treatment of antenatal parasitic infections may enhance childhood immunity to vaccine-preventable diseases.

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CHARACTERIZING COMPLEXITY IN PRE AND POST-TREATMENT CYTOKINE RESPONSES TO *SCHISTOSOMA HAEMATOBIUM* IN AN ENDEMICALLY-EXPOSED COMMUNITY

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Schistosoma haematobium infection is endemic in sub-Saharan Africa and is associated with cognitive impairment and chronic morbidity. Resistance to *S. haematobium* infection develops in the context of host heterogeneity and exposure to multiple parasite life-cycle stages and may be promoted by anti-helminthic treatment. To investigate how parasite-specific cytokine profiles may contribute to epidemiological patterns of infection whole blood samples were collected from 198 permanent residents (aged 5-84 years) of rural Zimbabwe. Blood was cultured with *S. haematobium* egg and adult worm antigens for 48 hours at 37ºC. Parallel cultures conducted without antigen acted as negative controls. IFNγ, TNFα, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, IL-17A, IL-21 and IL-23 titres were quantified in culture supernatants by enzyme-linked immunosorbent assay (ELISA). Follow-up samples were collected 6 weeks, 6 months and 1 year after a single dose of praziquantel to address the hypothesis that treatment alters cytokine profiles and influences resistance to re-infection post-treatment. Factor analysis was used to identify patterns of cytokine responses before and after treatment and non-metric multidimensional scaling (NMS) was used to identify treatment-induced shifts in host cytokine profiles. Pre- and post-treatment cytokine variations were analysed in the context of host variables via analysis of variance. Data from each participant on their sex, age, co-infection status, residential and anti-helminthic treatment history was collected to inform the analyses. Prior to treatment parasite-specific IL-10/IL-21 responses were positively correlated with infection intensity and IL-17A responses were negatively correlated with infection intensity. These patterns were age-dependent and changed following treatment. A reduced risk of re-infection was associated with elevated schistosome egg-specific cytokine responses. This study presents the most comprehensive analysis of pre and post-treatment *S. haematobium*-specific cytokines to-date and uses novel analytical methods to allow cytokine profiles rather than individual cytokine dynamics to be characterised within a naturally-exposed population. Importantly we have identified a potential role for Th17-associated cytokines in schistosome immunobiology.

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EFFECT OF INDOOR RESIDUAL SPRAYING OF INSECTICIDES ON THE MALARIA SLIDE POSITIVITY RATE IN AN AREA OF HIGH TRANSMISSION INTENSITY IN UGANDA

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There is limited data on the effectiveness of indoor residual spraying (IRS) of insecticide on malaria morbidity in areas of high malaria transmission intensity in Africa. Uganda has recently implemented an IRS program in areas of high transmission intensity through support from the U.S. President's Malaria Initiative. We sought to evaluate the temporal relationship between IRS and the slide positivity rate (SPR) among patients with suspected malaria at one sentinel health facility between Nov. 2006 and Feb. 2011 in the Apac District of Uganda, where the entomological inoculation rate was estimated to be 1586 in 2001. During this period, 3 rounds of IRS were completed. Round 1: March-May 2008 with dichlorodiphenyltrichloroethane (DDT); Round 2: March-April 2010 with alpha-cypermethrin; Round 3: August 2010 with the carbamate Bendiocarb. Over the 52 month observation period a total of 83,829 patients were seen, 41,294 (49%) had suspected malaria, and 77% of those with suspected malaria underwent microscopy. Associations between 6 month periods (with the exception of only 4 months between the 2nd and 3rd rounds) related to IRS and relative changes in the SPR were estimated using Poisson regression after controlling for age and seasonality. The SPR was 45% during the 6 months prior to completion of the 1st round of IRS. The 6 months following completion of the 1st round was associated with a 7% relative reduction in the SPR (p=0.22) compared to the 6 months before completion of the 1st round. The 4 months following completion of the 2nd round was associated with a 12% relative reduction in the SPR (p=0.01) compared to the 6 months before completion of the 2nd round. The 6 months following completion of the 3rd round was associated with a 27% relative reduction in the SPR

($p<0.001$) compared to the 4 months before completion of the 3rd round. In this area of very high transmission intensity, the 2nd and 3rd rounds of IRS were associated with a significant decrease in the SPR. Our analysis will be updated following completion of the 4th and 5th rounds of IRS in 2011.

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IMPACT OF INDOOR RESIDUAL SPRAYING WITH LAMBDA-CYHALOTHRIN ON MALARIA PARASITEMIA AND ANEMIA PREVALENCE AMONG CHILDREN <5 YEARS IN AN AREA OF INTENSE, YEAR-ROUND TRANSMISSION IN MALAWI

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Relatively little is known about the impact of indoor residual spraying (IRS) in areas with intense, year-long malaria transmission such as sub-Saharan Africa. In Malawi, IRS with lambda-cyhalothrin has been applied annually in an area of intense year-long transmission since 2007. We evaluated the impact of IRS on parasitemia and anemia prevalence in children aged <5 years (under-5s) using a cross-sectional household survey conducted in 2009, 6 months after the second IRS spray round. We measured malaria parasitemia and anemia (hemoglobin <11gm/dl) in 899 under-5s and used binomial regression to assess the impact of IRS by comparing under-5s living in a household sprayed with IRS (direct IRS); not sprayed with IRS, but in an IRS area (indirect IRS); and not sprayed with IRS and not in an IRS area (no IRS). In the IRS area, 77% of households reported receiving IRS. Adjusting for bednet use, house construction and socioeconomic status, receiving direct IRS and indirect IRS were significantly associated with a 33% and 46% reduction in parasitemia, and a 21% and 30% reduction in anemia prevalence, respectively.

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EXAMINING THE COMMUNITY EFFECT OF INSECTICIDE-TREATED BED NETS USING SURVIVAL ANALYSIS

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Malaria is a significant cause of child death in sub-Saharan Africa (SSA), causing 715,000 deaths in 2008. Randomized controlled trials across a range of malaria transmission settings have shown insecticide treated mosquito nets (ITN) to reduce child mortality. This reduction in mortality risk occurred not only in children using ITNs but also in children living in villages with high ITN coverage. Mathematical modeling suggests that this protective effect occurs at 50% household ITN usage in the population. Using nationally representative household surveys from 10 countries in SSA we created a retrospective cohort of children aged 1-59 months from complete birth histories, with monthly information on household ITN ownership, proportion of households in the community owning an ITN, age of the child, and malaria transmission season to model the effect of at least 50% of households in the community owning at least 1 ITN on all-cause child mortality. We also included the proportion of children aged 4-59 months receiving 3 doses of diphtheria-pertussis-tetanus vaccine and the proportion of births in the past 2 years delivered at a health facility as indicators of community-level access to healthcare; as well as calendar year, wealth quintile, age of the mother at first pregnancy, education of the mother, and relative birth weight as covariates. Living in a community with >50% ITN coverage is associated with a 32% decrease in the risk of all-cause child mortality. This protective effect is much greater than the 20% decrease associated with household ITN ownership. As expected from previous research, there is a significant community effect when ITN coverage reaches 50% of households in the community- that is children are protected from all-cause child mortality if they live in a community with high ITN coverage, regardless of whether they own an ITN or not. Unprotected children benefit from a reduced vector population, suggesting that investment in other malaria interventions is warranted once ITN coverage exceeds 50%.

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COST-EFFECTIVENESS STUDY ON IMPACT OF LONG-LASTING INSECTICIDE TREATED BEDNETS (LLIN) PROVIDED TO EITHER VULNERABLE GROUPS OR COMMUNITY WIDE ON ANEMIA IN CHILDREN IN SOUTHEAST NIGERIA

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In an integrated lymphatic filariasis and malaria project, LLIN were distributed to all households in 4 local government areas (LGAs) in two states in Southeast Nigeria starting in 2008, with a mop-up round in 2009. Two LGAs received LLIN for vulnerable groups (VG), i.e. pregnant women and under fives; the other two were targeted for full coverage (FC). Anemia rates in children were collected at baseline (2007) and annually for two years by cluster survey. The cost of all inputs in the LLIN distribution were tracked and are included in the analysis, except the costs of the LLINs and the monetary value of the volunteer health workers' time. Together, the cost data and health indicators were used to analyze the cost-effectiveness of the two types of LLIN distribution, from the perspective of funds saved and benefits gained. A total of 171,680 nets were distributed in the FC arm, and 57,251 in the VG arm. Costs were $51,507 and $28,795 in the two arms, respectively. Thus the cost per net distributed was $0.30 in the FC arm, and $0.50 in the VG arm. Net use in all ages increased from 2% (both arms) in 2007 to 62% (FC) and 14% (VG) arms respectively in 2008. The equivalent change in children <5 was 3% (both arms) to 61% and 25% respectively. The cost per 1% increase in net use was $23 ($25 in under 5s) in the FC arm and $99 ($88 in under 5s) in the VG arm. Hemoglobin status in children under 10 improved significantly in both the VG and the FC groups, from 2007 to 2009. The cost per 1% change in hemoglobin (g/dL) for children under 10 was $7,211 for full coverage and $3,311 for vulnerable coverage. The total cost of nets distributed in the VG arm LGAs was lower than that of the full arm LGAs, and achieved a higher percentage increase in hemoglobin. Mean cost to distribute an LLIN was lower in the FC arm, but the cost benefit as measured by improvement in anemia in children was greater in the (targeted) VG. Further analysis of impact and cost effectiveness of these two strategies on malaria prevalence in children and adults is pending.

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AGE-SPECIFIC INCIDENCE OF MALARIA BEFORE AND AFTER SCALING UP OF MALARIA CONTROL STRATEGIES IN BANDIAGARA, MALI

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Recently reported decreases in malaria incidence in many African countries have been attributed to the scaling up of prompt and effective antimalarial treatment using artemisinin-based combination therapy (ACT) and the widespread distribution of insecticide-treated nets (ITN). At a malaria

vaccine testing site in Bandiagara, Mali, ACT was introduced in 2004, and since 2007, ITNs have been distributed free of charge to children after they complete their childhood immunization schedule. We are measuring malaria incidence in an ongoing longitudinal cohort study. Three hundred children aged 0-6 years were enrolled in July 2009 and an additional 100 children aged 7-14 years were enrolled in 2010. Malaria incidence is measured through passive surveillance in the form of expeditious, free medical care and active surveillance through monthly scheduled clinic visits and quarterly blood draws. Ninety percent report ITN use. Preliminary analyses show an incidence of 1.10 malaria clinical episodes per child per season, using a sensitive but non-specific definition of clinical malaria as treatment-seeking behavior with any level of positive parasitemia. Age-specific incidence rates were 0.8, 1.2 and 1.3 for children aged from 0 to 2, 3- 4 and 5- 6 years, respectively. Survival analysis showed that older children experienced malaria illness earlier than younger children. Fifty per cent of children aged 3- 6 years old had their first malaria episode by 8 months from the study start compared to 6 months for children aged less than 3 years (log rank, p= 0.010). Previously, we reported an annual incidence of 1.92 malaria clinical episodes per child per season in 1999-2001, indicating that the implementation of ACTs and ITNs in this setting was followed by a decline in malaria incidence.

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THE ASSOCIATION BETWEEN MALNUTRITION AND THE RISK OF MALARIA IN A COHORT OF HIV-INFECTED AND UNINFECTED UGANDAN YOUNG CHILDREN

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In sub-Saharan Africa, malaria, malnutrition and HIV infection remain major causes of morbidity and mortality in children under five years of age. Few studies have investigated the relationships across malnutrition, malaria and HIV in this age group. Moreover, there is conflicting data on whether or not malnutrition is a risk factor for malaria, and how HIV may modify the malaria-malnutrition relationship. From August 2007 to January 2008, we recruited a cohort of 100 HIV-unexposed, 203 HIV-exposed (born to HIV-infected mothers) and 48 HIV-infected children 6 weeks to 1 year of age living in a high malaria transmission area in rural Uganda. Children were followed up until 2.5 years of age and seen for all their medical conditions in the study clinic. All children were provided with insecticide-treated bed nets. Daily trimethoprim-sulfamethoxazole (TS) prophylaxis was prescribed for HIV-exposed breastfeeding, and HIV-infected children. Height and weight were measured at every visit and stunting was defined as height-for-age z score < -2. Malaria was diagnosed when a child presented with fever and a positive blood smear. The incidence of malaria was compared using negative binomial regression controlling for potential confounders with the measure of association expressed as incidence rate ratio (IRR). The overall incidence of malaria was 3.64 cases per person year. Stunting was an independent risk factor for malaria (IRR 1.20, 95% CI. 1.04-1.39, p=0.01) as was increasing age (IRR=1.41 per 1 year increase, 95% CI. 1.10-1.81, p=0.01), while Urban vs. rural residence was associated with a decreased risk of malaria (IRR=0.42, 95% CI. 0.34-0.52, p<0.001). There was no association between HIV infection and malaria (IRR=0.86, 95% CI. 0.65-1.15, p=0.31), but HIV-infected children were more likely to be stunted (RR=1.50, 95% CI.1.31-1.72, p<0.001. This study suggests that stunting may be associated with an increased risk of malaria regardless of child's HIV-status.

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HEALTH WORKER FACTORS ASSOCIATED WITH CORRECT PRESCRIBING OF ARTEMISININ COMBINATION THERAPY FOR UNCOMPLICATED MALARIA IN RURAL TANZANIA

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Improving malaria case management is partially dependent on health worker adherence to clinical guidelines. We assessed health worker factors associated with correct antimalarial prescribing practices in two sites in rural Tanzania. We conducted repeated cross-sectional health facility surveys and collected information on patient consultations and health worker characteristics. Using logistic regression, we assessed health worker factors associated with correct prescription for uncomplicated malaria defined as prescription of artemisinin combination therapy (ACT) for patients with fever and *P. falciparum* asexual parasitemia on reference blood slide. In this analysis, we included 229 patients with uncomplicated malaria who were seen in a health facility with ACT in stock and 113 health workers practicing in 31 health facilities. Overall, 69% of patients were treated with an ACT. The only health worker factor significantly associated with correct prescription was having 3 or more years of work experience (adjusted odds ratio 6.3; 95% confidence interval 1.7-22.7; p=0.006) while receipt of training on ACT use, receipt of supervision visits, years of pre-service training and availability of job aids were not significantly associated with correct prescription. In conclusion, in this analysis, years of work experience was associated with correct ACT prescription for uncomplicated malaria. Targeted interventions to improve health worker performance are needed to improve overall malaria case management.

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MICRORNAS IN THE PARASITIC NEMATODE *BRUGIA*

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microRNAs are small non-coding RNAs that play key roles in regulating gene expression in animals, plants and viruses. To identify microRNAs in the filarial nematode *Brugia pahangi*, deep sequencing was carried out on small RNA libraries prepared from the third stage larvae or adult worms. Over 120 *Brugia* microRNAs were identified (68% of which were supported by a star strand). Some microRNAs were specific to either stage, but the majority were shared between L3 and adult worm. A small number of additional microRNAs were also identified on the basis of homology based computational searches. Most *Brugia* microRNAs are not conserved in other helminth species. On the basis of library reads, some microRNAs were very highly expressed in the parasite. To further investigate microRNA expression profiles throughout development, microarrays were probed with RNA isolated from six different life cycle stages of *B. pahangi*. Analysis of these has revealed a panel of microRNAs that are either up or down-regulated following the transmission of the L3 from mosquito to mammalian host. In addition a number of microRNAs that differ in expression level between adult males and females were identified. To more fully define the roles of specific microRNAs we are applying existing target prediction programs to the parasite data and adapting techniques reported to allow direct experimental target identification. Candidate parasite microRNA/target interaction will then be verified by transgenic expression using *C. elegans*. Determining if antisense oligonucleotide inhibition approaches are feasible in these parasites is of particular interest and preliminary findings indicate that the uptake of inhibitory oligonucleotides might be achievable in *B. pahangi*.

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TRANSCRIPTOME ANALYSIS OF *BRUGIA MALAYI* LIFE CYCLE STAGES BY DEEP SEQUENCING

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Developing new interventions for the control of parasitic nematodes continues to be a significant challenge. Genomics and post-genomics approaches play an increasingly important role for providing fundamental molecular information about these parasites, thus enhancing basic as well as translational research. Using Illumina high-throughput sequencing, we have undertaken a comprehensive genome-wide survey of the developmental transcriptome of the human filarial parasite *Brugia malayi*. Over 100 million paired-end reads were generated from polyA-tailed mRNA from seven life cycle stages: eggs and embryos, immature MF (of less than 3 days of age), mature MF, L3, L4, adult male and adult female. While deep sequencing data are highly informative in identifying novel transcribed elements and splice variants that help improve the genome annotation, the present study aims to characterize transcriptome changes along the progression of filarial life cycle to further our understanding of the molecular biology of the parasite. Examining the developmental transcriptome profiles of *B. malayi* revealed major transitions in RNA expression from eggs through larval stages to adults. Using statistical approaches, we identified groups of genes with distinct life stage dependent transcriptional patterns and functional categories over-represented in each of these groups. Global transcriptional differences were further evaluated between pairs of stages with particular emphasis on (i) MF maturation, (ii) late larval development, (iii) sex differences, and (iv) intrauterine reproductive processes. Overall, our analysis provides a first comprehensive view of the life cycle transcriptome of *B. malayi*, revealing the dynamics of gene expression during parasite development.

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CROSS-REACTIVITY OR CROSS-SENSITIZATION: MOLECULAR MIMICRY BETWEEN COCKROACH AND HELMINTH GLUTATHIONE S-TRANSFERASES AND ITS IMPLICATION TO THE ALLERGY-HELMINTH INTERFACE

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Although helminth infections have been shown to modulate allergic responsiveness, there are equally compelling data to show that they are associated with the induction of atopy and asthma. Similarities among helminth proteins and allergens are thought to be involved in helminth-driven allergic sensitization. We investigated the molecular and structural similarities between Bla g 5, a major cockroach glutathione-S-transferase (GST) allergen, and the GST of *Wuchereria bancrofti*. These two proteins were found to be 30% identical with a remarkable level of structural conservation based on predicted 3D models. Serological analysis of filaria-infected and -uninfected controls showed that filarial infection was associated with elevation of IgE, IgG and IgG4 anti-Bla g 5, and there was a significant correlation between IgE, IgG, and IgG4 antibodies to Bla g 5 and those to WbGST ($P<0.003$). Pre-incubation of sera from cockroach allergic individuals with WbGST could partially deplete (~ 70%) anti-Bla g 5 IgE, IgG and IgG4 antibodies. Mapping of the IgE binding epitopes for Bla g 5 identified four antigenically relevant epitopes and revealed that the two major N-terminal epitopes were highly conserved in WbGST. Moreover, incubation of sera of filaria-infected patients with the corresponding Bla g 5 peptides inhibited WbGST binding. Finally, mice infected with *Heligmosomoides bakeri* (Hb) developed anti-HbGST IgE and became allergic to Bla g 5 based on skin test reactivity but not to Bla g 4, another important cockroach allergen that has no helminth homologue. Interestingly, when Hb-infected mice were sensitized IP with a mix of Bla g 5 and Bla g 4, there was modulation only of the Bla g 5 allergic response. These data demonstrate that structural similarities can result in allergic cross-sensitization and/or specific cross-modulation depending on the timing of helminth infection and allergen exposure. These findings have important implications for not only understanding the helminth-allergy interface but for the development vaccines to helminth parasites.

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ULTRACONSERVATIVE PROTEINS: BINDING OF MONOCLONAL ANTIBODIES RAISED AGAINST *CAENORHABDITIS ELEGANS* PROTEINS TO SUBCELLULAR COMPONENTS IN THE PARASITIC NEMATODE *BRUGIA MALAYI*

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Few well characterized antibodies are available for studies of subcellular components and organelles in parasitic nematodes. We used immunohistology to explore binding patterns of 21 monoclonal antibodies (mAbs) to known proteins of the model nematode *Caenorhabditis elegans* in the filarial parasite *Brugia malayi*. For 3 mAbs no homologous proteins were identified in the *B. malayi* genome and no structures were labeled in adult *B. malayi*. For another 8 mAbs homologous proteins are present in the *B. malayi* genome, but no staining was detected. Ten mAbs produced distinctive staining patterns in adults as follows: anti-synaptobrevin (SNB-1, a component of synaptic vesicles) labeled the nerve ring of intrauterine microfilariae; anti-EHD1 (RME-1, a marker for recycling endosomes) stained ovaries and early embryos; anti-caveolin (CAV-1) labeled lateral chords and embryonic cells; anti-cytochrome P450 (CYP-33E1) labeled lateral chords, ovaries and testis; anti-HSP-60 (a chaperonin) labeled mitochondria, especially in lateral chords; anti-PAS-7 (part of the 26S proteasome) stained single, non-syncytial cells in the hypodermis and lateral chords; anti-APA-2 (alpha-subunit of the adaptor complex involved in clathrin-mediated endocytosis) labeled hypodermis, lateral chords, ovaries and intrauterine microfilariae; anti-cadherin (HMR-1) labled hypodermis, lateral chords and ovaries; anti-ERM-1 (a cytoskeletal linker in the ezrin-radixin-moesin family of apical membranes) labeled the inner uterus membrane and developing embryos; anti-SAX-7 (an adhesion molecule of plasma membranes) labeled cell membranes in stretched intrauterine microfilariae. These results show that many mAbs that bind to key subcellular structures in *C. elegans* also bind to specific structures in filarial worms. Further work will be needed to confirm whether these antibodies bind to shared epitopes in homologous proteins that have been conserved across some 350 million years of evolution. Epitopes and proteins shared between these distantly related species are likely to be present in other nematodes and critically important in nematode biology.

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THE INVOLVEMENT OF THE *WOLBACHIA* SURFACE PROTEIN FAMILY MEMBERS IN THE ENDOSYMBIOTIC RELATIONSHIP WITH THEIR *BRUGIA MALAYI* HOST

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The molecular basis for the symbiotic relationship between *Wolbachia* and their filarial host *Brugia malayi* remains unknown mystery. There is considerable interest in the filaria - *Wolbachia* relationship due to the dependence of the worms on the endosymbiont for survival and development. Our initial studies indicate WSP-0284, a member of the WSP protein family, potentially plays a role in the symbiotic relationship of the two organisms. The present study was designed to determine whether other members of this family of surface proteins show similar involvement. The genome of *Wolbachia* contains three unrelated WSP proteins and eight additional outer membrane / WSP-like proteins. All these WSPs are members of the outer membrane protein family known to be involved in bacteria-host interactions. We expressed five additional members of the WSP protein family as recombinant proteins and have shown using an ELISA-based assay that 3/5 bind specifically to *B. malayi* crude extracts. Notably, immunoelectron localization studies of two WSP members, WSP-0152 and WSP-0432, indicated that these proteins were not only present on the surface of *Wolbachia* but also in the various host tissues. In particular, anti-WSP-0432 antibodies recognized the protein in the eggshells surrounding the developing microfilaria, and more distinctively in the pseudocoelomic fluid surrounding the adult female worm gonads. To further determine whether WSP-0432 might be involved in the *Wolbachia*-*Brugia* symbiotic relationship and indirectly identify its putative interacting *B. malayi* host protein(s), we used an immunoprecipitation pull down assay followed by mass spectrometry. Interestingly, the most abundant protein that was pulled down was Fructose-bisphosphate aldolase, with many of the others interacting complex proteins also belonging to the glycolysis pathway. The ongoing studies aim to verify the binding of WSP-0432 to their putative interacting host proteins as well as uncovering the possible physiological role of these interactions during the endosymbiotic relationship.

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TRANSCRIPTOMIC AND PROTEOMIC APPROACHES TOWARDS UNDERSTANDING CRITICAL REGULATORS OF MOLTING IN *BRUGIA MALAYI* L3 LARVAE

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Molting of infective L3 larvae into L4s *in vitro* provides a platform to dissect the molecular basis of development. Previously, we provided preliminary microarray data analysis of gene expression early in L3 development to L4. We now provide detailed analyses throughout the L3 to L4 developmental cycle using microarray analysis that reveals a program of ordered gene expression that relate clearly to the lethargus, ecdysis and apolysis of the cuticle. In particular, expression data of >17,000 genes over 10 day period demonstrates clearly upregulated clusters of genes that are likely involved in the transition from vector to human host, in cuticle biosynthesis and its degradation. The most striking observation was the patterns of altered expression of cysteine proteases - cathepsins (CPL-1, -4 and -5) - and serine protease inhibitors (SPN-1, SPN-2) among other genes surrounding the molting process. While we were able to inhibit partially the CPL gene expression in the L3 by RNAi (using dsRNA), the ability to block molting by dsRNA was inconsistent. Nevertheless, chemical inhibition of cathepsin activity inhibited (75-80%) molting of L3 to L4 larvae. Parallel analysis of proteomic data revealed an interesting bias in the cysteine protease expression between L3 and other stages of the parasite. A tight regulation of the collagens and their associated biosynthetic genes could be observed during the molting process. Hierarchical clustering of microarray data and phylogenetic analyses of collagens from *Caenorhabditis elegans* and *Brugia malayi* suggest that Group 2 collagens are specifically regulated during the molt compared to other collagen groups. Interestingly, there were clusters of up- and down-regulated *Wolbachia* genes that also appeared to be developmentally regulated around the molting process. Furthermore, L3-L4 transcriptome data identified ~3000 genes that have not been identified in the whole proteome. Currently ongoing studies using stealth siRNA targeting CPL and serpins and their inhibition of activity will be discussed

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PLEOMORPHISM OF *WOLBACHIA* ENDOBACTERIA IN *BRUGIA* IS INFLUENCED BY WORM AGE AND TETRACYCLINE TREATMENT

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Most filarial species contain *Wolbachia*, obligatory endobacteria that are crucial for parasite development and reproduction. Tetracycline class antibiotics reduce *Wolbachia* loads and affect microfilaria production and worm viability. *Wolbachia* distribution and density vary in different filarial life cycle stages. Past ultrastructural studies of *Wolbachia* in filarial worms suggested that the bacteria have a Chlamydia-like life cycle with small dense bodies as potential infectious forms. We used immunohistology, in situ hybridization, and transmission EM to study effects of age and treatment on the morphology and distribution of *Wolbachia* in L4 and adult *Brugia* parasites recovered from ip-infected gerbils. Parasite material included *B. malayi* recovered at 3, 5, 8, and 12 weeks post infection (wpi), *B. pahangi* recovered at 12 and 112 wpi, and *B. malayi* recovered 5 and 8 wpi after a 2 wk-course of daily tetracycline (5mg/kg) that started at 3 wpi. *Wolbachia* were detected in large numbers in all examined worms with exception of 112 wpi *B. pahangi*, which contained fewer bacteria. Three major forms of *Wolbachia* were observed: (1) Typical bacteria (0.5-1 µm, sometimes dividing) were abundant in the lateral chords and the hypodermis of worms 12 wpi or younger, and they were also observed in the reproductive system of females > 8 wpi. Typical *Wolbachia* were present in lower numbers in treated worms, and they were rare in 112 wpi *B. pahangi*. (2) Large, non-dividing forms (0.9-1.2 µm) were the dominant form in 112 wpi *B. pahangi*, and they were also seen in 12 wpi worms. They were rare in worms less than 12 wpi, and not observed in treated worms. (3) Small (0.2-0.3 µm), electron dense structures were single or grouped (but not clustered). These putative infective bodies were abundant in worms younger than 12 wpi. These forms were absent in 112 wpi *B. pahangi* and only occasionally observed in worms at 12 wpi. Small forms were often present in vacuoles in treated worms, especially at 8 wpi. These results confirm the occurrence of different forms of *Wolbachia* in filarial worms and indicate that the frequency of these forms is related to worm age and external factors such as tetracycline treatment. Further studies will be needed to determine the specific functions of the different morphological forms of *Wolbachia*.

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INCIDENCE OF FATAL LEPTOSPIROSIS - PUERTO RICO, 2010

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Leptospirosis, a potentially fatal infection caused by any of >200 *Leptospira* genus serovars, is endemic in Puerto Rico. Although as many

as 100 cases are reported each year by law to the Puerto Rico Department of Health (PRDH), under-reporting is thought to be high. In January, 2010, the CDC Dengue Branch initiated enhanced surveillance for deaths due to acute febrile illnesses (AFI), which included testing of specimens taken during autopsy of AFI fatalities, an independent review of death certificates at the Vital Registry of Puerto Rico, and comparison of dengue and leptospirosis death rates. Confirmed cases were defined as having: (i) *Leptospira* antigen detected in tissue via immunohistochemistry; (ii) *Leptospira* genome detected by PCR in DNA extracted from tissue or serum; or (iii) detection of anti-*Leptospira* IgM by a private laboratory; suspected cases had "leptospirosis" listed on the death certificate as a cause of or factor contributing to death. Of 101 AFI fatalities that were reported in 2010, 14 (13.9%) were confirmed to be due to leptospirosis; 1 fatal leptospira/dengue virus co-infection was identified. Review of death certificates revealed 9 additional suspected cases; a positive diagnostic test result was found through medical chart review in 1 of these 9 cases. Thus, we identified 15 confirmed and 8 suspected leptospirosis deaths in Puerto Rico in 2010. Of these 23 deaths, the median age was 49 years (range: 19-84), and 18 (78.2%) were male. Cases resided in both rural and urban regions of the island. Medical chart review of all cases is on-going. Although the peak number of deaths occurred in October, lack of comparable leptospirosis surveillance made it impossible to determine if these cases represented an outbreak or baseline levels of disease. In comparison, 38 deaths due to dengue were identified through the same surveillance. In summary, deaths from leptospirosis occurred at a rate of 0.64 deaths per 100,000 residents of Puerto Rico in 2010, which, compared to deaths from dengue, suggests a large burden of disease.

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LEPTOSPIROSIS IN AMERICAN SAMOA 2010 - EPIDEMIOLOGY, ENVIRONMENTAL DRIVERS, AND RISK PREDICTION

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Leptospirosis has recently been identified as an emerging disease worldwide, including in the Pacific Islands. The environmental drivers of leptospirosis transmission vary geographically, and include climate change, extreme weather, land use, international trade, animal reservoirs, and farming practices. We undertook a seroprevalence study to better understand the drivers of emergence in American Samoa. Antibodies indicative of previous infection with leptospirosis were found in 15.5% of 807 participants Three serovars that were previously unknown in American Samoa predominated. Questionnaires and geographic information systems (GIS) data were used to assess behavioural and environmental risk factors. Many risk factors were found to be consistent with previous findings (male gender, outdoor occupations, low income, water exposure), but we additionally demonstrated that there was significant risk associated with living at lower elevation (OR = 1.53), and having higher numbers of piggeries around the home (OR = 2.63). An absolute risk prediction chart was generated using four variables: gender, occupation, knowledge about leptospirosis, and 'piggeries within 250m that have higher elevation than the house'. These variables were chosen because they were significantly associated with leptospirosis, likely to be of practical use for identifying sub-populations at-risk, and for informing potential public health interventions. Our findings suggest that a multi-faceted approach to combating the emergence of leptospirosis is required. Modifying individual risk and managing the evolving environmental drivers of risk need to be considered. These findings are likely to apply to other Pacific Islands with similar climate, culture, lifestyle, and animal-keeping practices. With climate change, the predictions of increasing frequency and severity of cyclones in the Pacific could potentially worsen flooding risk, and exacerbate the disease burden from leptospirosis. Communities need to manage rapidly evolving environmental drivers of risk, and our study will inform their ability to do so.

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EVALUATION OF A DUAL PATH PLATFORM (DPP) ASSAY FOR THE RAPID DIAGNOSIS OF LEPTOSPIROSIS

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Confirmation of leptospirosis by the gold standard MAT requires paired sera and is not widely available. The lack of an adequate and easily implementable diagnostic test hinders effective interventions. We developed a rapid serodiagnostic assay using immunodominant *Leptospira* immunoglobulin-like proteins in a dual path lateral flow platform. This study aims to evaluate the assay's sensitivity, specificity, and reproducibility in the setting of endemic transmission of urban leptospirosis. We measured sensitivity in sera from severe (370) and mild (60) laboratory-confirmed leptospirosis from active hospital and outpatient-based surveillance, respectively, in Salvador, Brazil, and confirmed sera (121) from a reference laboratory in Curitiba, Brazil. We measured specificity in blood bank donors (130) from Salvador, healthy residents (120) of a slum community within Salvador with high endemic transmission of leptospirosis, febrile outpatients (70), and confirmed cases of dengue (65), hepatitis A (64), and syphilis (50). Three blinded evaluators independently scored visual results. Evaluator agreement ranged from very good (kappa 0.86; 95% CI, 0.83-0.90) to excellent (kappa 0.94; 95% CI, 0.92-0.97). Sensitivity was 85% (95% CI, 81-89%) and 61% (95% CI, 42-78%) for acute-phase severe and mild leptospirosis from Salvador, respectively, which was similar to whole-*Leptospira* IgM ELISA (82% [95% CI, 76-86%] and 38% [95% CI, 18-62%], respectively). During the 1st seven days of illness, sensitivity was 77% [95% CI, 66-85%] and 56% [95% CI, 35-75%] for severe and mild leptospirosis. In severe disease convalescence, sensitivity was equivalent (98% [95% CI, 94-99%]) to ELISA (99% [95% CI, 95-99%]). Sensitivity for acute-phase Curitiba sera (58% [95% CI, 46-69%]) was similar to ELISA (66% [95% CI, 55-77%]); whereas it was lower (81% [95% CI, 65-91%]) than ELISA (100% [95% CI, 91-100%]) in convalescence. The specificity was ≥94% for dengue, hepatitis A, syphilis, febrile outpatients, and Salvador blood donors. However, specificity was lower (85%; 95% CI, 77-91%) for healthy residents of a slum within Salvador with high endemic transmission of leptospirosis. The DPP assay performs as well as IgM ELISA for the diagnosis of leptospirosis and can be easily implemented in hospitals where the disease is a major public health problem. However, performance may need to be improved for use in diagnosing milder clinical forms of leptospirosis.

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RICKETTSIOSES: FORGOTTEN CAUSES OF FEBRILE ILLNESSES IN SENEGAL

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Pathogenic rickettsiae, transmitted to humans by arthropod vectors such as ticks are emerging as important causes of acute febrile illness among human populations in Africa. During 2010, we investigated (1) the origin of unknown fever in patients from different districts with a negative arbovirus test result focusing on potential rickettsial infections

and (2) rickettsial strains found in ticks collected from domestic animals in order to assess the risk of these diseases in Senegal. We conducted a laboratory-based jaundice and febrile illnesses study in 2010. Patients' blood samples were assessed for evidence of arboviruses (Yellow-fever, Dengue, West-Nile, Rift-Valley-fever, Chikungunya and Crimean-Congo haemorrhagic fever) and rickettsiae by serological methods. In addition, we assessed ticks collected from domestic animals living in Barkedji (north of Senegal) for the presence of rickettsial agents. A *Rickettsia* genus-specific qPCR assay (Rick17b) targeting a fragment of the17-kD antigen gene consensus sequence was used to screen tick samples and then a *R. africae*-specific qPCR assay (Rafri) was used to test all *Rickettsia*-positive tick samples. Phylogenetic analysis of four rickettsial genes (17-kDa gene, gltA, ompB and ompA) from rickettsial DNA obtained from ixodid ticks was also conducted. All human blood sample tested (196) were negative for arboviruses. Spotted fever and typhus group rickettsiae-specific antibodies were identified from patients with an acute febrile syndrome (20% and 5% respectively). Among 811 specimens collected belonging to six tick species (*Hyalomma marginatum rufipes, H. impeltatum, H. anatolicum anatolicum, H. truncatum, H. dromedarri* and *Rhipicephalus evertsi* evertsi) a total of 174 monospecific pools were constituted and 32% of these pools were positive using Rick17b qPCR assay. All *Rickettsia*-positive samples were negative by Rafri qPCR assay. Phylogenetic analyses of the *Rickettsia* sequences generated from gltA (1124 bp), ompA (591 bp), and ompB (1367 bp) from 7 tick samples demonstrated that they aligned strongly (99.7 to 100%) with *R. aeschlimannii* MC16. These results show the important role of rickettsial diseases among acute febrile illness patients in Senegal. In addition, these results indicate that future work on the clarification of the role of *R. aeschlimannii*, a pathogenic rickettsia for humans, is necessary in north Senegal.

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SENTINELS FOR HUMAN INFECTION: SPOTTED FEVER-GROUP *RICKETTSIAE* IN CANINES

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Rocky Mountain spotted fever (RMSF) is an important tick-borne disease in the United States caused by *Rickettsia rickettsii*. It has been hypothesized that in addition to *R. rickettsii*, other rickettsial species may cause disease and may cross-react with R. rickettsii in serologic tests. There is serologic evidence that other spotted fever group rickettsiae (SFGR), such as *R. amblyommii* and *R. parkeri* may be associated human illness. Additionally, Amblyomma americanum ticks have been found to be infected with *R. amblyommii* and *R. parkeri* in the southeastern United States. Historically, Tennessee has reported one of the highest incidence rates of Rocky Mountain spotted fever in the U.S. Past studies in Tennessee have focused on identifying *Rickettsia* spp. in tick species throughout the state, but little is known about the diversity and prevalence of SFGR in hosts in Tennessee. Sera from 865 canines were collected by veterinarians throughout Tennessee and tested for antibodies to four Rickettsia spp. by enzyme immunoassays (EIA). Indirect immunofluorescent assays (IFA) were performed on all specimens positive by EIA, to confirm specific reactivity to *R. rickettsii, R. amblyommii, R. parkeri*, or *R. montanensis*. Of 275 canine specimens positive for *Rickettsia* spp. by EIA, 41.5% were reactive to *R. montanensis* antigen by IFA, 50.5% were reactive to *R. rickettsii*, and 1.5% were reactive to *R. parkeri*. The seroprevalence of antibodies against *R. rickettsii* was highest in western Tennessee where there is a high incidence of severe RMSF in humans. By using the One Health model with dogs as sentinels, we identified circulation of a variety of rickettsial species that could cause infections in humans. These data suggest that many human infections identified as RMSF in Tennessee may be caused by other rickettsiae. Ensuring the availability of species-specific clinical tests is important to ensure accurate diagnosis of human illness.

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EVALUATION OF DIAGNOSTICS OF *CHLAMYDIA TRACHOMATIS* INFECTION IN KAHE MPYA SUBVILLAGE, ROMBO DISTRICT, TANZANIA: A LATENT VARIABLE MODELING APPROACH

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Trachoma, caused by *Chlamydia trachomatis,* is the leading infectious cause of blindness. Polymerase chain reaction (PCR) is considered to be very sensitive for the diagnosis of *C. trachomatis* infection, but too expensive and time-consuming to be used routinely in a large treatment programme. The method currently used to assess whether populations require control intervention, and to conduct the evaluation of such an intervention, is clinical examination for active disease (Trachomatous Inflammation-Intense (TI) and -Follicular (TF)). We used Latent Markov models to assess diagnostic test accuracy before and after a round of mass treatment with azithromycin in a Tanzanian community (n=956; baseline prevalence of PCR, TI and TF positivity 9.5 %, 13.0 % and 14.6 % respectively), in the absence of a gold standard diagnostic. We defined the true health states as latent statuses of a dynamic latent (unobservable) variable and estimated 3 sets of parameters: 1) transition probabilities which allow for correlation between a respondent's true health state at times *t* and *t*-1; 2) probabilities of response to the 3 diagnostic tools conditional on the latent health status at each time point (i.e. sensitivities and specificities); 3) latent status prevalences (i.e. distribution of the true health states) at each time point. Some key results are: At the individual level, sensitivities and specificities for PCR, TI and TF to identify a) infected and diseased individuals and b) diseased but not infected individuals remained constant during baseline, 2, 6, 12 and 18 months after treatment, for both children <10 yrs and individuals ≥10 yrs. All 3 diagnostic tools were identified as highly specific markers of infection and disease for both age groups. Sensitivities for a) infection and active disease as well as b) active disease without infection, varied among the 3 diagnostic tools at the individual level. Results of this research will help trachoma programme managers identify useful markers to evaluate trachoma control interventions in similar low prevalence settings.

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NATURAL SELECTION IN THE CHOLERA ENDEMIC GANGES RIVER DELTA REGION

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Vibrio cholerae has likely played an important role in human evolution, especially in the Ganges River Delta, the historical and current epicenter of cholera. Observational data support this hypothesis. In particular, individuals with blood group O are at increased risk of severe cholera, and the lowest prevalence of type O in the world is found in the Ganges River Delta. In previous work, we showed that in Bangladesh, cholera aggregates within families independent of blood group, and that the gene *LPLUNC1*, a component of the innate immune system, is associated with susceptibility. Here, we report the first genome-wide study of the non-migrating ethnic group occupying the Ganges River Delta. Using

the Illumina 1M SNP array, we genotyped 36 Bengali mother-father-child trios from Dhaka, Bangladesh. Our results show that Bengalis are a homogenous population group on a distinct branch of the human genetic tree from the 11 populations of HapMap3. Using the Composite of Multiple Signals method, we identified 322 signals of natural selection in the Bengalis (average size 180kb with 2.5 genes; ~163 have 0 or 1 genes). Using INRICH, a new tool designed for genome-wide datasets, we found two especially interesting patterns. First, we repeatedly identified potassium channel genes in strongly selected regions. Second, we identified exceptionally strong enrichment ($p=2x10^{-4}$) for a module of co-expressed genes linked to the gene IKBKG, part of the immunity / inflammation NF-κB complex. Infectious diseases can exert strong selective pressure, and tests for natural selection are a powerful way to find genes influencing susceptibility. We show that, by leveraging massive public datasets and powerful new computational tools, we can identify multiple candidate genes using just 108 individuals. We are now evaluating our candidate genes for association with cholera susceptibility, using transmission disequilibrium testing (TDT) of parents / cholera-affected child trios.

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THE CARTAGENA PROTOCOL IN THE CONTEXT OF RECENT RELEASES OF TRANSGENIC AND *WOLBACHIA*-INFECTED MOSQUITOES

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In the last two years, the first environmental releases of both genetically sterile and *Wolbachia*-infected mosquitoes have been carried out with the goal of controlling dengue fever. Genetically sterile mosquitoes are governed by the Cartagena Protocol on Biosafety - the fundamental document of the United Nations on the international movement of living modified (LM) organisms. Their release provides insight on the suitability of the Protocol for LM mosquitoes. *Wolbachia*-infected mosquitoes are not covered by the Protocol; however their genetic novelty and potential ability to spread across international borders highlight issues relevant to self-propagating LM mosquitoes to which the Protocol does apply. We highlight weaknesses of the Protocol that should be addressed prior to an open release of mosquitoes with gene drive systems intended to spread disease-refractory genes on a wide scale. One weakness, highlighted by recent exports of LM mosquito eggs from the United Kingdom, is that a major section of the Protocol does not apply to LM mosquitoes that are initially intended to undergo laboratory and/or cage studies in the receiving country, and to be released into the environment if these studies are successful. This means that, under the most likely release scenario for any variety of LM mosquito, the exporting country is exempt from performing and financing a risk assessment. Another shortcoming is that, although the Protocol technically applies to non-signatories to the Protocol, these countries may not feel obliged to abide by a Protocol they did not agree to, even if the released transgenes are expected to spread on an international scale. Releases of LM mosquitoes in the Cayman Islands also highlight confusion over the applicability of the Protocol to movements between signatories and non-signatories, which should be clarified. Lessons learned from these releases should guide the Protocol to address the unique biosafety concerns posed by new varieties and future releases of LM mosquitoes.

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WOLBACHIA INFECTIONS OF *AEDES AEGYPTI* AND THEIR POTENTIAL TO CONTROL DENGUE TRANSMISSION

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Wolbachia is a very common intracellular bacterial infection of insects that is maternally inherited and present in up to 70% of all insect species. It does not occur naturally in the major insect vectors of disease however. Recently we have been able to transfer different strains of *Wolbachia* into *Aedes aegypti* where it is maintained and maternally transmitted between generations. It induces a number of effects in the mosquito host including a direct interference effect with dengue viruses, greatly reducing the ability of the mosquito to transmit virus. In recent work we have shown that *Wolbachia* can invade cage populations of wild type *Aedes aegypti* and can also establish itself in wild mosquito populations in open field trials in Australia, opening the way to consider *Wolbachia* as a new tool for the area wide control of dengue.

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SPATIAL-TEMPORAL VARIATION OF *AEDES AEGYPTI* PRESENCE AND ABUNDANCE IN IQUITOS, PERU: IMPLICATIONS FOR DENGUE VIRUS TRANSMISSION

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Spatial-temporal variation of *Aedes aegypti* populations affects both the transmission of dengue (DEN) virus and the effectiveness of vector control efforts. Using extensive *Ae. aegypti* surveillance data collected throughout Iquitos, Peru (1999 through 2002), we evaluated the temporal variation in the abundance of *Ae. aegypti* adult females, and pupae. Results were adjusted for spatial variation in mosquito abundance across the city, as well as differences in surveyor efficiency using linear mixed models. Significant seasonal variation in risk of infestation was observed for both *Ae. aegypti* adults and pupae. Abundance was lowest during July/August and peaked in January. The degree of spatial aggregation within houses also varied seasonally. During periods of low *Ae. aegypti* abundance the population was highly aggregated within houses and became more dispersed when abundance rose. Risk of houses being infested with *Ae. aegypti* adult females was positively correlated with average daily minimum temperature (p=0.02), average wind speed (p=0.0001), and negatively correlated with mean temperature (p=0.02), elevation of the collection site (p=<0.0001) and the water level of the Amazon River (p=<0.0001). The risk of houses being infested with more than 20 pupae was inversely correlated with the river level (p=<0.0001). In contrast the risk of houses being infested with less than 10 pupae was positively correlated with the level of the river (p=0.008). These results indicate that temporal variation in both the abundance and distribution of the *Ae. aegypti* vectors are likely to influence seasonal patterns in DEN virus transmission and disease dynamics.

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INFLUENCE OF HUMAN AND MOSQUITO DENSITIES ON THE PROPAGATION OF DENGUE VIRUS ACROSS HOUSES IN ENDEMIC AREAS

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Heterogeneity in the density of houses, people and *Aedes aegypti* production is a defining feature of dengue endemic cities. However, using this variation to focalize vector control requires a better understanding of the mechanisms through which human and mosquito densities influence viral propagation. At the household level, increased human density favors viral introduction and local propagation through social ties, in addition to increasing the average number of different people exposed to infectious vectors; however, more people reduce the average number

of mosquitoes exposed to infectious people due to frequency dependent mosquito biting. Given *A. aegypti*'s endophilic nature and limited dispersal, increased housing density may favor the infectious exposure of both hosts to each other, whereas increased seroprevalence will reduce only the average number humans exposed to infectious vectors. We used an agent based simulation to explore how the interplay between these processes affects dengue's reproductive rate, given observed ecological variation within endemic areas. Simulations were parameterized based on 7 censuses of *A. aegypti* pupae in 58 patches (2-4 blocks each) of 3 Colombian cities and their respective distributions of residents. Using commonly reported values for survival, biting, dispersal and herd immunity, simulated reproductive rates were comparable to the infection rates observed in a pilot study of clustering of dengue infection in children carried out in the same neighborhoods. Variation across patch-surveys was largely driven by infectious humans infecting many mosquitoes rather than infectious mosquitoes infecting many humans. Increased housing density ameliorated the effects of herd immunity on reducing the reproductive rate. We suggest that given the natural variation in *A. aegypti* densities in areas where domestic water stores generate most vectors, targeting the most productive containers per se is less efficient in reducing the long term transmission rate than reducing vector production in areas with highest human and housing densities.

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THE SPATIAL DIMENSIONS OF DENGUE TRANSMISSION AND EVALUATION OF MOSQUITO INTERVENTIONS

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A significant challenge for the evaluation of new dengue prevention strategies is determining the spatial scale required to detect an epidemiologic effect; i.e., reduction in virus transmission or disease. We use results from entomological and epidemiological studies, across different spatial scales, to examine relationships among mosquito movement, human movement, and dengue virus (DENV) transmission. 25 mark-release-recapture studies indicate that *Aedes aegypti* do not disperse far (typically < 100m) and spatial analysis indicate that a household is the proper spatial scale for measuring *Ae. aegypti* density. When entomological outcomes are the goal, area for treatment is determined more by inter-house variation in mosquito abundance than mosquito movement. Spatial dimensions of DENV transmission can be large and challenging to define due human movement (which affects mosquito-human contact rates and facilitates rapid virus spread) and heterogeneity (spatial and temporal) in virus transmission. Results from contact cluster investigations in Iquitos, Peru (testing blood from people in houses recently visited by a DENV-4 infected person) support geographically dispersed networks of DENV transmission. Attack rates were 17% in DENV positive clusters and 6% in control clusters with more than 50% of contact households >100 m from the index case house and peaks in frequency at 45 m, 265 m, and 1,636 m. Spatially-explicit semi-structured interviews and GPS data-loggers applied to 1,200 Iquitos residents indicate that when not in their own home, most people are in another residence and the average number of sites visited increase with age. We are developing spatially explicit network models to explore the structure of Iquitos human-vector contact networks and their implications for DENV transmission and prevention. Vector control assessments that seek epidemiologic outcomes will need to take human movement into account when selecting the spatial scale for application, or run the risk of obtaining ambiguous results because exposure to virus occurs outside the treatment area.

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EVALUATING ECOLOGICAL NICHE MODELS

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Ecological niche models are being used to predict the geographic range and distribution of medically-important species, including disease vectors, i.e., mosquitoes and sand flies. Ecological niche models predict geographic distribution using species' location records and environmental data (e.g. precipitation, temperature, and land cover). Because of the on-line availability of disease and vector data (e.g., mosquitomap.org) and environmental data (e.g., worldclim.org), models can be assembled quickly. Because most data are not collected for modeling purposes, the data are frequently not adequate to build a good model. While models are traditionally evaluated using statistical methods, other methods of evaluation are equally important such as 1) additional sampling in predicted areas of presence/absence, 2) comparison with disease, host, and other data sets, and 3) comparison of model results to known environmental factors affecting relative population distributions and abundance. This presentation reviews several ecological niche models (published and unpublished) built using the Maxent program for a variety of species, and discusses the methods used to evaluate the models. Models examined were for *Trichinella* spp., *Culex tritaeniorhynchus*, *Aspergillus* (as measured by aflatoxin levels), *Aedes aegypti*, and *Ae. albopictus*. The examples illustrate that steps to reduce clustering in the input data are essential to create good models. Methods to eliminate clustering include removing duplicate records for the same location, additional sampling for under-sampled areas, and resampling the data on a grid. The example models demonstrated that clustered sampling can result in a good to excellent statistical score but poor predictive results. It is therefore critical to evaluate modeling results using several evaluation methods.

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NOVEL AGE BIOMARKERS FOR AFRICAN AND ASIAN MALARIA VECTORS: CHANGES IN PROTEIN EXPRESSION IN HEADS AND THORACES

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Estimation of mosquito age would greatly assist assessments of the efficacy of vector control interventions against malaria. For example, successful vector control with insecticide treated nets (ITNs) and indoor residual sprays (IRS), which reduce transmission by reducing mosquito longevity, would be indicated by a mosquito population dominated by young mosquitoes. However, traditional methods of age grading mosquitoes involve difficult dissections to observe changes occurring in the reproductive system of the females, and only reliably distinguish young nulliparous females from older parous females. In this study, we have shown that mosquito age can also be determined from changes in protein expression occurring in mosquito heads and thoraces.The head and thorax proteome of two cohorts each of *Anopheles gambiae* s.s and *An. stephensi* was compared at different age points (1d, 9d and 17d for *An. gambiae s.s*) and (1d, 9d, 17d and 34d for *An. stephensi*). Proteins were extracted, separated and quantified using Differential in Gel Electrophoresis (DIGE). One way ANOVA was applied to determine

significant changes in protein spot volumes relative to age. For *An. gambiae*, the expression of 6 protein spots changed significantly with adult age for both cohorts (*P*=0.01). However, 14 spots changed with age in both *An. stephensi* cohorts. The abundance of two *An. gambiae s.s* and two *An. stephensi* proteins increased with adult mosquito age while the remainder of the proteins for both species decreased in expression with age. Importantly, two of these spots were shared between the two species. Currently, 2 of these proteins have been identified by mass spectrometry and recombinant forms of these proteins are being used as antigens for antibody production. We propose to develop a cost-effective ELISA age prediction assay from these antibodies to allow for rapid determination of the age of field collected *Anopheles* mosquitoes.

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DESIGNING FOR CHANGE: HOW AN EFFECTIVELY DESIGNED COMMUNITY-BASED BEHAVIOR CHANGE INTERVENTION SUBSTANTIALLY IMPROVED NEWBORN SURVIVAL IN SHIVGARH, INDIA

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It has been shown that simple, low cost interventions such as early and exclusive breastfeeding and keeping babies warm can substantially reduce neonatal mortality in community settings in high mortality regions. Despite this, only modest gains in newborn survival have been made over the past decade. The success of these interventions is contingent upon individual and normative changes in behavior at a community level. Often, these interventions are developed and implemented without taking into account the local realities and cultural context of the communities they are intended to benefit, leading to sub-optimal acceptance and adoption. We developed an essential newborn care intervention package based on the behavior change management framework after an assessment of high-risk newborn care practices, their underlying rationale, and people who had a role in influencing these practices. The intervention was epidemiologically targeted and hybridized scientific evidence with local 'wisdom' and socio-cultural reasoning paradigms in order to improve the understanding and acceptance of the intervention within the community. Further, we harnessed the power of influencers and social networks in order to accelerate normative shifts in behavior. Our approach led to a 54% reduction in neonatal mortality in a period of 16 months, assessed using a cluster randomized controlled trial design in Shivgarh, India. The behavior change management framework can be used to design and implement community-based interventions that are effective from an epidemiological standpoint, and at the same time, suitably adapted to the local socio-cultural context in order to maximize adoption.

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IMPACT OF THE AVAILABILITY OF INTEGRATED COMMUNITY CASE MANAGEMENT ON HEALTH CARE SEEKING BEHAVIOR IN RURAL ZAMBIA

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The provision of integrated community case management (iCCM) for common childhood illnesses by community health workers (CHW) represents a strategy that many developing countries are undertaking in order to reduce mortality of children under 5 years old. In our recently completed cluster, randomized controlled trial, the Zambia Integrated Management of Malaria and Pneumonia Study (ZIMMAPS), we also sought to assess how the availability of iCCM for malaria and pneumonia influenced care seeking behavior. In ZIMMAPS, CHWs were randomized to control [provision of artemether-lumefantrine (AL) to febrile children and referral of children with non-severe pneumonia to the nearest rural health center (RHC) for treatment] and intervention arms [performance of RDTs for febrile children, treatment of RDT-positive children with AL, and treatment of children with non-severe pneumonia with amoxicillin]. We conducted baseline and post-study household surveys on health care seeking practices among women aged 15 - 45 years who had at least one child ≤5 years. The same villages were used in both baseline and post-study surveys. A total of 440 and 441 caregivers were interviewed in the baseline and post-intervention surveys respectively. For children presenting with fever, there was a significant increase in the proportion seeking care from a CHW from baseline to post-study in both intervention [48.3% vs. 81.0%, p<0.0001] and control groups [51.3% vs. 77.9% p<0.0001]. There was a corresponding decrease in the proportion seeking care at RHCs between the baseline and post-intervention surveys in both intervention [35.6% vs. 13.4%, p<0.0001] and control groups [32.5% vs. 13.8%, p<0.0001]. For children presenting with difficult/fast breathing, there was an increase in the proportion who sought care from CHWs from baseline to post-study [50.8% vs. 74.3%, p=0.02 However, in the control group this shift was not found. Our study suggests that the provision of iCCM by CHWS can profoundly influence local health care seeking behaviors. Providing skills and supplies to CHWs is likely to increase the utilization of the services and reduce overload of staff of the public health facilities.

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UTILIZING A PROBLEM BASED APPROACH TO TACKLE GLOBAL HEALTH PROBLEMS: A CASE OF UNIVERSITY STUDENTS IN KIBERA SLUMS

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Kibera is the largest slum in Kenya and in Africa, and is home to 170,070 inhabitants. There are problems that compound access to health care including lack of transport, insecurity, insufficient health facilities and health workers, overcrowding and lack of basic essential amenities like water. The design liberations project assumed a multidimensional problem based approach addressing a broad topic of "Challenges of accessing health care in slum areas". A group of 30 students in university of Nairobi and 30 students from Stanford university from various academic disciplines were brought together in 9 groups. The groups studied the broad topic from perception of nurses, clinicians, policy makers, community health workers, mothers, adults, insurance agencies and the government. The groups used questionnaires and observation techniques to collect data on the broad topic from their identified study informants. The results of the research were then analyzed to develop problem areas which could be addressed by mobile technology and applications developed for these areas. Applications included 'baby bank' to allow pregnant mothers to save money for delivery; 'mnote' a mobile based application to facilitate the work for community health workers; 'mmaji' to assess on the quality and price of water supply in the slum; a pharmacy application to compare and authenticate quality of medicine among other applications. The project successfully addressed needs of the slum residents regarding health, using a bottom-up approach to address problems ranked in hierarchy of needs by key stakeholders within the slum. The project re emphasizes on the need for mutisectoral collaborations as a strategy to address global health problems, need for community participation in developing global health solutions and serves as an example of a simple north south research and technology partnership.

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IMPROVING ACCESS TO SKILLED BIRTH ATTENDANCE IN RURAL AREAS OF NINE SUB-SAHARAN AFRICAN COUNTRIES: RESULTS FROM A PAIR-MATCHED COMMUNITY INTERVENTION TRIAL

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Despite global commitments to achieve the Millennium Development goals (MDGs), progress towards MDG 5 remains slow in sub-Saharan Africa, where virtually no reduction in maternal deaths occurred between 1990 and 2005. We introduced an integrated package of health and infrastructure interventions in high-poverty rural sites in 9 sub-Saharan African countries. A quasi-experimental design and implementation research were used to evaluate the effect of this integrated package of interventions on rates of skilled birth attendance (SBA). Birth histories were collected from 15-49 year old women in randomly selected households in the 9 intervention sites and 9 pair-matched non-intervention sites. Data are available on 3,132 births, including 626 first-time births. The effect of the integrated model is evaluated by comparing the pre-post change in SBA rates in the intervention and non-intervention sites. Implementation research documented the package of interventions including equipped and staffed clinics; roads, water and electricity; referral services; emergency transport; user fees; traditional birth attendant engagement; and incentives. We find that the package was successfully implemented after 18 months in 8 of 9 intervention sites. After three years, SBA rates among all births had increased from 34% to 57% in the intervention sites (p-value=.0001) and from 28% to 42% in non-intervention sites (p-value=.001). Gains in the intervention and non-intervention sites do not differ by a statistically significant amount (odds-ratio=1.3, CI = 0.9 - 1.9). Among first-time births greater differences were observed, with a doubling of SBA rates in intervention sites (39% to 81%, p-value=.004); in non-intervention sites gains were more modest, from 42% to 59% (p-value=.023). Gains in the intervention sites are statistically greater than in the non-intervention sites (odds-ratio= 3.0, CI = 1.1 - 8.6). Despite limited progress towards MDG 5 over the past two decades, these data provide encouraging evidence of recent progress. The implementation of an integrated comprehensive model was associated with dramatic gains in SBA in a relatively short period, particularly among first-time mothers.

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USING GPS DATA TIMESTAMPS AND OTHER MOBILE ICT METHODS TO IMPROVE COMMUNITY-BASED WORKER ACCOUNTABILITY: A RURAL BANGLADESH EXPERIENCE

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Supervisory systems for field-based workers can be difficult and expensive to implement. In contrast to office-based staff, community workers are expected to cover large geographic areas, and only have occasional interactions with supervisors during a work day. Inexpensive Global Positioning System (GPS) units and GPS-enabled smartphones allow novel approaches to performance monitoring. Since 2001, we have conducted randomized controlled trials in a contiguous 436 km2 area of rural northwest Bangladesh, with a field staff of ~750. ~300,000 GIS waypoints are maintained for geospatial analyses and study monitoring. We developed innovative ways of using portable technologies such as Geographic Information Systems (GIS) and mobile phones to monitor and maintain staff accountability and performance across a wide geographic area. We used timestamp signatures embedded in GIS waypoints collected by GPS to assess and map team workers in the field over an 11-week period in 2009. Other examples of performance-assessment methods used in this site include GIS mapping of supplementation adherence, community refusals to participation, and reported vaccination receipt. GPS and location-capable smartphones were also evaluated to quantify the time savings for "expensive" research physicians. Analysis of GPS timestamp data identified poor-performing field workers, who spent a median of 1.89 hours of an expected 5 hour day collecting waypoints. After supervisory counseling, GPS data was used to map worker movements, generating both temporal and spatial data to ensure that expected daily performance benchmarks were being met. For research physician performance, an overall a 32% increase in daily efficiency was achieved using a GIS system, generating 1 extra day's worth of data each week. In conclusion, timestamp and location data from mobile devices, often regarded as uninformative data tags, can improve worker accountability and performance. Innovative use of GPS/GIS technologies can improve program or research efficiency, in addition to allowing for geospatial analyses.

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HEALTH IN HARMONY - HEALTHCARE IN BALANCE WITH THE ENVIRONMENT

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In much of the world, the pursuit of basic human needs and the integrity of the natural environment are in conflict. Loss of biodiversity, poverty, and lack of access to health care are linked and require simultaneous, inclusive solutions. Health In Harmony (HIH) supports Project ASRI in creating a comprehensive community health program that directly links environmental and human health in Indonesia. Five years ago, villages in the project area shared their priorities for improving quality of life. HIH then built upon their suggestions and worked to integrate high-quality, affordable health care with specific strategies to protect threatened rain forests. In the teaching medical clinic, Indonesian physicians and nurses are trained by volunteer physicians; more than 15,000 patients have been served. Public health programs include a team of community health workers and a DOTs program. These vital health care services are connected to several conservation strategies, which serve to link human and environmental health. An incentive program rewards rain forest protection with discounted health care. The reforestation program asks patients to bring in seedlings in exchange for mosquito nets or to pay for their medical care. The organic farm training program has given community members increased crop yields, better nutrition, and a healthier relationship with the natural environment, as well as an alternative method of payment for health care through organic farm labor. In addition, the "Goats for Widows" initiative provides widowed women with two goats to breed and a market for the goats' fertilizer, the organic gardens. Health in Harmony's comprehensive approach to health care provides innovative solutions to preserving the tropical forest, empowering individuals to improve the health of their communities.

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ESTABLISHING INTEGRATED COMMUNITY MANAGEMENT OF MALARIA, PNEUMONIA AND DIARRHEA IN SELECTED TWO LOCAL GOVERNMENT AREAS, AKWA IBOM STATE NIGERIA

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Integrated Community case management (iCCM) of malaria is seen as an essential strategy in achieving Roll Back Malaria coverage targets. Coverage is a key outcome measure but does not help envision the challenging steps needed to establish iCCM. One challenge is deciding and convincing stakeholders to include rapid diagnostic tests in iCCM. Most control efforts have been based within the health services. Formative research in two selected Local Government Areas has shown poor access to malaria interventions is related to distance from health facility, poverty, financial constraints, and perceptions of health services quality. Therefore, iCCM is needed to improve coverage. A team from Jhpiego (affiliate of Johns Hopkins University) and local government health services are piloting an iCCM program based on national guidelines from Nigeria's National Malaria Control Program. This presentation outlines the major organizational, logistical and attitudinal challenges facing the start-up of iCCM. These have included procurement process of rapid diagnostic test kits and anti-malarial drugs, providers' and community poor acceptance of RDTs, community disposal of waste and sharps from RDTs, and providers' and volunteers request for incentives and motivation as program are seen as a burden. Challenges in procurement process include difficulty in sourcing RDTs that come with a complete set of ready to use components. In Onna Local Government communities there is a belief that 'blood of someone alive cannot be buried' because it is believed that blood is life and such burial of blood in a used cassette would mean burying the person alive. To address such challenges we held community dialogue and agreed that used cassettes will be sent to health facility by the CDDs for appropriately burning before burial. This was more acceptable to the community members. We also worked with other malaria partners to identify reliable sources of RDTs. Finally stakeholders meetings helped address reluctance by the health ministry to allow RDT use at the community level. In conclusion we have learned the need for consensus building among partners on roles and extent of services to be provided by volunteers and for community education and dialogue prior to the initial start-up iCCM provision. Without attention to these start-up processes we cannot expect to reach our endpoint coverage indicators.

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IN SEARCH OF PUMP HANDLES: RISK FACTORS FOR DISEASE EARLY IN THE 2010 HAITI CHOLERA EPIDEMIC

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On October 21, 2010, cholera was confirmed in Haiti. Within 1 month, laboratory-confirmed cases were reported from all 10 Haitian departments; Artibonite was one of the earliest and most heavily affected departments. We conducted a case-control study of risk factors for cholera in Artibonite. We enrolled 49 cases - persons ≥5 years old with acute, watery diarrhea admitted to the Cholera Treatment Unit in Petite Riviere between October 25 and November 9, 2010 - and two age- and neighborhood-matched controls per case. We interviewed participants about multiple exposures, including water-related exposures and foods, and conducted household visits. Drinking water was tested for free chlorine as an objective measure of chlorine treatment. Few case-patients (31%) or controls (23%) had an improved drinking water source as defined by WHO. Similar percentages of case-patients (79%) and controls (74%) lacked safe water storage. Although comparable percentages of case-patients (52%) and controls (51%) reported not treating their drinking water before the outbreak, case-patients were significantly more likely than controls to not treat their drinking water during the outbreak (41% vs. 16%, mOR= 3.5, 95% CI: 1.5, 8.7). Stored water from a lower percentage of case-patients than controls had ≥ 0.1mg/liter of free chlorine (27% vs. 39%, mOR= 0.4, 95% CI; 0.1, 1.1). A higher percentage of case-patients (61%) than controls (48%) lacked access to a latrine and, therefore, practiced open defecation (mOR= 2.0, CI: 0.7, 6.1). This study demonstrated that not treating drinking water was the most important factor associated with symptomatic cholera. This finding provides evidence that safe drinking water is a critical need in Haiti. The increase in reported frequency of treating drinking water during the outbreak, particularly among controls, suggests that cholera prevention messaging effectively reached part of the population. The cholera epidemic should galvanize both governmental and non-governmental organizations to address Haitians' need for safe water and adequate sanitation.

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A RAPID QUALITATIVE ASSESSMENT OF CHOLERA RESPONSE EFFORTS, ARTIBONITE DEPARTMENT, HAITI, NOVEMBER, 2010

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On October 21, 2010, Haiti confirmed its first cholera outbreak; Artibonite Department reported 9,694 hospitalized cases and 595 deaths in the first 3 weeks. The Haitian government and non-governmental organizations initiated a communication campaign about cholera, including preparation of homemade sugar-salt solution (SSS) for treatment, through radio, television, and community workers on October 22. Water treatment products and oral rehydration salts (ORS) were distributed. Household surveys in Artibonite revealed that 55% of families had water treatment products and 36% had ORS. A survey of cholera decedents' families suggested that nearly half did not know cholera was treatable. To understand discrepancies between response activities and household preparedness, from November 12-17, we conducted 7 focus group discussions (median size=8 persons) and 5 semi-structured interviews with women regarding cholera prevention and treatment, household water treatment methods, and hygiene. Data were analyzed for dominant themes and concepts using ATLAS-ti software. Analysis revealed that most respondents feared cholera and lacked understanding about cholera prevention, transmission, and spectrum of illness. Discussants expressed a desire for in-depth cholera education. Product distribution events were described as chaotic and stressful, with inequitable distribution of supplies and insufficient education about specific products. Many discussants could describe neither proper dosing for water treatment products, nor correct SSS preparation. Most knew proper ORS preparation, and all discussants noted an insufficient supply. Discussants noted that water treatment products lasted only a few days and replacement supplies were unaffordable. Three weeks into the outbreak, despite intensive communication and product distribution, there were substantial cholera knowledge gaps and insufficient supplies for cholera prevention and treatment. This qualitative assessment rapidly identified correctable deficiencies in cholera response efforts.

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ESTIMATED INCIDENCE OF *VIBRIO CHOLERAE* IN THE CATCHMENT AREA OF A DIARRHEAL DISEASES HOSPITAL IN BANGLADESH

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Evaluating cost-effectiveness of interventions to prevent cholera in Bangladesh depend on estimated community-based incidence. ICDDR,B conducts pathogen specific diarrhea surveillance by testing a 2% sample of patients hospitalized with diarrhoea in it's Dhaka Hospital. Estimating cholera incidence in the catchment area of this hospital using only the hospital data underestimates the cholera burden because many patients seek treatment elsewhere. We estimated the incidence of cholera in Dhaka Hospital's catchment area by adjusting the hospital-based incidence by the proportion of severe diarrhoeal patients within the hospital catchment area who were admitted to this hospital. We defined the catchment area of the hospital as those neighborhoods where more than two-thirds of admitted patients resided. To estimate the proportion of severe diarrhoea cases in the hospital catchment area who were admitted to Dhaka hospital, we conducted a house-to-house survey in randomly selected areas. In the catchment area survey, severe diarrhoea was defined as patients admitted to a healthcare facility, or received intravenous rehydration, or died as a result of frequent loose or watery stools in the previous 12 months. Hospital-based incidence of cholera was calculated by dividing the laboratory confirmed *Vibrio cholerae* cases in the Dhaka Hospital admitted from defined hospital catchment area by the total catchment population. The total population in the hospital catchment area was projected as 9.7 million applying 4.1% annual growth rate to 2001 population census. In the catchment area survey, we visited 38,000 households and identified 895 severe diarrhoeal cases including 2 deaths. The proportion of severe diarrhoeal patients who were admitted to Dhaka Hospital was 0.63 (95% CI: 0.54-0.69). In the hospital surveillance, 339 (18%) cases were positive for *V. cholerae* O1 from March 2010 through February 2011 among the enrolled patients admitted from hospital catchment area during this period. We extrapolated a total of 16,950 *V. cholerae* cases among all admitted patients from hospital catchment area. The population-based incidence of *V. cholerae* was estimated as 275 per 100,000 population (95% CI, 251-317) in the catchment area of Dhaka Hospital. This study provides a credible estimate of cholera incidence in Dhaka, which can be used to assess the cost effectiveness of cholera prevention activities including vaccine.

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PERCEPTION AND PRACTICE ON HANDWASHING LINKED TO CHILD FEEDING IN RURAL BANGLADESH

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Encouraging caregivers to wash their hands with soap before preparing food and before feeding a child may prevent illnesses and improve child growth. This formative study explored community perceptions and practices related to handwashing with soap at these two key times. We conducted this study in 50 rural villages using survey questionnaires, video observations, motivational exercises, in-depth interviews and focus group discussions with caregivers of 6-24 month old children and community members to collect baseline data on handwashing linked to child feeding. Of 350 surveyed respondents, the perceived important methods for ensuring the safety of food for children were to wash utensils with soap and water (61%), wash vegetables, fish, and meat (56%), wash hands with soap before feeding a child (55%), clean the food preparation area (47%), and wash hands with soap before food preparation (40%). Although 18% of respondents reported washing hands with soap before preparing food and 35% during the last child feeding episode, during the video observations, out of 12 opportunities to wash hands with soap before food preparation, participants washed with water alone 5 times, and did not wash their hands 7 times. Out of 27 opportunities to wash hands with soap before feeding a child observed on video, participants washed with water alone 12 times, and did not wash their hands 15 times. The majority of surveyed respondents (60%) cited the unavailability of soap and water near the cooking place as a physical barrier to handwashing before food preparation. In the motivational exercise, most caregivers ranked 'nurture' as the best motivator to encourage washing hands with soap, and 'disgust' as the second best motivator. Although a good proportion of respondents had knowledge that handwashing with soap before food preparation and feeding a child contributes to food safety, this knowledge did not translate into practice. Video observations demonstrated that caregivers in this community do not usually wash their hands with soap before preparing food or feeding a child; the physical absence of soap contributes to a lack of handwashing with soap. Although nutrition and hygiene improvement programs offer opportunities to deliver combined behavior change interventions, to improve handwashing around food preparation in child feeding these approaches will need to address the physical absence of soap, and should employ themes of nurture and disgust.

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ACCOUNTING FOR BIASES IN A HOUSEHOLD WATER TREATMENT INTERVENTION TRIAL IN THE CONGO

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The effectiveness of household water treatment (HWT) devices against diarrheal disease is often measured with intervention trials. It can be difficult to generalize these results outside the study population, and compliance with HWT is often incomplete. We constructed a quantitative microbial risk assessment (QMRA) model to address these issues. The QMRA model simulates a placebo-controlled trial (Boisson et al. 2010, PLoS One 5(9):e12613) of an HWT filter, and accounts for bias due to: A) incomplete compliance with filtration, and B) unexpected antimicrobial activity by the placebo filter. The QMRA model simulates a chain of events in children < 5 years over 12 months with a time unit of 1 day: 1) determine concentrations of 3 marker pathogens (diarrheagenic E. coli, Giardia, and rotavirus) in drinking water; 2) calculate daily doses of pathogens, using pathogen concentrations and water consumption; 3) convert doses to probabilities of infection, using a different dose response function for each pathogen; 4) assign infection to individuals, using probabilities of infection; 5) assign disease, using morbidity ratios. After calibrating the QMRA model to the results of the published trial, the model was used to estimate device effectiveness under different compliance scenarios. Four levels of compliance were considered: low, 65% of children treating 33% of their water; medium, 65% of children treating 67% of their water; high, 65% of children treating 100% of their water; and perfect, 100% of children treating 100% of their water. Compliance was a major driver of effectiveness. Assuming a perfect placebo and low, medium, high, or perfect compliance, the median preventable fraction of reported disease was 14%, 30%, 50%, and 87%, respectively. Adjustment for the imperfect placebo increased the median preventable fraction of disease by 8 percentage points assuming low compliance, but by 22 percentage points assuming medium compliance. The precise level of compliance can greatly affect measurements of HWT effectiveness; such trials should carefully measure compliance.

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ENVIRONMENTAL INDICATORS OF DIARRHEA IN VELLORE, INDIA

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Diarrhea is an important cause of morbidity and mortality in resource-poor settings. This study aims to characterize environmental drivers in transmission of enteric infections in 80 rural and 160 urban households in Vellore, India. Diarrheal episodes were investigated with microbiologic analysis of stool in an ongoing 1-year open cohort study. Information on demographics, hygiene, human/animal interactions, and water sources was collected by questionnaire. Household water contamination was tested using fecal coliform counts. Fly densities were measured in 2 seasons using fly ribbons placed in kitchens. PCR for enteric pathogens were performed on flies. From 8/6/2010- 1/31/2011, there were 91 episodes of diarrhea over 198,795 total person days (PD) with substantial fluctuations in monthly incidence from 0.15 to 0.92 per 1000PD. Fecal coliforms were present in 67% and 74.6% of household water samples from rural and urban areas respectively. Stool pathogens isolated in 24 of 77 (31%) of samples included *E. coli*, *Shigella* spp., *Vibrio* spp., *Giardia spp.*, *Cryptosporidium spp.*, and rotavirus. 43 of 60 (72%) fly samples were positive for pathogens including *E. coli*, *Salmonella* spp., norovirus, and rotavirus. Fly densities were 2.56 times higher during the dry season compared to monsoon ($p<0.001$). The absence of animals in living quarters and indoor latrine use were protective of high fly densities. The adjusted relative risk of diarrhea associated with the 75th% of fly densities was 1.15 [95%CI:1.02,1.29]. Risk factors for increased duration of diarrhea included family size, private well or indoor house-tap use, untrimmed nails, and increased fly densities, while rural living, indoor latrines, no animals in living quarters, and better education were protective. Flies harbored enteric pathogens including norovirus, a poorly documented pathogen on flies. Several modifiable environmental risk factors for diarrhea were identified including water sources, living conditions, hygiene, and fly densities.

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EVALUATION OF HOUSEHOLD MICROBIAL WATER QUALITY TESTING IN A PERUVIAN DEMOGRAPHIC AND HEALTH PILOT STUDY USING THE PORTABLE COMPARTMENT BAG TEST (CBT) FOR *E. COLI*

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The Joint Monitoring Programme of the UN relies on household surveys to determine the kind or source of drinking water supply present. A classification system is used in lieu of actual testing for the microbial water quality due to the unavailability of simple and affordable methods of testing. Demographic and Health surveys (DHS) represent an opportunity to examine household water quality on a large scale if low-cost water quality testing is available. A novel water quality field test was developed by the investigators of this study, field deployed and evaluated. In the new Compartment Bag Test (CBT), a 100-ml water sample is supplemented with a bacteriological medium to support the growth of E. coli, poured into a sterile bag having internal compartments of specified volumes and incubated overnight. If E. coli grows, the water in that compartment turns blue. From the number of E. coli-positive compartments, bacteria concentration is calculated as a Most Probable Number per 100 ml. The purpose of this study was to evaluate the performance of the method in assessing household drinking water quality in the context of a DHS. The pilot study included three regions of Peru with a total of about 750 households surveyed over a 14 week period. Results in the field were compared to the results in reference laboratories analyzing aliquots of the same water samples. Data from the first 10 weeks show that: 1) there was no significant difference between the laboratory (membrane filtration and CBT) and field (CBT) results (Repeated Measures ANOVA $p=0.44$, Friedman Nonparametric Repeated Measures ANOVA $p=0.07$); 2) there was no significant difference between the laboratory or field CBT results of the same water samples; 3) field surveyors and laboratory technicians could easily learn and perform the test. These results suggest that the CBT for E. coli is an effective, simple and affordable method to quantify fecal bacteria in household drinking water as an indicator of safety that can be incorporated into DHS and similar household surveys around the world.

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SCHOOL-BASED MALARIA CONTROL: IMPACT OF INTERMITTENT PREVENTIVE TREATMENT ON MALARIA MORBIDITY AND COGNITIVE FUNCTION IN UGANDAN SCHOOL CHILDREN

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Delivery of intermittent preventive treatment (IPT) of malaria in schools is a promising option for extending malaria control activities to older children. However data on the effects of IPT on the general health and school performance of schoolchildren remain few. We are currently conducting a randomized, single-blinded placebo controlled trial evaluating the impact of two different IPT regimens versus placebo on malaria morbidity and cognitive function among primary school children living in a high malaria transmission area in rural Uganda. In February 2011, 740 children age 6 years to 14 years who met the selection criteria were enrolled and randomized to one of the three study arms: i) Dihydroartemisinin-Piperaquine(DP) given once a school term (every 4 months), DP given once a month, and iii) Placebo. Baseline evaluations included a detailed history, physical exam, cognitive function testing, heamogram (Hb) estimation, stool examination for helminth infections and blood smear exam for presence of malaria parasites. Primary outcomes are the incidence of malaria (fever + parasitemia) and mean cognitive function test scores (using the Raven's matrices and code transmission tests). Secondary outcomes include risk of parasitemia, hospital admissions, adverse events, missed school days, school performance and mean change in Hb levels. An interim analysis of 734 participants completing 3 months of follow up (targeted follow up is 1 year) is presented here. One third (30%) of the children had asymptomatic parastaemia, 37% reported using a bed-net and 9% had helminth infection at baseline. The overall incidence of malaria was 0.08 episodes per person per year; risk of asymptomatic parasitemia was 18% and 31% of children missed at least one school day. Adverse events were reported in 28% of the children and no serious adverse event was reported. Detailed, un-blinded results will be presented at the meeting.

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SCALE-UP OF HOME-BASED MANAGEMENT OF MALARIA BASED ON RAPID DIAGNOSTIC TESTS AND ARTEMISININ-BASED COMBINATION THERAPY IN A RESOURCE-POOR COUNTRY: RESULTS IN SENEGAL

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Effective case management of malaria requires prompt diagnosis and treatment, ideally within 24 hours. In Senegal, case management of malaria in public health facilities was scaled up nationwide with artemisinin-based combination therapy (ACT) in 2007 and rapid diagnostic tests (RDTs) in 2008. Home-based case management may improve access in remote areas with limited access to health facilities. In 2008, 20 villages > 5 km from the nearest health facility participated in a home-based management pilot in which volunteer Home Care Providers (HCP) were trained to manage uncomplicated malaria using RDTs and ACTs, demonstrating the feasibility of integrated use of RDTs and ACTs in the community. Home-based management (PECADOM) was scaled up in 408 villages beginning in 2009. During 2009, 6697 suspected cases were managed by HCP, 92.5% (6198) of whom were tested with an RDT. Among those tested, 34.5% (2144) were positive, 96.1% (2061) of whom were reported successfully treated. Home Care Providers referred 3377 patients to health posts for management: 3324 with a negative RDT, 41 infants < 2 months, 36 pregnant women, and 76 severe cases. There were no deaths among these patients. In 2009 compared to 2008, in districts in which PECADOM was introduced, reported all-cause hospitalizations decreased by 21.7%, malaria hospitalizations by 41.6%, all deaths by 13.2%, and deaths attributed to malaria by 61.4%. Given the simultaneous scale-up of other malaria control interventions, including insecticide-treated nets, during the same period, we used Pearson's correlation coefficient (r) to evaluate the association between HCP/100,000 persons and the absolute decrease from 2008 to 2009 in malaria deaths and hospitalizations per 100,000 persons. We found a moderate positive correlation; for hospitalizations $r = 0.54$ ($p = 0.006$) and for deaths, $r = 0.52$ ($p = 0.008$). Home-based management of malaria including parasitologic testing and treatment based on test results may be a promising strategy to improve the access of remote and rural populations to effective management of uncomplicated malaria and to identify patients needing referral to health facilities, plausibly contributing to a decrease in malaria-related hospitalizations and deaths.

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WHERE COULD INTERMITTENT PREVENTIVE TREATMENT IN CHILDREN (IPTC) BE IMPLEMENTED AND WHAT IS ITS POTENTIAL IMPACT?

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Intermittent Preventive Treatment in children (IPTc) is a promising tool for control of malaria in areas of seasonal transmission. A WHO Technical Expert Group (TEG) recently reviewed evidence for the effectiveness of IPTc with a view to making a policy recommendation. To support the work of the TEG we determined the epidemiologic and geographic situations in which IPTc would be appropriate, and estimate its potential public health impact. First, a series of literature reviews were undertaken to identify monthly malaria data for full calendar years. Several definitions were considered to characterize sites with sufficient seasonality for IPTc implementation. Second, spatial predictors of 'IPTc seasonality' were explored, including rainfall, and climate-driven predictors from a spatial transmission model. Third, based on the continental maps produced by the best spatial predictors, the burden of malaria in under-fives in these areas was estimated using a range of approaches. Lastly, the number of cases that might be averted by IPTc with a range of efficacies and coverage was estimated. Our analyses suggest that the occurrence of 60% or more of the annual number of malaria cases within four consecutive months is the optimal definition of sites suitable for IPTc. The two best predictors for identification of 'IPTc areas' outside the areas for which epidemiological data were available were (i) >60% of the annual rainfall and (ii) >60% of the predicted proportion of total annual transmission from a mathematical model, both within 3 consecutive months of the year. The maps produced by these two predictors identified two potential 'IPTc' areas: (i) large areas of the Sahel and sub-Sahel and (ii) some areas in southern and eastern Africa. We estimate that at least 35 million under-fives live in areas suitable for IPTc. There is considerable uncertainty in making burden estimates, but our lowest estimate suggests that 21.5 million malaria cases and almost 100,000 deaths occur each year in areas suitable for IPTc. Assuming 70% efficacy and 70% coverage, IPTc could approximately halve the malaria burden in these areas and avert many thousands of unnecessary deaths.

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NO REBOUND INCIDENCE OF MALARIA AMONG HIV-EXPOSED CHILDREN AFTER DISCONTINUATION OF TRIMETHOPRIM-SULFAMETHOXAZOLE PROPHYLAXIS IN RURAL UGANDA

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As per WHO guidelines, infants born to HIV-infected mothers should be placed on trimethoprim-sulfamethoxazole (TS) prophylaxis until HIV infection is excluded. We recently showed that TS prophylaxis in HIV-exposed, uninfected children from cessation of breastfeeding until 2 years of age yielded a 39% reduction in malaria incidence. However, it is not known whether prior TS prophylaxis results in a "rebound" malaria incidence. To test this hypothesis we compared the incidence of malaria between 2-4 years of age in the following 3 groups from a cohort of children enrolled at 1.5-9 months of age: 1) HIV-unexposed children (n=88) never taking TS, 2) HIV-exposed, uninfected children (n=80) randomized to stop TS after breastfeeding cessation (median age 10 months, range 6-22 months), and 3) HIV-exposed, uninfected children (n=46) randomized to stop TS at 2 years of age. All children were given a long lasting insecticide-treated net at enrollment. Malaria was diagnosed when a child presented with fever and a positive thick blood smear and was treated with highly efficacious artemisinin-based combination therapy (ACT). Incidence of malaria was compared using a negative binomial regression model controlling for location of residence, the measure of association being an incidence rate ratio (IRR). Incidence of malaria between 2-4 years of age was similar for HIV-unexposed children who had never received TS (5.96 episodes/PY; reference group) compared to HIV-exposed children who received TS through breastfeeding (6.07 episodes/PY; IRR=1.01, p=0.92) and HIV exposed children who received TS through 2 years of age (5.84 episodes/PY; IRR=0.98, p=0.85). Malaria incidence was also similar when comparing HIV-exposed children randomized to stop TS at 2 years of age compared to those randomized to stop after breastfeeding cessation (IRR=1.03, p=0.74). Prior use of daily TS prophylaxis was not associated with a "rebound" incidence of malaria. However, the incidence of malaria between 2-4 years of age was very high in this cohort despite the use of ITNs and prompt and effective ACT.

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ACCESSIBILITY AND AFFORDABILITY OF ANTIMALARIALS IN A RURAL DISTRICT IN KENYA AFTER IMPLEMENTATION OF A NATIONAL SUBSIDY SCHEME

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Poor access to prompt and effective treatment for malaria contributes to high mortality and severe morbidity. In Kenya, it is estimated that only 12% of children receive antimalarials for their fever within 24 hours. The first point of care for many fevers is a local pharmacy or chemist. The role of the medicine retailer as an important distribution point for malaria medicines has been recognized and several different strategies have been used to improve the services that these retailers provide. Despite these efforts, many mothers still purchase ineffective drugs because they are less expensive than effective artemisinin combination therapies (ACTs). One strategy that is being piloted in several countries is an international subsidy targeted at antimalarials supplied through the retail sector. The goal of this strategy is to make ACTs as affordable as ineffective alternatives. The program, called the Affordable Medicines Facility - malaria was rolled out in Kenya in August 2010. In December 2010, we evaluated the affordability and accessibility of malaria medicines in a rural district in Kenya. We did a complete census of all public and private facilities, chemists, pharmacists, and medicine retailers within the Webuye Demographic Surveillance Area. We assessed availability, stock-outs, types, and prices of antimalarials. There are 12 private clinics, 13 public facilities and 84 medicine retailers (registered and unregistered). The average distance from a home to the nearest public health facility is 2km, but the average distance to the nearest medicine retailer is half that. Quinine is the most frequently stocked antimalarial and more shops stocked sulphadoxine pyramethamine (SP) than ACTs. No shops had chloroquine in stock and only 5 shops were selling artemisinin monotherapy. The mean price of any brand of artemether lumefantrine (AL, the recommended first line drug in Kenya) was $2.5. Brands purchased under the AMFm program cost 40% less than non-AMFm brands. Artemisinin monotherapies cost on average more than twice as much as AMFm-brand AL. SP cost only $0.5, a fraction of the price of an ACT. AMFm subsidies have reduced the price of AL, but the price difference between effective and ineffective therapies is still large.

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ACCESS AS A COMPONENT OF EFFECTIVE COVERAGE OF ARTEMISININ-BASED ANTI-MALARIA TREATMENT IN RURAL TANZANIA

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Following declining efficacy of previously used antimalarials, National Malaria Control Programs across sub-Saharan Africa have adopted artemisinin-based anti-malaria combination therapy (ACTs) as first line treatment. Despite excellent efficacy of ACTs, real-world effectiveness of these drugs is reduced by factors related to health systems. The INDEPTH Network's Effectiveness and Safety Studies in Africa (INESS) programme is investigating access, diagnostic targeting, provider compliance, patient adherence and costing to build a complete picture of ACT effective coverage at district scale.This presentation will share findings related to the access component of artesunate-lumefantrine (Alu) in two Health and Demographic Surveillance Sites (HDSS) in rural Tanzania. Linked to the routine HDSS update rounds, continuing surveys were conducted over a one year period to determine access to authorized providers of the official first-line ACTs within 24h and 48 hours of fever onset and reasons for choices and failed access. We surveyed 2,250 individuals resident in 8,874 randomly selected households from two HDSS districts. We analyzed access and care seeking pathways by age, sex, season, household socio-economic status, distance to public and private health providers and community health insurance. We also determined population fever prevalence and parasitemia as well the proportions treated with various other antimalarials in a continuous longitudinal monitoring system. We will share the latest results on access to ACTs from the INESS platform and show how this contributes to understanding effectiveness of ACTs in real-world health systems. We will discuss options on how to improve access and consecutively effective coverage of ACTs in rural Tanzania.

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FORECASTING DEMAND FOR ARTEMISININ COMBINATION THERAPIES UNDER THE AFFORDABLE MEDICINES FACILITY - MALARIA (AMFM)

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The first phase of the Affordable Medicines Facility - malaria (AMFm) is ongoing in Ghana, Kenya, Madagascar, Niger, Nigeria, Tanzania and Zanzibar, and Uganda. By subsidizing the purchase of artemisinin combination therapies (ACTs) to both private and public sector first-line buyers, the program aims to increase widespread usage of ACTs - the only effective treatment for *falciparum* malaria in much of the world - by dramatically reducing the cost to the consumer. Because the global market for ACTs is constrained by the availability of raw materials, long production cycles, and limitations in funding, accurate prediction of the impact of AMFm on global ACT demand is essential to avoid interruptions in supply. However, the scale and uncertainties surrounding this enormous market intervention make development of such a forecast extremely challenging. In 2011, UNITAID sponsored the formation of a consortium tasked with providing periodic forecasts of global ACT demand. The Clinton Health Access Initiative, in collaboration with MIT-Zaragoza and Boston Consulting Group, has developed a novel method for forecasting the uptake of AMFm. This method uses a patient-based approach to estimate the overall demand for antimalarial treatment in the private sector of each country participating in the subsidy. Based on analysis of antimalarial consumption data from previous ACT-subsidy pilot studies, along with data from ACT Watch, DHS/MIS, and in-country support teams, we model the impact that shifts in consumer demand have on AMFm private sector ACT uptake. The forecast estimates AMFm private sector demand for 45.5MM and 68.9MM treatments, comprising 16.5% and 25% of the global ACT market, in 2011 and 2012, respectively. This estimation has been outpaced by demand from first-line buyers during the initial phase of AMFm, indicating that additional market forces are influencing AMFm uptake. Ongoing refinement of this ACT demand forecast, incorporating new data on first-line buyer and consumer behavior, will help to ensure a sustainable supply of these life-saving medications.

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LYMPHATIC FILARIASIS: TREATING A NEGLECTED TROPICAL DISEASE IN THE UNITED STATES

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Lymphatic filariasis (LF) is a mosquito-borne parasitic disease caused primarily by infection with *Wuchereria bancrofti.* Although LF affects an estimated 120 million people in 81 countries, the majority of filarial infections are asymptomatic. However, approximately 40 million persons

are affected by lymphedema or hydrocele, the long-term sequelae of LF. A global effort to eliminate LF is based on annual mass drug administration using antifilarial drugs; in 2008 alone, 496 million people received treatment. In the U.S., there are over 4 million immigrants from LF endemic countries and an estimated 10,000 people have LF. Like many orphan diseases, treatment for LF is readily available in endemic countries, but in the U.S. it is only available through the Centers for Disease Control and Prevention (CDC). CDC has partnered with the Palm Beach County Health Department to implement a pilot program to test and treat immigrants from LF endemic countries. The program aims to offer immigrants the opportunity to be tested and if needed treated within a single clinic visit at no cost to the patient. This pilot program took over three years to establish because both the rapid diagnostic test and the drug treatment although widely used in the global LF elimination program, are not FDA approved and require IRB approval for use in the U.S. A total of 12 clinics within four health centers in the Palm Beach County health department are participating in the program. Each patient from an LF endemic country is offered the opportunity to be tested. Currently, 433 patients have been tested, 425 (98.2%) stated their country of origin was Haiti. Thirty two (7.4%) tested positive and 31 patients were treated; the one untreated patient was ineligible due to pregnancy. The median age of the people positive was 15 years (range 6 - 40). No adverse events were reported. The staff and patients are very supportive of the program. This program provides access to LF treatment to a U.S.-based population that was exposed in the their countries of origin. This program could serve as a model for other orphan parasitic diseases.

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INCIDENCE AND GEOGRAPHICAL DISTRIBUTION OF SERIOUS ADVERSE EVENTS FOLLOWING MASS ADMINISTRATION OF IVERMECTIN IN CAMEROON FROM 1999 TO 2009

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Mass treatment with ivermectin started in Cameroon in 1987, followed by community-based ivermectin treatment and Community Directed Treatment with Ivermectin (CDTI) implemented by the National Onchocerciasis Control Program (NOCP) in 1999. In 1999, a cluster of serious adverse events (SAEs) was reported in an area endemic for loiasis. This prompted the institution of a surveillance system in Central Africa to promptly address SAEs.The present study evaluated the annual incidence and risk factors of post-ivermectin SAEs from 1999 to 2009 in Cameroon. Treatments data were obtained from the Cameroon NOCP. Medical files of subjects presenting with SAEs were analysed, and geographical coordinates of their communities of residence were collected. A total of 9,057,076 treatments was administered in the 38 health districts from 1999 to 2009. During this time, 382 SAEs were recorded, giving a cumulative incidence of 4.2 cases/100,000 treatments. The outcome was fatal in 11 cases for a mortality rate of 1.2 deaths per million treatments. The annual incidence of SAEs decreased from 8.6 cases/100,000 in 1999 to 0.9 cases/100,000 in 2009. Nearly all (95%) of the SAEs occurred following the first ivermectin treatment. A mean period of 32.9 hours (ranged 2-168 hours) elapsed between the treatment and the first symptoms. Nearly all (91.6%) of the subjects with SAEs were found to have *Loa loa* microfilariae in post-treatment blood samples. Furthermore, all SAEs occurred in regions predicted to be highly endemic for loiasis. This study confirms previous data demonstrating that loiasis is the main risk factor of SAEs following CDTI and that the risk is greatest after the first treatment. The current maps predicting Loa loa endemicity will be useful in the identification of at-risk areas, and will be necessary to guide the NOCP in Central Africa during the planning and implementation of CDTIs for onchocerciasis and lymphatic filariasis control in untreated areas. In areas treated for some years, it may be helpful to test ivermectin naive individuals for *L. loa* before treatment.

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THE HISTOPATHOGENESIS OF IVERMECTIN-INDUCED LOIASIS-ASSOCIATED PATHOLOGY IN PRIMATES

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Understanding the pathogenesis of the severe adverse effects that occur in patients with high circulating loads of *Loa loa* microfilaria can suffer when treated with ivermectin has been a major goal of those associated with the distribution of this important drug for the control and elimination of onchocerciasis and lymphatic filariasis in areas of Africa endemic for loiasis. The tissues examined from splenectomized baboons carrying very high loads of circulating microfilariae (>100,000 mf/ml) revealed a number of tissue changes and clinical changes in these animals consistent with those reported in humans suggesting that the baboon model is a useful model for studying and understanding the pathogenesis and can allow for development of therapeutic approaches to managing the human condition. These changes in the baboons after ivermectin treatment included parasitic thrombi, fibrin deposition and damage to vascular endothelium. Petechial hemorrhages were commonly found at autopsy in the CNS and other tissues, as was acute damage in the tissues surrounding these vascular lesions. Chronic inflammatory responses were seen in the liver that occasionally were associated with microfilarial death and tissue eosinophilia; these are believed to be inherent to the long term presence of high loads of Loa loa and not due to the ivermectin treatment. All of the 12 animals studied shown evidence of considerable regeneration of splenic tissues, new organs that were very actively involved in degeneration and destruction of microfilariae. Histological evidence of dermal Mazzotti reactions were also common post ivermectin therapy. These finding suggest that the adverse clinical responses seen after ivermectin treatment in hosts with high levels of circulating microfilariae are vascular based lesions and that there is a tendency for these involve the central nervous system.

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IMPACT OF A COMMUNITY-BASED LYMPHEDEMA MANAGEMENT PROGRAM ON PERCEIVED DISABILITY, PRODUCTIVITY AND QUALITY OF LIFE AMONG LYMPHEDEMA PATIENTS IN ORISSA STATE, INDIA

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Lymphatic filariasis (LF) infects an estimated 120 million people worldwide, causing lymphedema and hydrocele in over 40 million. India comprises over 40% of the world's LF burden, with millions of people in need of lymphedema management. A community-based lymphedema management project in Orissa State, India, was begun in 2007 by the Indian non-governmental organization, Church's Auxiliary for Social

Action (CASA) in consultation with the Centers for Disease Control and Prevention, and has enrolled and treated over 21,000 lymphedema patients. To assess the impact of this program on disability, productivity, and quality of life, a random sample of 375 patients was followed over their first 12 months of enrollment. Each patient was interviewed at enrollment and at 1, 2, 3, 6, and 12 months thereafter. Perceived disability and quality of life were measured using the WHO Disability Assessment Schedule II (WHO DAS-II), a well-validated, 36-question tool, which assesses 6 domains of functioning: cognition, mobility, self-care, getting along with others, life-activities, and participation in society. Mean disability scores (simplified, non-weighted scoring) at each time point were compared using paired T-tests in SAS 9.2. Statistically significant decreases in disability were observed in all 6 WHO DAS-II domains within 3 months of enrollment, and were most pronounced at 6 months post-enrollment. At 6 months, the decreases in self-reported disability (compared to baseline) were 17% for cognition (P<.0001), 6% for mobility (P=.01), 13% for self-care (P<.0001), 21% for getting along with others (P<.0001), 7% for life activities (P=.01), and 15% for participation in society (P<.0001). At 12 months, the magnitude of each change was smaller than at 6 months, but remained statistically significant for all domains except mobility (3% reduction compared to baseline (P=.13)). When asked how many days in the past month they were totally unable to attend to their daily work due to their lymphedema, patients reported 3.3 fewer days lost at 6 months (95% CI 2.4-4.3) and 2.4 fewer days lost at 12 months (95% CI 1.3-3.2). These data demonstrate a beneficial impact of this lymphedema management program on quality of life and productivity, and emphasize how community-based lymphedema management can provide psychological support and increase productivity for those suffering from lymphedema.

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TOGO'S NATIONAL LYMPHOEDEMA MANAGEMENT PROGRAMME: EVALUATION OF PROGRESS OF PATIENTS AFTER THREE YEARS

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In 2007, Togo's Ministry of Health, with technical assistance from the Centers for Disease Control and Prevention, started a National Lymphoedema Management Programme. In each health structure in the country, including non-endemic districts, at least one person was trained in lymphoedema management following the recommendations from the World Health Organization. The aim was to train patients in self management of their lymphoedema. A cohort of patients was followed each year during three years to evaluate the impact of the program on the physical well-being and quality of life of the patients. Data were collected annually. The primary indicators used to evaluate the project include included changes in treatment behavior, incidence and duration of acute attacks, and quality of life. Data for 107 patients of the 166 originally selected by convenience sample in 6 districts were available for paired analyses of 2007 and 2010 responses. Fifty three percent of the patients were female, and the median age was 46 years (6-90). In 2010, 95 % of the patients followed were using at least one promoted treatment compared to 25 % in 2007 (OR=38.5, 95% CI: 11.33, 233.4). Patients were also more likely to report current use of each of the three promoted treatments -limb elevation (18% to 77%, OR=16.75, 95% CI: 6.67, 54.08), exercise (4% to 79%, OR=81.00, 95% CI: 16.05, 1638.00), and washing (10% to 93%, OR=89.00, 95% CI: 17.67, 1798.00). Patients were less likely to report no current lymphoedema treatment use (31% to 3%, OR=26.47, 95% CI: 0.010, 0.22). However, between 2007 and 2010, there were no significant improvements in patient self-sufficiency (as measured by activities limited by lymphoedema symptoms) or stigma There was no significant change in median number of acute attacks per year (2007 median: 2, 2010 median: 2, p=0.15). However, there was a significant decrease in median duration of acute attacks (2007 median: 6 days, 2010 median: 4 days, p=0.05). Although we have heard very positive feedback from the patients themselves, according to our survey, very little improvement was made on patient outcomes, although a high rate of loss to follow up limits our analysis and better tools are needed for quality of life assessments. We recommend that to assess the impact of national lymphedema management programs on patient outcomes, objective measures are needed to supplement self-reported data.

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AFRICAN PROGRAMME FOR ONCHOCERCIASIS CONTROL: IMPACT AND COSTS BY 2010

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Onchocerciasis causes a considerable burden of disease in Africa, mainly due to skin and eye disease. Since 1995, the African Programme for Onchocerciasis Control (APOC) has coordinated annual mass treatment with ivermectin in endemic countries. In this study, we estimated the effect of APOC on population health and the associated costs up to 2010. Using data from pre-control mapping studies, we estimated the pre-control prevalence of infection in APOC areas. Next, using data from APOC's mass treatment records and the micro-simulation model ONCHOSIM, we estimated the decline in infection, blindness, visual impairment and severe itch between 1995 and 2010. The associated burden of disease was expressed in disability adjusted life years (DALYs). Data on costs made by APOC, non-governmental development organizations, beneficiary governments and the Mectizan Donation Program (MDP) were obtained from reports, where available. We estimated that between 1995 and 2010, mass treatment with ivermectin caused a considerable decline in the overall prevalence of infection with O. volvulus (from 39% to 14%), troublesome itch (from 14.5% to 3.0%), visual impairment (from 1.0% to 0.7%) and blindness (from 0.4% to 0.2%) in APOC areas. We estimated that 1.5 million DALYs were lost due to onchocerciasis in 1995. Due to population growth, this loss would have been 2.2 million DALYs in 2010 if there had been no mass treatment. However, in 2010 only 0.7 million DALYs were lost. Overall, we estimated that APOC has cumulatively averted about 8.8 million DALYs due to onchocerciasis up to 2010. The total associated costs were estimated at about US$2.4 billion (US$2.2 billion for MDP only). In conclusion, APOC has had a great impact on population health in Africa, at a relatively limited cost.

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ELIMINATION OF ONCHOCERCIASIS TRANSMISSION IN MT. ELGON FOCUS OF EASTERN UGANDA HAS BEEN ATTAINED

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The Mount Elgon onchocerciasis focus is a highly isolated, having an area of 250 km^2, and located in eastern Uganda on the border with Kenya.

The vector is *Simulium neavei s.s.*, and its larvae develop in a phoretic association with the fresh water crab *Potamonautes granviki*. Annual ivermectin mass drug administration (MDA) was launched in Mount Elgon in 1997. In 2007, ivermectin MDA was changed to twice yearly treatment after the government of Uganda moved from a policy of control to elimination, and intensified interventions. MDA coverage has been over 90% of eligibles since the 1997 and was unaffected by the switch to twice per year treatment. In early 2008, vector elimination activities using the larvicide temephos (Abate®) at 0.2-0.4 ppm were applied once a month at identified breeding sites for a year, then every other month for a period of 6 months, and then stopped. Baseline crab trapping and examinations for *S. neavei* infestation and black fly landing catches were conducted monthly since 2007. Sentinel village (SV) examinations with skin snips to measure microfilardermia (mf) prevalence were conducted in 1994 and 2011. Serologic testing (OV16 antibody) of children below the age of 14 years was conducted in 2010. The Abate campaign drastically reduced crab infestation with larvae and pupae of *S. neavei* from 30.2% pre-control in 2007 to 0% by September, 2008, and has remained so since. Adult fly biting rate reduced from 5 flies per man hour in 2007 to 0 in July 2008, and has remained at zero through April 2011. Mf prevalence in SVs dropped from over 50% in 1994 to 0.003% in 2011. Only one child out of 3051 was positive for antibody. We conclude that transmission of onchocerciasis has been interrupted, recommend halting of all interventions, and commencement of post treatment surveillance activities.

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AEDES AEGYPTI SALIVARY PROTEINS MODULATE DENDRITIC CELL IMMUNITY TO DENGUE VIRUS INFECTION

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Hematophagous arthropod saliva has been shown to possess a variety of effector functions that facilitate the acquisition of a blood meal. Mosquito saliva contains molecules with anti-inflammatory, anti-haemostatic, and immuno-modulatory capabilities. Arbovirus-infected mosquitoes expectorate saliva and virus immediately prior to blood feeding and this saliva may have the potential to aid the establishment of arbovirus infection within the vertebrate host. One such molecule in the saliva of *Aedes aegypti*, the primary vector of dengue virus (DENV), is Aegyptin, a protein shown to inhibit platelet aggregation. The effects of Aegyptin on the immune response profile of primary human monocyte-derived dendritic cells (moDCs) were examined using multiplex cytokine immunoassays. Aegyptin in combination with DENV type 2, strain 16803, demonstrates increased production of the anti-inflammatory cytokine IL-10 at time points beyond 12 hours, raising secretion levels in relation to those of mock infected or virus-only infected cells. The increase in IL-10 levels positively correlates with increases in moDC secretion of the chemokine IP-10 in groups treated with this combination of Aegyptin and virus. Further, the production of IL-4, a cytokine necessary in the shift toward a TH2 response, decreased over time, with particularly notable decreases in virus infected groups as compared to controls. Lowering levels of IL-4 taken in conjunction with the increases in IP-10 and IL-10 secretion levels could indicate that DENV in combination with Aegyptin creates an environment more permissive to infection, where immune cells suspected to serve as DENV replication sites, such as dendritic cells and macrophages, are being recruited to the site of infection (IP-10), suppressed in their antiviral, TH1 functions (IL-10), and minimally activated to a TH2 immune state (IL-4). Further characterization of the roles of other specific mosquito-expectorated proteins in the establishment and course of arboviral infection is needed.

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REDUCED ANTI-HEMOSTATIC QUALITIES OF *AEDES AEGYPTI* SALIVARY EXPECTORATE FOLLOWING DENGUE-2 VIRUS INFECTION

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Dengue virus is mainly transmitted by *Aedes aegypti* mosquitoes and the virus must disseminate into the salivary glands in order to be transmitted to the vertebrate host via salivation during probing and/or feeding. The saliva contains a diverse cocktail of pharmacologically active compounds that are deposited with the virus in bite site of the vertebrate host. This is where the mosquito's saliva can alter the local environment, perhaps in a way that facilitates the establishment of an infection. In order to determine if dengue virus is altering the composition of that cocktail by altering the expression of various salivary components, we have analyzed the protein composition of *Ae. aegypti* saliva in bloodfed needle-inoculated dengue-2 infected mosquitoes and uninfected bloodfed control mosquitoes via 2-D gel electrophoresis. Using naturally-expectorated saliva, the resultant salivary proteins were precipitated, desalted, and reconstituted for further proteomic analysis. We have found a global down-regulation of the majority of the proteins in the saliva, with the exception of the most abundant proteins, the various isoforms of D7 and apyrase. Previously identified proteins such as C-type lectin 1 and salivary serpin 4 were down-regulated 10-fold in infected saliva, and a previously unreported low-density lipoprotein receptor was found to be secreted and also reduced 10-fold in infected saliv*A. aegypti*n, adenosine deaminase, C-type lectin 2, and an inosine-uridine preferring nucleoside hydrolase were reduced 5-fold. The majority of these down-regulated proteins have been shown to be involved in the mosquito's anti-hemostatic response, perhaps leading to an increase in viral inoculum by the infected mosquito to compensate for the increased hemostasis or increased viral dissemination due to feeding interruptions. This research indicates the need to not only review the components of mosquito saliva that are being inoculated alongside the virus, but also the effect the virus has on the composition of those components in the saliva.

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VIRAL AND IMMUNOLOGICAL DETERMINANTS OF DENGUE VIRUS FITNESS AND DISEASE SEVERITY

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The relative contributions of viral evolution, pre-existing immunity, and host genetic factors to dengue virus (DENV) fitness and disease severity remain unclear. In Managua, Nicaragua, we observed an abrupt increase in disease severity across three epidemic seasons of DENV-2 transmission in two independent studies of pediatric dengue. Sequence analysis of full-length genomes of viruses isolated from patients identified a genetically distinct clade of DENV-2 circulating in later epidemic seasons. Viruses from the replacing clade replicated more productively in human and mosquito cells and had longer-lasting viremia in patients, which supports the emergence of a more-fit virus. However, association analyses revealed that the abrupt increase in disease severity occurred across years, irrespective of clade. Analysis of immunological profiles in children from both studies demonstrates a role for pre-existing DENV immunity, as DENV-3 immunity is associated with severe disease in our cohort study. Further, both waning immunity to DENV-1 and a specific interaction between DENV-3 immunity and viruses from the replacing clade appear to be playing a role in

increasing severity across seasons. In sum, our data demonstrate that it is the interplay between viral genetics and host immunity that is the major driver in determining risk of severe dengue disease.

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A MODEL OF LETHAL DENGUE VIRUS 2 INFECTION IN C57BL/6 MICE DEFICIENT IN THE IFN-A/ß RECEPTOR

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The four serotypes of dengue virus (DENV1-4) cause dengue fever and dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS), the most prevalent arthropod-borne viral diseases in humans and a major public health problem worldwide. Animal models for dengue are needed to study the mechanisms underlying disease pathogenesis as well as the complex immune response to primary and secondary DENV infections. We have previously demonstrated that a mouse-adapted DENV2 virus, D2S10, caused mortality after intravenous infection at a high dose in 129/Sv mice doubly deficient in interferon (IFN)-α/β and IFN-γ receptors (AG129). Here, we characterize a more virulent DENV2 strain, D220, that was obtained via ten alternate passages of D2S10 between mosquito cells and AG129 mouse serum. D220 is lethal after intravenous infection of AG129 mice with a 10-fold lower dose than D2S10. In C57BL/6 mice that are deficient in only the IFN-α/β receptor (A-B6), D220 is 80% lethal with 10^6 plaque-forming units (pfu); in 129/Sv mice carrying the same deficiency (A129), the lethal dose is higher and 100% of mice die at 10^7 pfu. However, when anti-DENV antibody is administered 24 hours prior to infection, D220 causes 100% lethality with 10^5 pfu in both A-B6 and A129 mice. The mortality induced by D220 in A-B6 and A129 mice appears due to a vascular leakage phenotype similar to that previously described with D2S10 in AG129 mice, and occurs 3.5-5 days post-infection. Further characterization of infection kinetics and phenotype is underway. Full-length sequencing of the viral genome revealed that, compared to D2S10, D220 carries four amino acid substitutions; likely most important are non-conserved substitutions at position 122 in the viral envelope (E) protein and position 228 in the non-structural (NS) 1 protein. The susceptibility of C57BL/6 mice further opens a broad range of immunological methods and genetically deficient mice that are readily available in this strain background. The development of this virus enables study of the mechanism of dengue pathogenesis, testing of antiviral compounds, and investigation of the immune response to DENV in less immunocompromised mice.

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MECHANISM OF ACTION OF THERAPEUTIC MONOCLONAL ANTIBODIES IN A DENGUE MOUSE MODEL

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Dengue hemorrhagic fever and dengue shock syndrome (DHF/DSS) are life-threatening complications following infection with one of the four serotypes of dengue (DENV). Epidemiological evidence has suggested that the greatest risk factor associated with the development of DHF/DSS is prior infection with a different serotype. Recently, we have published that antibodies alone are sufficient to enhance a sub-lethal DENV2 infection and cause lethal disease in AG129 mice, with features similar to human DHF/DSS. Subsequently, we identified a monoclonal antibody (MAb) targeting the E-domain II (DII) fusion loop, E60, which is therapeutic 24 and 48 hours following an antibody-enhanced lethal disease when aglycosylated to prevent binding with FcγR. Here we characterize a panel of nine human or mouse-human chimeric MAbs and their aglycosylated variants and correlate their therapeutic and prophylactic potency *in vivo* with various *in vitro* characteristics, including epitope specificity, mechanism of neutralization, neutralizing titer, and affinity. These nine MAbs target five different epitopes on the E protein and are moderately to strongly neutralizing *in vitro.* Initial results indicate that these aglycosylated MAbs are effective as therapeutics following a lethal infective dose of DENV. However, two of these Mabs, which target two distinct epitopes, are also completely therapeutic following an antibody-enhanced, lethal infection. Further analysis indicates that both neutralizing potency and MAb affinity correlate with antibody-enhanced therapeutic efficacy with MAbs targeting the EDII fusion loop but not with MAbs targeting different epitopes, including the EDIII C-C′ loop, EDIII A strand, or EDI/II dimer interface. These modified antibodies also provide a unique tool to study the early kinetics of a lethal DENV infection. These studies should further our understanding of ADE and the mechanism of action of therapeutic MAbs that are effective in preventing lethal disease.

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EMERGENCE OF A NEW LINEAGE OF DENGUE-2 VIRUS WITH INCREASED PATHOGENESIS IN PERU

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Dengue fever is an arboviral disease caused by four antigenically distinct but related viruses. Dengue infection occurs in more than 100 countries with an estimated 50-100 million infections annually. The clinical spectrum of dengue disease is broad: most dengue infections are inapparent or present as a debilitating but self-limiting undifferentiated febrile illness; however, a small number of cases progress to more severe forms of the disease (dengue hemorrhagic fever and dengue shock syndrome). While historically the largest burden of severe disease has fallen on Southeast Asia, there is an increasing burden of severe disease on the Americas as well. Over the past 20 years there has been an 8.3 fold increase in number of dengue hemorrhagic fever (DHF) cases in the Americas, and in late 2010/early 2011, the Amazonian city of Iquitos, Peru, experienced its largest DHF outbreak ever. The DHF outbreak in Iquitos corresponded with the introduction of a dengue-2 virus (DENV-2) belonging to the Asian/American II lineage. While DENV-2 had previously circulated in Iquitos, this was the first time strains of this lineage had circulated there. In order to better understand the increased disease severity associated with this virus, we compared replication kinetics, vectorial capacity and whole genome sequences of DENV-2 isolates collected during the recent DHF outbreak to isolates previously collected in the area. Our phylogenetic analyses based on complete genome sequences confirmed the introduction of the new lineage of Asian/American genotype. Sequence analyses revealed a high degree of conservation in the 5′- and 3′- untranslated regions, but considerable differences at the nucleotide and amino acid levels were observed within the open reading frame. Additionally, replication was compared in cultured cells, where lineage II strains produced a significantly higher output of progeny in human liver cells, but not in mosquito cells. Understanding the genetic relationships and phenotypic differences of this emergent lineage may provide valuable insight into DENV emergence and guide monitoring of future outbreaks.

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REPLICATION DYNAMICS OF DENGUE VIRUS TYPE 1 FROM TWO HAWAII OUTBREAKS IN LOCAL *AEDES ALBOPICTUS* MOSQUITOES

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Dengue virus is a globally expanding pathogen accounting for increasingly severe epidemics, whose genetic diversity is partially responsible for differential transmission and epidemic potential. Dengue is transmitted primarily by the anthropophilic mosquito *Aedes aegypti*, and occasionally, as in Hawaii where the former is rare, by the Asian tiger mosquito *Aedes albopictus*. To understand the importance of viral strain in transmission by vectors, we took advantage of the well-documented outbreak history of dengue in Hawaii and infected local mosquitoes with 4 different virus strains isolated from Hawaii as early as 1943. Mosquitoes from a wild-founded Oahu *Ae. albopictus* colony were allowed to feed on infected blood (50% human blood and 50% supernatant from viral C6/36 culture) containing one of four strains of dengue virus type 1 (DENV-1) or a negative control. The strains included three isolates from the 2001 Hawaii outbreak: two viruses linked to Tahiti where the 2001 epidemic was characterized by high transmission and severe disease, and one virus linked to an attenuated outbreak in American Samoa at the same time. The final virus was an isolate from the 1943 Hawaii outbreak, representing an earlier form of DENV-1. Up to four replicates of each treatments were fed to 4-5 day-old *Ae. albopictus* mosquitoes (colony generation F7) resulting in 1401 blood-fed females. A subset of mosquitoes were sacrificed immediately post blood meal, at 5 hours post feeding, and again at 1, 4, 7, and 14 days post-infection. At each time point, 2-4 mosquitoes per replicate were dissected into midgut, salivary glands, and remaining carcass. An additional 2-5 mosquitoes were collected whole for additional quantification of virus infection. Total RNA was extracted from each tissue type, transcribed to cDNA and quantified for DENV by qPCR for the NS5 gene. Although all virus treatments resulted in high rates of mosquito infection, dissemination to the salivary glands by day 7 were highest for one of the Tahitian derived strains, and lowest for the American Samoan derived strain and another of the Tahitian strains. The 1943 strain was intermediate. Results for the other time points will also be presented. The identification of virus strain-specific differences in mosquito infection dynamics suggests an important role for differential viral fitnesses in epidemic dynamics. Support was provided by NIH-RR018727, NIH-AI065359, NIH-RR003061, and DOD-06187000.

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CONSTRUCTION AND CHARACTERIZATION OF CHIMERIC JAPANESE ENCEPHALITIS/DENGUE VIRUS TYPE 4 VACCINE CANDIDATES

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Japanese encephalitis virus (JEV), a member of the flavivirus genus, is a leading cause of viral encephalitis worldwide and vaccination remains one of the most effective ways to prevent disease. A safe, live-attenuated vaccine would be ideal because of the potential for low cost production, lifelong immunity following a single dose, and the possibility of combination with a live-attenuated dengue vaccine. Here we describe the construction of six different chimeric JEV/dengue virus type 4 (DEN4) vaccine candidates. The chimeric viruses were generated by replacing the membrane precursor (prM) and envelope (E) structural genes of DEN4 or DEN4Δ30, which contains a 30 nucleotide deletion in the 3′-untranslated region (UTR), with those of JEV strain India/78. This strategy has successfully produced vaccine candidates for other flaviviruses, and special attention has been paid to the nature of the capsid (C)/prM cleavage junction, which contains the viral protease and furin cleavage sites, and plays an important role in virus viability. Therefore, chimeric cDNA molecules were constructed containing either a JEV, DEN4, or West Nile virus C/prM junction. All six recombinant JEV/DEN4 chimeras were recovered in C6/36 mosquito cells from transcripts produced *in vitro*, followed by terminal dilution in Vero cells to acquire biologically-cloned and Vero cell-adapted viruses. These viruses were sequenced and shown to have acquired a single NS4B gene mutation, such as P101L, T105I, L112S or V109A/A240V, which have previously been identified as being important for Vero cell adaptation of DEN4 and other DEN4 chimeric viruses. Mutations were also identified in E (F167S, M240L, V253F, Q264H, K312R, S364P, G413R, I430T, and M475V), NS2A (M168V), NS3 (S158L and R202I) and the 3′-UTR among the various subsets of the chimeric viruses, and may also be important for Vero cell adaptation. These six chimeric JEV/DEN4 vaccine candidates are currently being evaluated in mice to determine the level of neurovirulence and neuroinvasiveness compared to the wild-type JEV parent.

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HEPATITIS C VIRUS REPLICON SENSITIZES HOST CELLS TO TRAIL INDUCED APOPTOSIS BY UP-REGULATING DR4 AND DR5 THROUGH A MEK1-DEPENDENT PATHWAY

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shown that HCV infection can sensitize host cell to TRAIL-induced apoptosis, while the mechanism by which HCV regulates TRAIL pathway remains to be determined. Here we demonstrated that HCV replicon sensitized Huh7 cells to TRAIL-induced apoptosis by up-regulating two TRAIL receptors death receptor 4 (DR4) and death receptor 5 (DR5). Elimination HCV replicon from HCV replicon cells reversed the up-regulation of the expression of DR4 and DR5 and decreased the sensitivity to TRAIL. We found that HCV replicon enhanced Sp1-mediated transcription of DR5 gene and mutation of Sp1 binding sites on 5′-flanking promoter region of DR5 or knockdown of Sp1 by specific siRNA decreased expression of DR5. Furthermore, we found that PD98059, an inhibitor of MEK1, inhibited the enhancement of expression of DR4 and DR5 mediated by HCV replicon, and over-expression of MEK1 in Huh7 cells enhanced the promoter activity of both DR4 and DR5. Also we found phosphorylation of MEK1 increased and knock down of MEK1 by siRNA reversed the increased expression of DR4 and DR5 in HCV replicon cells. This finding may help to further unravel the pathogenesis of HCV and provide new therapeutic interventions of HCV infection.

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IDENTIFICATION OF NEUTRALIZING EPITOPES ON CHIKUNGUNYA VIRUS ENVELOPE PROTEIN

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In order to obtain comprehensive monoclonal antibody (MAb) epitope maps at the resolution of individual amino acids, we developed a novel technology, Shotgun Mutagenesis Epitope Mapping. This approach offers the capability of mapping both linear and conformational epitopes, even for structurally complex proteins such as oligomeric and glycosylated Envelope proteins. Integral Molecular is using this technology to generate detailed and comprehensive epitope maps of the immunodominant Envelope protein (E2/E1) of Chikungunya virus (CHIKV). A comprehensive mutation library for the CHIKV S27 strain Env protein was created in which every residue was individually mutated to a defined substitution, expressed

in human cells, and analyzed for its effect on antibody reactivity. For each MAb tested, Shotgun Mutagenesis identified amino acids on Env that are critical for antibody binding. These residues will enable generation of detailed epitope maps that can be visualized on the E2/E1 protein structure. Our goal is to map epitopes on CHIKV Env protein, determine how they contribute to neutralization of infection, and how they relate to protein function. We expect that this approach will help define the range of immunodominant structures on CHIKV Env and identify novel neutralizing antibody epitopes that can be used for therapeutics, diagnostics, and vaccine development.

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ZIKA VIRUS FROM FEVER SYNDROMIC SURVEILLANCE IN CAMBODIA

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In collaboration with the Cambodian Ministry of Health, US Naval Medical Research Unit 2, Cambodia has conducted fever syndromic surveillance study since 2006. Patients are currently being enrolled from 11 in 5 provinces in south central and northeastern Cambodia. Upon enrollment, respiratory specimens, whole blood and serum were collected. Testing was performed for viral, bacterial and parasitic pathogens at a centralized laboratory in Phnom Penh. Dengue fever is tested for by serological (IgM) and molecular methods (real time PCR). The real time PCR utilized in this study is a universal flavivirus screen (reported previously) that targets the NS5 gene. In August 2010, a 3 year-old child was enrolled with clinical complaints of fever, headache, sore throat, and cough. Serological tests for dengue from both the acute and convalescent specimens were negative. The serum was positive by the flavivirus screen but negative by dengue and Japanese Encephalitis specific PCR tests. Nucleic acid sequencing of the amplicon isolated by gel purification produced a 100bp fragment with 100% sequence identity to Zika virus. This is the first case of Zika virus identified in Cambodia.

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ISOLATION AND CHARACTERIZATION OF A TICK-BORNE ENCEPHALITIS VIRUS STRAIN FROM *IXODES PERSULCATUS* TICKS FROM MONGOLIA

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Tick-borne encephalitis virus (TBEV), a member of the family flaviviridae, causes one of the most important inflammatory didease of the central nervous system (CNS), namely severe encephalitis in Europe and Asia. In Mongolia TBE is known since the 1980s. The numbers of human cases have been increasing during the last years. Endemic areas of TBE associated with severe CNS diseases have been reported mainly in the provinces (Aimak) Selenge and Bulgan in Northern Mongolia close to the Russian border.We report the first isolation and preliminary genetic characterization of a TBE virus strain from ticks collected in Mongolia. 68 ticks (Ixodes persulcatus) were collected by flagging in the Bulgan district near Khylganatt in the North of Mongolia in July 2010. Ticks were homogenized individually and supernatants were used for nucleic acid extraction (QIAGEN Viral RNA Extraction Kit). Extracted RNA was screened for TBEV-specific sequences by real-time RT-PCR. Two out of 68 (2.9%) tested ticks RNAs were reactive. The real-time RT-PCR positive tick supernatants were inoculated into Vero cells. A TBEV strain (MucAr M14/10) could be recovered from one of the two positive tick supernatants. The second positive tick supernatant with a lower TBEV RNA content proofed negative in cell culture. By conventional RT-PCR targeting the complete genome of the TBE virus strain could be amplified. Sequence comparison of the complete genome and of particular genes with other TBE virus strains of different subtypes revealed the highest homology on the nucleotide level and on the amino acid level to three strains of a subclade of the Sibirian subtype of TBE virus, the strains Zausaev (AF527415), the strains IR99-2m7 (AB049351) and Lesopark (GU121966), respectively. The data imply that TBE virus in Mongolia was introduced only recently by anthropogenic activities like road and/or railway construction.

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IMPACT OF THE JAPANESE ENCEPHALITIS (JE) IMMUNIZATION PROGRAM WITH LIVE, ATTENUATED SA 14-14-2 JE VACCINE IN NEPAL

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Japanese encephalitis (JE) cases have been reported in Nepal since the mid-1970s. In 2006, the Ministry of Health and Population introduced an immunization program to control JE. By 2009, immunization campaigns had been conducted in 23 JE-endemic districts. Campaigns targeted children 1-15 years of age (11 districts) or the whole population ≥1 year of age (12 districts) with a single dose of live, attenuated SA 14-14-2 JE vaccine. To evaluate the impact of the program, we analyzed acute encephalitis syndrome (AES) and laboratory-confirmed JE case surveillance data collected from 2004-2009 through Nepal's routine surveillance system. Expected AES and JE incidence rates and observed post-campaign rates in each district were compared. For AES, the observed post-vaccination incidence of 7.5 per 100,000 population in the 23 districts where JE immunization campaigns were conducted was 58% (95% CI 56%-60%) lower than the expected incidence of 17.9 per 100,000 if no campaigns had occurred. The greatest impact was in the four high-risk western Terai (plain) districts where the observed incidence of 6.6 per 100,000 was 84% (95%CI 83%-85%) lower than the expected incidence of 41.5 per 100,000. For JE, the observed incidence of 1.3 per 100,000 population in the post-campaign period in the 23 districts was 72% (95%CI 69%-75%) lower than the expected incidence of 4.6 per 100,000. As with AES, the impact on JE was greatest in the four high-risk Terai districts; the observed incidence of 1.9 per 100,000 was 84% (95%CI 81%-86%) lower than the expected incidence of 11.7 per 100,000. Although this analysis was limited by availability of only short-term post-campaign data in some districts, it demonstrated that the SA 14-14-2 JE vaccination program has had a clear impact on AES and JE incidence in Nepal. As additional surveillance data are available, further analysis will provide greater accuracy in the assessment of campaign impact. An ongoing routine infant immunization program will be essential to ensure the achievements in JE control are maintained.

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DEVELOPMENT AND CHARACTERIZATION OF A NONHUMAN PRIMATE MODEL FOR EBOLA VIRUS: SEQUENTIAL SAMPLING STUDY OF EBOLA ZAIRE VIRUS IN NONHUMAN PRIMATES BY AEROSOL EXPOSURE

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Ebola virus (EBOV) is a single-stranded negative-sense RNA member of the *Filoviridae* that causes hemorrhagic fever (HF). Since its discovery in 1976, this zoonotic virus has caused epidemics with high case fatalities. Although EBOV causes sporadic outbreaks in sub-Saharan Africa, it is of significant concern from a biodefense perspective because of case reports of EBOV infections outside of Africa and because the virus may be spread on aerosols. To develop a standard animal model, we have employed aerosol exposure of rhesus macaques to Zaire Ebola virus in a sequential sampling study. The results presented herein are derived from a robust sequential sampling study (28 animals) of EBOV HF in rhesus macaques challenged by a lethal dose of aerosolized EBOV and sampled on Days 1, 3, 4, 5, 6, 7 and 8. The parameters measured include: clinical symptoms; weight and temperature; complete blood counts (CBC); blood chemistry; cytokines; viral levels; coagulation; and pathology. Clinical signs included nonresponsiveness, gastrointestinal changes (no output), diarrhea, reduced food consumption, dehydration, rash, dyspnea, weakness, and depression. CBCs demonstrated that white blood cells, lymphocytes, monocytes, and platelets decreased, whereas basophils increased. Blood chemistry measurements showed increases in blood urea nitrogen, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and creatinine. Measurements of cytokine/chemokine levels showed strong increases in hepatocyte growth factor, MIG, INF- α, IL-1RA, IL-6, IL-10, IL-15, eotaxin, MIP-1β, and MCP-1. Pathology analyses demonstrated viral staining in macrophages and dendritic cells and progression to liver, spleen, and lymph nodes in the first 3-5 days after exposure. Thereafter the virus was found in kidney, adrenal gland, thymus, bone marrow, pancreas, and gastrointestinal tract. Activation of the coagulation cascade and the production of d-dimers and fibrin deposition was prominent. To attempt to validate this model the observed disease course was compared to available data from intramuscular Zaire Ebola virus challenges and the limited data available from reports of human disease.

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IMMUNE RESPONSE INDUCED BY LIVE-ATTENUATED JAPANESE ENCEPHALITIS VACCINE (JE CV) NEUTRALIZE RECENT WILD-TYPE JAPANESE ENCEPHALITIS VIRUS (JEV) ISOLATES FROM SOUTHEAST (SE) ASIA AND INDIA

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During clinical development of JE-CV, the neutralization ability of vaccine-induced antibodies was assessed against the vaccine virus (JE-CV) and against well characterized wild-type (wt) viruses isolated between 1949-1991. We sought to assess whether JE-CV-induced antibodies can also neutralize recent wt JEV isolates, representative of currently circulating genotype 1 and 3 JEV strains. Sera from 12-18m/o children who received a single dose of JE CV in a phase III study in Thailand and the Philippines (ClinicalTrials.gov NCT00735644) were randomly selected and pooled. Pooling was based on Day28 post-vaccination neutralizing antibody titers assessed by PRNT50 to JE-CV. Eight serum pools of differing titer ranges were prepared from 7-20 samples / pool, all samples used in pooling except one were from children who were JE-naïve before vaccination. Two recent isolates were obtained from the WHO Flavivirus Diagnostics Reference Laboratory for Asia at the Center for Vaccine Development University of Mahidol, Thailand: JEV-SM1 from a mosquito in Thailand, 2003; and JEV-902/97 from a clinical case in Vietnam, 1997. A single analyst performed 3 independent PRNT50 assays against these 2 isolates, as well as against 4 JEV tested previously during the development program, including JE-CV. Results were compared using geometric mean titer and median values of the 3 independent tests. All positive titer serum pools from JE-CV-vaccinated children neutralized the 2 recent JEV isolates. Within each serum pool GMT and median titers against isolates were similar generally within one 2-fold dilution, ranging from 84-980 for the low titer-high titer pools. Neutralization titers against recent wt strains were comparable to those against previously tested JEV, including the vaccine virus. Consistent with previously generated data on the neutralization of wt JEV isolates, immune responses induced by JE-CV neutralize recently isolated virus from SE Asia and India.

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YELLOW FEVER EPIDEMIOLOGICAL SITUATION AT 2010 IN BURKINA FASO

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A resurgence of yellow fever has been noticed in Africa (1) and this situation is particularly serious in West Africa. In Burkina Faso, since 2004, there have been outbreaks of this disease. The control strategy of yellow fever recommended by WHO and applied in the country is based on four key pillars including enhanced surveillance of the disease. We intend to review the situation of the surveillance of yellow fever in 2010 at the National Reference Laboratory of Yellow Fever in Centre MURAZ of Bobo Dioulasso. The serum/plasma of patients with fever and jaundice of all the health districts of the country are received at the laboratory according to the protocol defined for the national surveillance. The samples are accompanied by a form of investigation and kept at +4°C in coolers during transport. ELISA was used to search for specific IgM yellow fever. For the year 2010, 970 samples of febrile jaundice were received from 13 health regions. 935 (96.40%) samples were adequate (N ≥ 90%). Vaccine recipients were among 401 (41%) and those not vaccinated 347 (36%). The transmission of samples was done on time (≤ 7days) for 670 (69.07%) cases. 11 aliquots have been diagnosed positive for IgM specific for yellow fever and sent to the Pasteur Institute in Dakar, 8 (0.82%) were confirmed positive and 03 (0.31%) classified doubtful. Of these 08 positive samples, 07 cases were from the same region (Cascade) and none were vaccinated. In spite of the carried out efforts, cases of yellow fever are diagnosed in Burkina Faso, especially at not vaccinated subjects. it is important in spite of the monitoring to carry out vaccination campaigns apart from the programs of response.

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INVESTIGATION OF WEST NILE VIRUS RNA IN BLOOD DONORS BY REAL-TIME RT-PCR

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West Nile Virus (WNV), a member of the family Flaviviridae, is an enveloped RNA virus. Primary reservoir hosts of this virus are birds, but the virus can cause various infections in humans and other mammals. WNV infection is generally asymptomatic, but this virus may cause a wide range of different clinical forms from mild WNV fever to neurodegenerative diseases with high mortality. The most common and natural way of transmission of WNV infections is mosquito bites, but that are shown the humans can be infected by this virus with different routes. The most

important non-mosquito transmission route is contaminated blood and blood products. WNV seropositivity has been reported in various regions of Turkey and around the province of Ankara so far. Seven patients with West Nile fever were reported in the western regions of Turkey in August 2010 and three of these patients resulted in death. In this study, we aimed to investigate the risk of WNV transmission through blood and blood products, especially for the region of Ankara in Turkey. For this purpose we included 729 serum samples in the study that are obtained from healthy volunteer blood donors. The vast majority of donors were male (97.5%) and resident in Ankara (96.3%). We investigated the presence of viral RNA in the serum samples by real-time RT-PCR. WNV RNA was not detected in serum samples. This result may be due to absence of patient with viremia among blood donors included in this study. Previous studies have shown that seroprevalence of WNV infection was 0.6 to 2.4% among blood donors in this region. For this reason, the risk of WNV infection in blood donors should not be ignored.

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WEST NILE VIRUS-INDUCED CYCLOOXYGENASE-2 PROMOTES INFLAMMATION IN THE BRAIN

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Inflammatory immune responses in brain initially triggered to clear West Nile Virus (WNV) promote blood-brain barrier (BBB) disruption, infiltration of immune cells and neuronal death. However, the mechanisms by which WNV modulates these inflammatory responses are unclear. We previously demonstrated that matrix metalloproteinases (MMPs) play an important role in WNV-induced BBB disruption. Cyclooxygenase-2 (COX-2) and its product prostaglandin E2 (PGE2) can initiate inflammation via cytokines and matrix metalloproteinases (MMPs). This study was aimed to identify and characterize the pathophysiological consequences of COX-2 expression in WNV-infected human brain cortical astrocytes (HBCA) and in mice brain. C57BL/6 mice were infected with WNV (NY99) at 10^2 PFU and COX-2/PGE2 levels were measured. Primary HBCA were infected with WNV at MOI-5 in the presence or absence of specific COX-2 inhibitor (NS398), and expression and activity profile of COX-2, cytokines and MMPs were analyzed. In mice brain, WNV infection increased the expression of COX-2 mRNA and protein at day 7 after infection, which correlated with peak virus titers. The expression of COX-2 in WNV-infected HBCA was 10- to 87-fold high from days 1 to 4 after infection, which coincided with peak expression of multiple MMPs, IL-1β, -6 and -8 and PGE2. Treatment of HBCA with NS398 decreased the expression and release of WNV-induced MMPs, IL-1β and cytokines by 60 to 88%. In conclusion, our data identifies astrocytes as one of the sources of COX-2-derived PGE2 that initiates multiple downstream pathological events such as cytokine and MMP production, thereby contributing to two major hallmarks of WNV-encephalitis, neuroinflammation and BBB disruption. The ability of COX-2 inhibitors to modulate WNV-induced COX-2 and PGE2 signaling should be further investigated in an animal model as a potential approach for the clinical management of WNVE.

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MUTATIONS IN THE PRM PROTEIN OF WNV INHIBIT SECRETION OF VLP BUT NOT VIRUS

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West Nile virus-like particle (VLP) mutants differing in amino acids (AAs) of the prM protein were produced and used to identify epitopes reactive with three human monoclonal antibodies. We discovered that four prM mutations (T20D, K31A, K31V, or K31T) reproducibly resulted in undetectable levels of VLP secretion. To determine the effects these prM mutations had on the virion, they were introduced into the West Nile virus (WNV) infectious cDNA clone. In all cases, infectious virus was recovered following transformation of C6/36 cells. Replication of the prM K31A and T20D viruses were similar to wild-type (wt) WNV in both mosquito (C6/36) and mammalian (Vero) cells, with no compensatory AA changes in either the prM or envelope (E) proteins. The prM K31T virus titer was reduced 10-fold when grown in both C6/36 cells and Vero cells as compared to wt WNV with a stable prM and E gene sequence. The prM K31V had reduced levels of replication in both Vero cells (10-fold) and C6/36 cells (100-fold). Sequencing revealed that after transfection of C6/36 cells (C=0), prM K31V incurred a compensatory mutation of prM, L33P. Our results suggest that while mutations in the prM can reduce or eliminate secretion of VLPs following transfection of COS cells, these same prM mutations have less or no effect on viral replication in both Vero and C6/36 cells. This difference may be due to the high level of prM seen in WNV VLPs grown in mammalian cells as compared to virus, or to the differences in structure and symmetry of the VLP compared to virus.

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BLACK FLY PHEROMONES AND THE MONITORING AND ERADICATION OF ONCHOCERCIASIS

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Onchocerciasis or river blindness disease is a parasitic disease caused by infection from the nematode Onchocerca volvulus. The parasite is transmitted to humans by black fly vectors of the genus Simulium. Most of the infections occur in central Africa, with significant incidence also in Central and South America. According to the World Health Organization an estimated 18 million people suffer from onchocerciasis. However, since the disease is endemic to only the poorest regions of the world it is difficult to gain exact reports and statistics about the disease. The current method for monitoring the spread employs human bait, which is neither optimal nor ethically sound. The need for a new monitoring method is very important. It was noticed that gravid flies are attracted to egg masses recently deposited by other flies of the same species. This paper will describe our efforts to isolate and identify the pheromone responsible for this attraction, which we then plan to develop as bait for a field trap for monitoring vector pressure. In the long term, field traps may be useful in eradication of the disease.

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FACTORS AFFECTING MASS DRUG ADMINISTRATION PROGRAM FOR THE ELIMINATION OF LYMPHATIC FILARIASIS IN A DISTRICT IN GHANA

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Lymphatic filariasis (LF) is the second most common vector-borne parasitic disease after malaria in many tropical countries. Worldwide, the WHO estimates more than 1.3 billion people in 81 countries are threatened and over 120 million people are currently infected. It is endemic in the northern and southern sectors in Ghana. The disease is targeted for elimination by 2020 and the key intervention is mass drug administration (MDA) using a single annual dose combination of ivermectin or DEC and albendazole for 5-6 years. The MDA has been running in Ghana since 2000. The aim of this study was to identify factors responsible for compliance and non-compliance to MDA. Ninety nine communities were stratified into 4 strata according to MDA coverage rates and 17 were randomly selected and studied. A six part pre-tested questionnaire was applied to all respondents and the key issues explored included knowledge

of MDA, reasons for compliance and non-compliance, mode of drug distribution and acceptance of MDA. Observed coverage of MDA of 65.4% in 2006 increased to 86.3% in 2008. Overall MDA acceptance was 99.7% but drug compliance was 86.0% among respondents. Some 14% of study respondents perceived the drug was good for their health. Reasons for non-compliance included travelling (14%) and view that the programme was not necessary (4%). Awareness that the drugs prevent LF was a major contributor to compliance. Door-to-door mode of drug delivery was the most preferred (82.1%). Drug compliance showed significant positive correlation with awareness of MDA. Door-to- door delivery using community volunteers was more successful than delivery from health centres. Efforts to eliminate the disease are however hampered by community ignorance, misguided education and adverse effects. High MDA acceptance could be sustained with simplified education using volunteers.

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FACTORS ASSOCIATED WITH *WUCHERERIA BANCROFTI* MICROFILAREMIA IN AN ENDEMIC AREA IN MALI

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Lymphatic filariasis (LF) due to *Wuchereria bancrofti* (Wb) is endemic in all 8 administrative regions of Mali and represents an important public health problem. Although mass drug administration (MDA) strategies have been implemented in most regions, a number of factors may influence Wb microfilarial load and thus potentially affect the efficacy of MDA at the individual or community level. These factors include spatial clustering, coinfection with *Mansonella perstans* (Mp) and bednet use. To determine the effect of these factors on Wb prevalence and microfilarial levels, cross-sectional data obtained during screening for an interventional study in Bougoudiana and Tieneguebougou, neighboring villages (<10 km apart) in the district of Kolokani, were examined. A total of 372 volunteers (235 males and 137 females) aged 14 to 65 (mean of 34 years), were questioned about bednet use and prior participation in MDA. Wb and Mp microfilarial (mf) loads were assessed by calibrated thick smear, and Wb circulating antigen (WbCAg) levels were determined using the TropBio™ ELISA. All volunteers were georeferenced for disease distribution analysis and mapping. The overall prevalence of Wb microfilaremia was 17%. Prevalence was significantly higher in Tieneguebougou than in Bougoudiana (23.2% vs. 10.7%, respectively; p=0.0015; Fisher's Exact test); however, positive and negative individuals were randomly distributed across the two villages (Moran's I spatial statistic = -0.01, Z score =0.1, P>0.05). Of the 196 subjects with detectable Mp microfilaremia, 47 (24%) had detectable Wb mf, as compared to 17/177 (9.5%) Mp-negative subjects (p<0.001, Fisher's Exact test). However, the geometric mean Wb load was comparable in the two groups (214 vs. 123 mf/ml; p=0.17, Mann-Whitney U test). Only 36% of subjects gave a history of bednet use at the time of the survey and 52% had received antifilarial therapy as part of MDA one year prior to the study. Neither a history of bednet use nor prior antifilarial therapy had an effect on prevalence of Wb microfilaremia, Wb mf load or WbCAg positivity. Thus, of the factors examined, only Mp infection had a significant influence on the prevalence of Wb microfilaremia. Whether the relationship between Mp and Wb is due to host factors or the biology of the parasites remains to be elucidated.

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LACK OF EFFECT OF FILARIAL INFECTION ON ASYMPTOMATIC MALARIA PARASITEMIA IN KOLOKANI, MALI

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Wuchereria bancrofti (Wb) and *Mansonella perstans* (Mp) are blood-borne filarial parasites that are endemic in many countries of west and central Africa, including Mali. Their geographic distribution overlaps considerably with that of malaria, and coinfection is common. Although prior studies have demonstrated effects of filarial infection on the immune response to malaria, the influence of filarial infection on asymptomatic carriage of malaria parasites is unknown. To address this question, *Plasmodium falciparum* (Pf) parasitemia was assessed monthly throughtout the transmission season in 83 asymptomatic subjects participating in a study of the effects of filariasis on clinical malaria in two villages in Kolokani, Mali. Filarial infection was defined by the presence of Wb or Mp microfilariae on calibrated thick smears performed between 10 pm and 2 am and/or by positive TropBio™ ELISA for circulating filarial antigen (CFA) in serum. There were no significant differences between the filarial-positive (FIL+) and filarial-negative (FIL-) subjects with respect to age, gender and hemoglobin status. At the beginning of the transmission season, 22/36 FIL+ subjects and 29/46 FIL- subjects had Pf parasitemia (p=NS). Geometric mean Pf parasitemia was also comparable between the two groups (179.3 vs 201.2, respectively). Although the prevalence of Pf parasitemia increased over the course of the transmission season in both FIL+ and FIL- groups, no significant differences were seen between the groups with respect to prevalence of Pf parasitemia or Pf parasite load. Thus, despite differences in immune responses to malaria parasites in the setting of filariasis, asymptomatic carriage of Pf appears to be unaffected.

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CAN SCHOOL-BASED SAMPLING BE USED IN PLACE OF COMMUNITY-BASED SAMPLING TO MEASURE CIRCULATING FILARIAL ANTIGEN FOR *WUCHERERIA BANCROFTI* IN AREAS WHERE SCHOOL ATTENDANCE IS LOW?

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Population-based surveys serve as the benchmarks for monitoring and evaluating the progress of lymphatic filariasis (LF) elimination programs. From the start of LF elimination efforts to surveys to determine if mass drug administration (MDA) can be safely discontinued, these surveys are crucial for measuring program success. Large-scale surveys conducted at the household level are expensive and time consuming; there is great interest in shifting to school-based sampling that would involve less travel for data collection teams, greater ease of planning, improved facility for systematic sampling, and an entry for integration with other NTDs whose assessments depend on sampling the community survey. The prevalence of CFA in the household survey (ICT-positive) was 0.44%, compared with 0.32% in the school survey, which corresponds to a chi-squared statistic of 0.26 (p=0.61). Both of the survey results indicate a CFA prevalence that is well below the MDA stopping threshold. Based on the results of this study we conclude that there is no statistical difference between children sampled in the schools or community. These findings also indicate the feasibility of integrating NTD programs, particularly schistosomiasis and trachoma, whoseschool-age children. When school attendance is high it follows that school-based sampling should yield similar results to household-based sampling; however, when school attendance is low

the equivalency of these sampling methods remains undetermined. The purpose of this study is to determine if school-based sampling of children yields statistically equivalent results to sampling children of the same age in the community when school attendance is <70%. To address this question, two concurrent surveys of children 6-7 years old, surveyed in the household, and 1st and 2nd graders, surveyed in the schools, were conducted in the Houndé health district in Burkina Faso, where school enrollment is 57%. All surveyed children were tested for the presence of circulating filarial antigen (CFA) using ICT cards. A total of 3145 children were sampled, 1542 from the school survey and 1603 from assessments target school-age children.

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EVALUATING PROGRESS TOWARD THE ELIMINATION OF LYMPHATIC FILARIASIS IN AMERICAN SAMOA THROUGH THE ASSESSMENT OF FILARIAL ANTIGEN AND ANTIFILARIAL ANTIBODY RESPONSES

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Significant progress has been made toward the global goal to eliminate lymphatic filariasis (LF) by 2020; however, demonstrating success will depend on careful monitoring after the implementation of mass treatment interventions. As treatment goals are met, programs face the decision of when it is appropriate to stop mass drug administration (MDA). Newly modified WHO guidelines provide a protocol for conducting transmission assessment surveys (TAS) to guide this decision. A TAS was conducted in American Samoa in February 2011. A total of 1,134 children (6-7 years old) were enrolled, representing 44.5% of the students in this age range. Of the children enrolled, 956 (84.3%) were tested by ICT for the presence of filarial antigen. Two positive children (0.2%) were found by ICT with no evidence of microfilaremia by blood smear or PCR. Since the number of positive children was below the established critical value for the TAS as performed in American Samoa, an official recommendation was made to stop MDA. After MDA is stopped, programs face a new challenge in carrying out surveillance to prevent the re-emergence of transmission. A history of LF recrudescence in American Samoa after previous MDA campaigns coupled with the efficiency of the vector (*Aedes polynesiensis*) make surveillance in this setting an issue of paramount importance. Evidence suggests that detection of antifilarial antibodies provides the earliest indicator of filarial exposure. Therefore, monitoring filarial exposure through the assessment of antifilarial antibody responses may provide a useful tool for detecting potential recrudescence. During the TAS, blood was collected on filter paper, dried and stored for testing. Using a newly developed multiplex platform which allows for the analysis of multiple antigens at one time, all of these samples will be tested with three available filarial antigens (Bm14, Bm33, Wb123). Results from this study will establish a baseline for surveillance and potentially provide insight into filarial exposure in an area that has been recommended to stop MDA.

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ONCHOCERCIASIS TRANSMISSION CONTINUES IN NYAGAK-BONDO FOCUS OF NORTHWESTERN UGANDA AFTER 18 YEARS OF ANNUAL DISTRIBUTION OF IVERMECTIN

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A single dose of ivermectin through community-directed treatment with ivermectin (CDTI) was recently estimated to eliminate onchocerciasis transmission in 15 to 17 years, allowing safe withdrawal of mass drug administration programs. In Nyagak-Bondo focus of northwestern Uganda annual mass treatment has been provided for 18 years. The objective was to determine whether annual treatment could be withdrawn without a possibility of recrudescence in Nyagak-Bondo focus. Baseline skin snip microfilariae (mf) and nodule prevalence data from 1993 from 6 communities in the Nyagak-Bondo focus were compared with data collected during 2011 follow up study in 7 communities from the same transmission zone. Three hundred adults were snipped at baseline in 1993, and 180 had been assessed for nodules. In 2011, 607 adults were examined for mf and nodules. From the same communities, mf baseline data from 58 children were compared with 2011 data from 145 children aged under 10 years. All communities in the transmission zone have been receiving regular annual mass treatment, and at an annual coverage of more than 85% of eligible population. Overall, mf prevalence among adults dropped from 83.1% (with community prevalences ranging from 90% to 100%) to 23.6% (range 3.4% to 40%, $p<0.0001$). Nodule prevalence dropped from 97% (range 96% to 100%) to10.9% (range, 2.3% to 20%, $p<0.0001$). In children mf prevalence decreased from 78.5% (36.4 to 100%) to 11.9% (0 to 36.8%), $p<0.0001$). Despite a dramatic decrease in onchocerciasis infection parameters in the Nyagak-Bondo focus after 18 years of annual treatment, 2011 infection rates in adults and children are too high to consider it feasible to halt ivermectin treatment at this time without risk of recrudescence.

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LONG LASTING INSECTICIDAL NETS (LLIN) ALONE APPEAR TO INTERRUPT TRANSMISSION OF LYMPHATIC FILARIASIS IN SOUTHEAST NIGERIA

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In West Africa Lymphatic filariasis (LF) is caused by the parasite *Wuchereria bancrofti;* in rural areas LF is transmitted by *Anopheles* mosquitoes. In southeast Nigeria, which includes Imo and Ebonyi states, potential coinfection with *Loa loa* parasites prevents use of the mass drug administration (MDA) strategy for LF elimination. The Carter Center is working with the ministries of health of those states to determine if mosquito vector control for malaria by means of LLIN will impact transmission of LF. In two study areas (Abakaliki and Ohaji Egbema local governments) baseline LF antigenemia in sentinel sites averaged 29%. From April-May 2008, 139,080 LLIN were distributed to reach all age groups in the two local government areas with an additional 32,600 nets in June 2008. Household (HH) cluster surveys showed that the proportion of HH with at least one LLIN increased from 3.3% in 2007 to 92.0% immediately after distribution in 2008, with an average of two nets within HHs owning at least one net. In six sentinel villages (three in each LGA)

mosquitoes have been collected by pyrethrum knockdown every month in one room of each of 30 HH since June 2007. Collected mosquitoes were immediately dissected to determine rates of LF infection (L1-L3 stage larvae). Eighty-three percent of the collections were *Anopheles* species (*An. gambiae* sl 75 percent and *An. funestus* 7 percent). We compared mosquito collection numbers and infection rates before/around LLIN distribution for the 12 month period June 2007-May 2008 with a 14 month period starting one year after LLIN distribution (June 2008-July 2009). Mosquito captures show a trend suggesting a decrease in mosquito abundance (decreasing from 5,098 to 1,395) and infection: one infection (L1) was found in the year after LLIN were distributed compared to 38 for the year before (Chi square 7, p<.01). No L3 have been detected since LLIN were distributed compared to 11 at baseline (P=NS). The study continues, but these early results suggest interruption of LF transmission can occur with LLIN alone, without accompanying MDA.

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PREVALENCE OF *WUCHERERIA BANCROFTI* INFECTION IN AMERICAN SAMOA AFTER SEVEN YEARS OF MASS DRUG ADMINISTRATION WITH DIETHYLCARBAMAZINE AND ALBENDAZOLE

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As part of the Pacific Program to Eliminate Lymphatic Filariasis (LF), the US Territory of American Samoa initiated mass drug administration (MDA) with diethylcarbamazine and albendazole in 2000 after a prevalence survey indicated 16.5% of residents were infected with *Wuchereria bancrofti*. Monitoring in sentinel sites indicated decreasing prevalence only after the fourth round of MDA when program strategies were modified to improve drug coverage. We conducted a population based prevalence survey in 2007 to determine the impact of seven annual rounds of MDA. Using geographical data, we took a simple random sample of households from all residential building structures on the island groups of Tutuila and Manua. All residents of selected households older than 2 years of age were eligible for assessment of circulating filarial antigen. Persons testing antigen positive were examined for microfilaria (mf). Overall 1,881 out of 2,216 registered, eligible residents were examined from 394 households. The prevalence of antigenemia among all ages 2 years and above was 2.26% (upper 95%CI 2.82%). We were unable to obtain an additional blood sample on 6/43 antigen positive individuals, but microfilaremia was detected in 5 of the remaining 37 antigen positive persons. Assuming those missed were mf positive and those antigen negative were mf negative, microfilaremia prevalence was 0.6% (upper 95%CI 0.89%). Among tested individuals age-eligible for participating in all 7 rounds of MDA, the mean reported number of times taking LF drugs was 4.02 (SE 0.08). Antigenemia was associated with increasing age (p<0.001) and reported noncompliance in MDA (p=0.042). After seven rounds of MDA, of which coverage of greater than 60% of the total population was achieved in only four, antigenemia has reduced from the baseline estimate. However, transmission may have not yet been interrupted. Targeted MDA or other alternative strategies should be considered.

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UPDATE ON THE ONCHOCERCIASIS ELIMINATION PROGRAM FOR THE AMERICAS (OEPA)

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Onchocerciasis in the Americas affects six countries (Brazil, Colombia, Ecuador, Guatemala, Mexico, and Venezuela) where it was originally endemic in 13 foci. OEPA is a regional initiative operating under PAHO Directing Council resolution CD48.R12 that calls for elimination by 2012 of new ocular morbidity attributable to onchocerciasis, and interruption of transmission. The OEPA partnership includes the endemic countries, The Carter Center, PAHO, the Gates Foundation, Lions Clubs, CDC, several universities, and the Mectizan[R] Donation Program. The strategy is ivermectin mass drug administration (MDA) at least twice each year to all endemic communities, reaching > 85% coverage. In 2010, 7 of the original 13 foci had stopped their MDA programs. As a result, the total number of ivermectin treatments administered in the Americas decreased by 28% from a peak of 852,721 in 2006 (when all 13 foci were under treatment) to 616,360 in 2010. Epidemiological indicators in 2010 showed that transmission was now interrupted in the Northcentral focus of Venezuela, and the Venezuelan Ministry of Health agreed with an OEPA recommendation to stop MDA there in 2011. As a result, only 5 foci out of 13 remain under MDA (2 in Venezuela, and 1 in Brazil, Mexico and Guatemala). MDA has ceased in Ecuador and Colombia, and may cease in 2012 in Mexico and Guatemala. WHO guidelines recommend that foci removed from MDA should conduct post-treatment surveillance for a minimum of 3 years before declaring transmission 'eliminated.' In 2010, for the first time since the start of the initiative, 3 foci (2 in Guatemala and 1 in Mexico) qualified for this "transmission eliminated" category. Certification of elimination, which can only be considered by WHO when requested for an entire country, could be requested by Colombia in 2012, followed by Ecuador in 2013. Brazil and Venezuela have all the remaining eye disease and the most active onchocerciasis transmission in the region. The difficult to access endemic area shared by these two countries on their frontiers in the Amazon region is the greatest hurdle to completing onchocerciasis elimination from the Americas.

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CAN MALARIA VECTOR CONTROL IMPACT FILARIASIS TRANSMISSION IN SUB-SAHARAN AFRICA?

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The Global Programme to Eliminate Lymphatic Filariasis (GPELF) was launched in 2000 and nearly all 42 endemic countries in the Americas, Eastern Mediterranean and Asia-Pacific regions have now initiated the WHO recommended mass drug administration (MDA) campaign to interrupt transmission of the parasite. However, nearly 50% of the LF endemic countries in Africa are yet to implement the GPELF MDA strategy, which does not include vector control. Nevertheless, the dramatic scale up in usage of insecticide treated /long lasting nets (ITNs/LLINs) and indoor residual spraying (IRS) for malaria in these African countries may significantly impact LF transmission because the parasite is transmitted mainly by *Anopheles* mosquitoes. Therefore, this study aimed to examine the magnitude and geographical extent of vector control activities in the 16 African countries yet to start MDA. National data on mosquito nets, ITNs/LLINs and IRS were obtained from published literature, national reports, surveys and datasets from public sources such as Demographic Health Surveys, Malaria Indicator Surveys, Multiple Indicator Cluster Surveys, Malaria Report, Roll Back Malaria and President's Malaria Initiative websites. The type, number and distribution of interventions were

summarised and mapped at sub-national level, and compared with known or potential LF distributions. These analyses found that vector control activities had increased significantly since 2005, with a three-fold increase in ITN ownership and IRS coverage overall. However, coverage varied dramatically across the 16 countries, and some regions reported >70% ITNs ownership and regular IRS activity, while others had no coverage in remote rural populations where LF was endemic. Although these African countries are behind in initiating MDA, and populations remain at risk, the continued global financial support, and expansion of vector control activities is promising. It is not beyond the scope of GPELF in reaching its target of global elimination by 2020, however, monitoring and evaluating the impact of these activities over the next decade will be critical to its success.

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MAPPING LYMPHATIC FILARIASIS DRUG COVERAGE AND CLINICAL CASES IN MALAWI

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In 2010, Malawi completed its second year of national mass drug administration (MDA) for the elimination of lymphatic filariasis (LF). The district health centres play a key role in the distribution of drugs, and as part of the National LF Programme, have started to collate data on the local population at risk, population treated and number of clinical cases associated with LF infection i.e. lymphodema and hydrocele, into a database. The aim of this study was to map MDA coverage and clinical cases at health centre level within endemic districts to identify high risk and vulnerable populations. Data from 10 districts with medium to low levels of endemicity were available for analyses, and the location (latitude and longitude) of each health centre was geo-referenced. The epidemiological drug coverage rates (population treated/total population at risk), number of lymphodema and hydrocele cases, and prevalence rates (%) were quantified and mapped using statistical and mapping software. Analyses found that the majority of health centres had adequate epidemiological coverage rates of >65%, and those with lower coverage were dispersed or in close proximity (i.e. 10km) to those with high coverage. However, one district had several health centres with very low drug coverage <50%, which were geographically clustered in one region. The reported number of lymphodema and hydrocele cases differed between the 10 districts and totals ranged from 30 to 91, and from 127 to 340, respectively. Not all health centres reported clinical cases, however, those that did reported between 1 and 22 lymphodema, and between 1 and 152 hydrocele cases, with generally low prevalence rates of $\leq$ 2%. This study shows that developing a geo-referenced database and district maps on MDA coverage and clinical cases can help to identify and monitor health centres with low drug coverage and high morbidity levels. This will enable interventions to be targeted appropriately and improve the prospects of LF elimination in Malawi.

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EFFECT OF HERPETIC CO-INFECTIONS IN CHILDREN WITH AIDS TREATED WITH HAART

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The objective of this study was to assess herpetic co-infection in Cambodian children whit AIDS in terms of risk factors and outcome. Two groups of children whit AIDS were retrospective analyzed with univariante (Chi-square, Fisher's tests and t-test) and multivariante analysis. A logistic regression was done to identify risk factors and factors influencing outcome. Statistical analysis was performed with the open source statistical package R. A *P*-value of <0.05 was considered statistically significant. From all of 75 children with AIDS 48 had herpes coinfection (7 herpes simplex, 17 herpes zoster, 28 chickenpox). Herpes co-infection was not observed in 29 children on HAART. In univariante analysis immune reconstruction syndrome (IRS) (OR=7,33; $CI_{95\%}$ =1,76 -35,22; *P*=0,003) and otitis media (OR=2,83; $CI_{95\%}$=0,99-8,24; *P*=0,05) were significant more frequently observed among herpes-coinfected AIDS children. IRS were significant in multivariante analysis too (*P*=0,007). If herpes infection during HAART occurs, those children are of increasing risk of IRS, otitis media and can develop adverse outcome during antiretroviral therapy. Therefore vaccination against varicella and prophylaxis use of antivirals (eg. Acyklovir) in close contacts whit herpes infected children is advisable.

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BREASTFEEDING AND VITAMIN D IN COMPARISON WITH OCCURRENCE OF INFECTIOUS EVENTS AMONG HIV EXPOSED CHILDREN IN RURAL UGANDA

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A lot of new infections due to the human immunodeficiency virus (HIV) in children were acquired through mother-to-child transmission (MTCT) of HIV. Presented study is prospective study with characteristics of quantitative and confirmative scientific approach. The major view forms formative research with focus of PMTCT program and access to the feeding of HIV exposed children (HIV+/ HIV-) relating to occurrence of infectious diseases with contemporaneous administration of vitamin D in rural areas of Uganda. The research surveyed on the definition of spread feeding approaches and described local performance associated with choice of feeding and the purpose in view was associated with impact on breastfeeding, HIV status and administration of vitamin D supplementation and occurrence of infectious event. We found, that breastfeeding in whole group of HIV exposed children under 3 months is low and prevalence of infections is high. Analysis by the event of diarrhea or pneumonia among HIV exposed children relating to breastfeeding and administration of vitamin D showed significant correlation in occurrence of diarrhea among HIV positive children 59% vs. 30,43% among HIV negative group (*P* = 0,02). Absence of HIV infection, breastfeeding and administration of vitamin D were associated with statistical significant decreasing of infectious event (*P* < 0,001). In this study was find significant impact upon infection presentation when using daily vitamin D supplementation in both children HIV negative even HIV positive. We suppose that these observation can be important for further research among HIV infected children with focusing on deeper understanding of action of vitamin D and HIV infection in consideration of prevention of infectious events among children living with HIV.

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ROLE OF MRSA AND ESBL-PRODUCING ENTEROBACTERIACEAE DECREASED DURING HAART: SEVEN YEARS FOLLOW UP IN CHILDREN WITH AIDS

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The aim of this study was to assess prevalence of resistant gram-positive organisms (MRSA, PRP) and multiresistant gram-negative bacteria (ESBL-producing *Klebsiella* spp., *Serratia* spp, *Enterobacter* spp, MDR-*Acinetobacter*, *Pseudomonas aeruginosa*) among respiratory isolates from Cambodian children with AIDS within 7 years follow up. All children with AIDS on HAART were screened every 6 month within 7 years for respiratory isolates of drug-resistant bacteria. Of 116 children 408 isolates were detected and tested for antibiotic resistance. We detected 206 gram-positive and 202 gram-negative: MRSA 47%, PRP 14%, ERY-R S. pyogenes 8%, ESBL Klepsiella spp. 10,5 %, ESBL plus Enterobacteriaceae spp., MDR-R *P. aeruginosa* 10 %, MDR-R *A. baumani* 8%. The proportion of MDR-R and MRSA decreased from 82% at the baseline to 33% after 7 years of HAART. HAART improve the immune response increasing the CD_4 absolute count and clearance of multiresistant gram negative bacteria and MRSA from respiratory tract of Cambodian children. ATB resistance during the 7 years follow up decreased despite the amount antibiotics for the treatment of opportunistic infections increased.

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ROLE OF HIGH SCHOOL STUDENTS' HEALTH LITERACY IN THE CONTROL OF THE HIV/AIDS EPIDEMIC IN SOUTH AFRICA

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South Africa's severe HIV/AIDS epidemic requires a health literate population to reduce the spread of infection, promote early screening and adherence to treatment. HIV counseling and testing, offered at no charge at health facilities is a critical intervention, but the uptake is low and targeting high school students is a feasible option to reach large numbers of youth in order to promote safe sexual practices. AIM. This study investigated the association between health literacy, HIV testing and sexual behaviour amongst rural and urban high school students in KwaZulu-Natal. In a cross sectional study, students (n=1076) at 10 KwaZulu-Natal public high schools completed a structured self-report anonymous questionnaire using the I-Change Behavioural Change Model as the theoretical framework, to measure awareness and motivating factors. Male students, mean age 17.08 years (SD 1.64) were older than females 16.47 years (SD 1.56) (P<0.005), but of 16.7% students who had tested for HIV, 127 were females(70.6%) and 53 males (29.4%) (P<0.005). More females than males thus supported HIV testing (P=0.002), reported self-efficacy to test (P=0.01) and intentions to test (P<0.005). In the model knowledge about HIV transmission (P=0.04), attitudes to HIV infected persons (P=0.03), perceptions of risk (P=0.01), and self-efficacy to be treated for HIV (P=0.007) predicted testing for HIV. Factors influencing sexual behaviour: Students who had used a condom at last sex were older (P=0.002), knew about HIV prevention (P=0.04) and were more positive about testing for HIV (P=0.03). Knowledge about HIV prevention and self-efficacy to test for HIV were also associated with sexual abstinence (P=0.048). Multiple partners decreased students' intentions to test (P=0.008). In conclusion, South Africa is placing renewed emphasis on HIV counseling and testing and these findings about health literacy awareness and motivating factors can assist in developing focused health promotion programmes which are gender and context specific.

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PERCEPTIONS AMONGST HIV-POSITIVE PEOPLE OF TAKING ANTIMALARIAL MEDICATION CONCOMITANTLY WITH ANTI-RETROVIRAL THERAPY: FINDINGS FROM A QUALITATIVE STUDY

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The double burden of malaria and HIV co-infection is faced by millions of people in sub-Saharan Africa. However, little research has addressed how affected individuals cope with prevention and treatment of these two diseases together. The pharmacokinetic safety and effectiveness of taking artemisinin-based combination therapy (ACT) for malaria concomitantly with anti-retroviral therapy (ART) for HIV is currently being examined through a clinical observational study in Muheza, Tanzania. We designed a qualitative study alongside the clinical trial to explore how affected individuals conceptualise co-infection and the prevention and treatment of malaria when HIV positive and taking ART. We are carrying out focus group discussions with HIV-positive people on ART as well as with HIV-negative people, for comparison. Findings will be triangulated with in-depth interviews held with key health workers delivering care to HIV-positive people in Muheza. Data collection will be completed in July 2011, and data will be analysed using an iterative, line-by-line approach based on the principles of grounded theory. Preliminary findings suggest that people hold a wide range of perceptions and experiences of taking concomitant treatments, and a variety of sources shape beliefs of danger or effectiveness of taking antimalarial medicines alongside ARVs, including religious or spiritual beliefs and information received from health workers. As data collection and analysis proceeds, these findings will further be situated in the local milieu of care and treatment. As such, we believe this study will help to inform public health interventions that aim to minimize the risks related to co-infection and co treatment of HIV and malaria.

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ACCEPTABLE OUTCOMES IN HIV/TB CO-INFECTED PATIENTS IN HAITI WITH CD4 COUNTS > 350 CELLS/MM^3 NOT STARTED ON ANTIRETROVIRAL THERAPY

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Ninety percent of the 10 million HIV/TB co-infected persons live in low- and middle-income countries, where there is often little infrastructure to regularly monitor long-term side effects associated with antiretroviral therapy (ART) and little availability of second-line treatment if antiretroviral resistance develops. Although there is clear evidence supporting the early initiation of ART in HIV/TB co-infected persons with CD4 counts less than 350 cells/mm^3, no studies to date have determined optimal timing for ART in HIV/TB co-infected patients with CD4 counts greater than 350 cells/mm^3. Partners In Health (PIH) runs comprehensive healthcare programs in support of the ministry of health in Artibonite, Haiti. Using PIH protocol, patients with HIV/TB co-infection and CD4 counts ≥ 350 are treated for TB and followed monthly. ART is started if CD4 count drops < 350 or if clinical symptoms deteriorate. In this retrospective study we reviewed medical records between, January 1, 2008 and January 1, 2011 for patients with CD4 counts greater than 350 cells/mm^3 not started on ART prior to or during tuberculosis treatment. Demographic data including

age, sex, weight, CD4 cell count at time of TB diagnosis, and ART status, as well as outcome measures of TB treatment success (cured or judged clinical improved by the health care provider overseeing their care), death, weight change, and days from initiation of TB treatment to initiation of ART were analyzed. Results are presented here of the first 20 patients. 85% were treated successfully; 90% were known to have survived until last follow-up date (a median 13.2 months from TB diagnosis). Average weight gain during TB treatment of 4.9kg was significant from no change ($p = 0.0034$). ART was started at a median 8.1 months from TB diagnosis in 35% of patients. Results from this pilot study suggest that outcomes for patients with HIV/TB co-infection and high CD4 counts in rural Haiti are acceptable in short term when patients are regularly followed in a comprehensive care clinic.

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LOW MAGNITUDE AND FREQUENCY OF HSV-2-SPECIFIC INTERFERON-Γ -PRODUCING CD4+ AND CD8+ T CELL RESPONSES DETECTED IN HIV-1 HETEROSEXUAL DISCORDANT COUPLES

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Herpes simplex virus type 2 (HSV-2), the most frequent cause of genital ulcer disease (GUD), has been shown to play a more important role than any other sexually transmitted infections (STIs) in driving HIV prevalence in Africa. In turn, HIV-1 infection leads to more frequent HSV-2 reactivations and shedding. The exact immune mechanisms involved in this virological negative immuno-synergy are unknown. In the present study we sought to assess whether HIV co-infection would affects HSV-specific T cell immunity. Nineteen HSV peptides, derived from HSV-2 glycoproteins gB and gD, were used to analyze the frequency and the magnitude of HSV-2-specific IFN-γ-producing $CD4^+$ and $CD8^+$ T cell responses in 30 HSV-2 seropositive patients and 17 HSV-2 seronegative individuals in a cohort of heterosexual Senegalese HIV-discordant couples, using ELISpot assay. The magnitude and frequency HSV-2-specific T cell responses was compared between 21 HSV-2 co-infected with HIV-1 and 9 HSV-2 mono-infected individuals. A significantly higher magnitude of IFN-γ-producing T cell responses were observed in HSV-2 infected patients compared to seronegative individuals (median, 61 vs. 0 spots/10^6 PBMC, $P = 0.001$). Moreover, twenty-four (80%) out of 30 HSV-2 seropositive patients showed significant HSV-2-specific IFN-γ-producing T cell responses compared with only 6 (35%) out of 17 HSV-2 negative subjects ($P < 0.001$). The HSV-2 mono-infected patients showed significantly higher magnitude of HSV-2-specific T cell responses compared to HSV/HIV co-infected patients (median, 140 vs. 42 spots/10^6 PBMC, $P = 0.024$). Our finding suggest that co-infection with HIV-1 in HSV-2-infected patients might be associated with reduced HSV-2 cellular immune responses. However, the interaction between HIV and HSV-2 appears complex, and precise longitudinal studies will be required to dissect their exact temporal relationship.

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HIV PREVALENCE IN RURAL SIERRA LEONE

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HIV prevalence is unknown in rural areas of Sierra Leone. HIV infection rates in Sierra Leone have an urban prevalence of approximately 20%. Intervention strategies can be implemented once this information is discovered. The purpose of this study is to document the prevalence of HIV infection in a rural area of Sierra Leone. Adult and Pediatric Patients presenting for medical care were randomly selected and underwent ELISA blood testing for the presence of HIV. Positive test results were sequentially screened with a Western Blot analysis to confirm true positives. 500 Adult patients and 100 pediatric patients were tested for HIV with 1 (0.2%) adult testing positive and 499 (99.8%) negative adult results obtained. 100 pediatric patients tested negative. Total population of patients treated was 1143. Total population of the chiefdom estimated at 2200. We were only able to screen 600 (27.3%) patients out of 2200, thus leaving a large section of the community untested. HIV prevalence is significantly less that in others areas of Sub-Sahran Africa. This presents a significant opportunity for primary prevention, intervention, and education to keep this devastating disease out of this area.

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SAQUINAVIR INHIBITS *PF*CRT-MEDIATED CHLOROQUINE TRANSPORT

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Antiretroviral protease inhibitors (APIs) such as ritonavir and saquinavir can directly inhibit the growth and development of HIV and malaria parasites. Data describing the antiplasmodial activity of APIs with many of the current antimalarial agents is still lacking and in the case of the artemisinin derivatives conflicting. However, studies with mefloquine and chloroquine (CQ) demonstrate that APIs act synergistically against *Plasmodium falciparum in vitro*. The activity of API/CQ combinations, however, appears to be related to the CQ sensitivity of parasites and the API under investigation. The rationale for these observations is not completely understood but is likely to be the result of a number of interplaying factors relating to the antimalarial action of each drug and the CQ resistance mechanisms employed by different parasite strains. The major determinant of CQ resistance in *P. falciparum* is the 'CQ resistance transporter' (PfCRT). PfCRT is an integral membrane protein located at the parasite's digestive vacuole. Its normal physiological function is unknown. However, specific mutations in this protein permit it to transport CQ away from its site of action within the digestive vacuole. As APIs are well known for their ability to inhibit proteins of the drug/metabolite transporter super-family, of which PfCRT is a member, inhibition of PfCRT-mediated CQ transport in resistant parasites may be associated with the synergy seen with these drugs against *P. falciparum*. In order to gain insights into the complex interplay of interactions occurring in CQ-resistant *P. falciparum* parasites treated with APIs we have used previously described transgenic parasites lines $C4^{Dd2}$ $C6^{7G8}$and $C2^{GC03}$, to examine the role of the CQ resistant PfCRT alleles in CQ/API interactions. We have also determined the effect of saquinavir, ritonavir and lopinavir on CQ accumulation in these parasites and in the *Xenopus laevis* oocyte PfCRT expression system. Our data demonstrate that the synergistic antiplasmodial action of saquinavir in combination with chloroquine against *P. falciparum in vitro* is dependent on PfCRT and that this antiretroviral protease inhibitor inhibits chloroquine transport mediated by the Dd2 chloroquine resistance-conferring form of PfCRT.

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BREASTFEEDING, HIV TRANSMISSION: CURRENT KNOWLEDGE AND GUIDELINES; REALITIES, CHALLENGES AND ETHICAL DILEMMAS IN AN HIV HIGH PREVALENCE AND RESOURCE LIMITED SETTING (RLS)

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Breastfeeding has been a universal and major determinant of child survival even before the HIV era. Recognition of HIV transmission through breast milk complicates promotion of breastfeeding (BF) especially in RLS where level of literacy and information dissemination is poor. International Guidelines regarding Infant feeding options and HIV in RLS have been changing leading to confusion and debate which needs to be well informed through current evidence from these settings. We describe realities, challenges and ethical dilemmas regarding infant feeding with reference to guidelines within a high HIV prevalence RLS country. Guidelines are distilled to focus on 'HIV-free survival' of the child as a primary outcome that is effectively counting an HIV infection as equivalent to a death, viewing only fatal outcomes in uninfected children as equivalent to an HIV infection. This approach ignores broader issues of nutritional status, community perceptions, maternal health status before conception, during pregnancy and after birth; growth and development of infected and uninfected children, their morbidity and mortality. Primary causes of infant deaths in RLS are; Infectious diseases, malnutrition and not being breastfed. Infant risk of becoming infected through breast milk is lower than risk of dying from other causes of not BF. On the other hand breast milk of an HIV infected mother is labeled as poison especially in RLS. Guidelines are informed by research contacted within ideal settings. Questions are raised regarding the appropriateness and implementation of these guidelines for both health care providers and mothers and communities in RLS. In Zimbabwe exclusive breastfeeding (EBF) up to 6 months has dropped from around 25% in 2005 to 6% in 2010 and the main reason is malnutrition among nursing mothers regardless of HIV status, whilst one in 3 under five children in Zimbabwe are malnourished. Realities and challenges of feeding options in the HIV era should be assessed and evaluated in relation to maternal and child nutrition and PMTCT coverage in RLS.

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STATE OF MALARIA DIAGNOSTIC TESTING AT CLINICAL LABORATORIES IN THE UNITED STATES, 2010

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The diagnosis of malaria can be a difficult undertaking in non-endemic areas such as the United States, where delays in diagnosis and errors in treatment occur too often. A nationwide survey of laboratories in the United States and its nine dependent territories was conducted in 2010 to determine factors that may contribute to diagnostic shortcomings. This survey explored the availability of malaria diagnostic tests, techniques used, and reporting practices. The survey was completed by 201 participants. Ninety percent of all respondents reported having at least one malaria diagnostic test available on-site in their laboratories. Nearly all laboratories performed thick and thin smears on-site; only 17% had access to rapid diagnostic tests on-site; and about 50% had access to molecular testing. Seventy-three percent reported fewer than five confirmed cases of malaria in their laboratory during the 12-month period preceding the survey. Twenty-eight percent stated that results of species identification took more than 24 hours to report. Only nine of 149 laboratories who performed testing 24 hours, 7 days a week complied with all of the Clinical and Laboratory Standards Institute (CLSI) guidelines for analysis and reporting of results. Though malaria diagnostic testing services were available to a majority of U.S. laboratories surveyed, very few were in complete compliance with all of the CLSI guidelines for analysis and reporting of results, and most laboratories reported seeing very few cases of malaria annually. The difficulty in adhering to the rigorous guidelines and lack of practice and proficiency may account for delays and errors in diagnosis. It is recommended that laboratories that infrequently process samples for malaria seek opportunities for practice and proficiency training annually, and take advantage of resources available to assist in species identification.

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MONITORING MALARIA PARASITE DYNAMICS IN VOLUNTEERS ENROLLED IN A MALARIA VACCINE TRIAL: A NEW TARGET PRODUCT PROFILE FOR RT-QPCR AND PFHRP-2

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Parasite biomarkers, PfHRP2/pLDH and PCR could potentially offer better diagnostic capability for malaria than microscopy. In this study, we report on malaria parasite dynamics for 112 days of passive follow-up as evaluated by microscopy, PfHRP-2, pLDH and PCR. We also report on comparison of the assays in predicting onset of clinical malaria. Blood samples were obtained from 30 adult research subjects enrolled in the FMP-10 blood stage malaria vaccine trial that was conducted between December 2008 and June 2009 at the KEMRI/Walter Reed Project Clinical Trial Center in Kombewa, Kisumu District, Kenya. Samples were obtained weekly first for the first three weeks and fortnightly thereafter during scheduled visits for a period spanning 112 days and analyzed after the end of observation period. Volunteers were asked to report for treatment whenever they fell sick. Presence of malaria was evaluated by microscopy, ELISA for PfHRP2 and pLDH antigens and RT-qPCR. Kaplan-Meier was used to evaluate proportions of study participants predicated to have parasitemia and clinical malaria by each assay at each scheduled visit. Survival proportions were highest when estimated by microscopy and pLDH and lowest by PfHRP-2 and RT-qPCR. Log rank (Mantel-Cox) test showed significant differences in the trend curves ($P<0.0001$). Of the 12 participants who developed clinical malaria, RT-qPCR/PfHRP2 correctly predicted 90% (11/12) while pLDH and Microscopy could only predict 50% (6/12). During the 112 days follow up, microscopy and pLDH ELISA detected 40 and 50 malaria events respectively, while RT-qPCR and HRP-2 ELISA detected 118 and 110 events respectively. Because of enhanced sensitivity of RT-qPCR and PfHRP-2 over conventional methodologies, there is need to re-define their target product profile from being diagnostic for purposes of disease management to monitoring parasite dynamics in the context of drug/vaccine clinical trials.

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COMPARISON OF THREE METHODS FOR THE DETECTION AND SPECIATION OF *PLASMODIUM* SPECIES IN CHILDREN AND PREGNANT WOMEN IN BANGOLAN, NORTHWEST REGION OF CAMEROON

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Effective treatment of malaria requires accurate laboratory diagnosis. Microscopy still remains the gold standard for the diagnosis of malaria. Rapid diagnostic tests (RDTs) and PCR assays are alternatives to microscopy and have been shown to be sensitive and specific. However, very few comparative studies have been reported on the three diagnostic methods in vulnerable groups. The sensitivity and specificity of microscopy, RDTs (SD Bioline kits; Pf/pan and Pf specfic kit) and PCR was used for detection and speciation of *Plasmodium falciparum* (Pf), *P. malariae* (Pm) and *P. ovale* (Po) in patients in Bangolan. A total of 54 children and 16 pregnant women were recruited for the study after obtaining an informed consent. Blood collected was used for thin and thick smears for microscopy, RDTs and blood spot on filter paper for DNA extraction and conventional PCR. Of the 70 patients diagnosed, parasitemia ranged from 520 -149600/µl. A total of 87.14% were positive by microscopy, 85.71% by RDTs and 90% by PCR. The distribution of *Plasmodium* species in the study population as identified by PCR was 72.86% Pf/Pm, 11.43% Pf/Pm/Po and 5.43%, Pm while 10% were negative. All the children were positive for malaria by microscopy though it could not clearly differentiate the various species. Of the 54 children, 94.44% tested positive with RDTs while 98.15% were positive with PCR. In pregnant women, the detection/speciation of malaria parasites was 62.5% by PCR, 50% by RDTs and 43.75% by microscopy. The Cohen's Kappa agreement between PCR and RDTs was K= 0.75 (CI = 0.28-1.22) whereas that for PCR and microscopy was K= 0.64 (CI= 0.18-1.10). PCR still remains the most specific and sensitive method. RDTs could be used for routine diagnosis of malaria in vulnerable groups as they have indicated a good concordance with PCR. Malaria infection in Bangolan is mostly due to mix infection predominantly *P. falciparum/P. malariae* and this could influence treatment outcome.

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SEVERE MALARIA AND CHILD NON-SICKLE CELL ANEMIA IN A PEDIATRIC EMERGENCY UNIT AT BONZOLA HOSPITAL: A PROSPECTIVE STUDY

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In order to adequately improve malaria survival in the different areas of Sub-Saharan Africa, there is a need to periodically generate contextual indicators on prevailing malaria case management. We explore cases and correlates (age, gender, onset of complaints, timing of admission, blood type, blood transfusion and survival/death) of severe malaria in Mbuji Mayi by reviewing medical records of all 0-5 year old children seen at Bonzola Hospital from May to July 2009. To be included, a child had to have a documented positive malaria diagnosis. To be considered severe, a malaria case had to show clinical signs of fever, diarrhea, throwing up, and asthenia and hemoglobin level of < 5mg/dl. We explored 1907 records, finding 907 malaria cases (50.9%) of which 188 severe non drepanocytaire cases (9.8%). Of these cases, 184 (97.8%) had hemoglobin level ≤ 5g/dl. There were 24 deaths (12.7%) of which about half occurred due to shortage of blood supply. The mortality rate was not different between females and males. However, younger children (< 3 years) died at a higher rate than their older counterparts. There is a need to increase the survival rate of younger children, notably by improving access to medical care and to blood supply.

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EVALUATION OF THE EXTERNAL QUALITY ASSURANCE PROGRAM IN 23 DISTRICTS IN UGANDA

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The World Health organization recommends parasitological diagnosis with microscopy or rapid diagnostic tests (RDT) for malaria and emphasizes with-holding antimalarials for patients with negative tests implying need for high accuracy for malaria laboratory diagnosis. Good-quality malaria microscopy requires technically competent personnel, high-quality supplies, microscopes, adequate workplace environment and an effective external quality assurance (EQA) system. The Stop malaria Project in collaboration with the Ministry of Health implemented and evaluated an EQA system in 22 districts in Uganda. The WHO based EQA System was introduced in health facilities following a refresher training in malaria diagnosis by microscopy and RDT's in 22 districts. Each health facility randomly collected two blood slides per day (a positive and a negative); of these 10 positive and 10 negative slides were randomly selected per month and sent for external reading by two expert malaria microscopists. Results for facilities were compared with those of expert district level microscopists. Discordant slides were tie broken by expert laboratory technologists at the Infectious Diseases Institute. Blinding of readers was done at all levels. The Kappa statistic was used to measure accuracy. Facilities received additional laboratory supplies to cater for stock outs. Facilities without microscopes received new ones and faulty microscopes were repaired. A total of 1876 blood slides were read; of these 141 were discordant at the district level. Forty five percent (45%) of the laboratories had excellent accuracy (excellent agreement, Kappa >0.80) 41% had good accuracy (good agreement Kappa of o.6-0.80, 5% had fair accuracy (Kappa = 0.57) while 9% had very poor accuracy (Kappa <4.0).Accuracy of reading blood slides varied across districts and this necessitates maintenance of EQA and external quality control systems in all districts to support the recent WHO malaria case management policy which emphasis parasite based diagnosis and discourage presumptive treatment with antimalarias.

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COMPARISON OF THE BINAXNOW® MALARIA RAPID ANTIGEN ASSAY TO REAL-TIME PCR AND GIEMSA-STAINED BLOOD SMEAR FOR THE DIAGNOSIS OF *PLASMODIUM* SPP. INFECTION

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Malaria continues to be a global health burden with approximately 250 billion cases and one million deaths annually. This is partially due to the fact that individuals living in resource-limited countries are the most vulnerable to infection and effective laboratory diagnostic methods and treatment in these areas is limited. Although laboratory tests such as real-time PCR and microscopic examination of whole blood smears are sensitive and specific, these methods are either expensive or require highly trained personnel. The goal of this study was to evaluate a recently FDA-approved immunochromatographic assay (BinaxNOW® Malaria, Inverness Medical, Princeton, NJ) and compare the performance of this rapid antigen test to real-time PCR and routine blood smear examination. Whole blood samples (n=157) submitted to our reference laboratory for malaria real-time PCR and/or routine blood smear were also analyzed by the BinaxNOW rapid antigen assay. Among the 157 samples tested by BinaxNOW, 101 (64%) were tested by PCR, 109 (69%) were analyzed

by blood smear, and 53 (33.7%) were tested by all three assays.When compared to real-time PCR, the BinaxNOW assay demonstrated a percent agreement, sensitivity and specificity of 92.1% (93/101), 77.4% (24/31) and 98.5% (69/70), respectively. When compared to routine blood smear, the BinaxNOW showed a percent agreement, sensitivity and specificity of 91.7% (100/109), 83.3% (25/30) and 94.9% (75/79), respectively. Interestingly, among the 4 samples that were BinaxNOW positive, smear negative, 3 (75%) were also positive by real-time PCR. These results indicate that the BinaxNOW Malaria assay could be used in emergency or point-of-care settings to rapidly rule-in malaria based on the high specificity (≥94%) and positive predictive value (≥96%) of this test. However, a negative result by the BinaxNOW assay does not rule-out malaria, and should be followed up with a blood smear or real-time PCR. The BinaxNOW assay requires less technical expertise than blood smear examination, is less expensive compared to PCR, and provides a more rapid turn-around time (15 min. *vs*. 1.5 h for blood smear *vs*. 2 h for PCR).

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A HIERARCHICAL SYSTEM TO ENSURE QUALITY OF MALARIA MICROSCOPY IN MALI

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In 2010, Mali's National Institute for Public Health Research (INRSP), with support from the Improving Malaria Diagnostics project financed by the US President's Malaria Initiative, rolled out an Outreach Training and Supportive Supervision (OTSS) program consisting of quarterly visits to health facilities to identify and correct barriers to quality malaria microscopy (MM) and malaria Rapid Diagnostic Tests (mRDTs). The baseline visits to 50 laboratories found that 38% lacked standard operating procedures (SOPs) for conducting MM; 84% had no SOPs for external quality assurance (EQA) of MM; 70% did not save slides for EQA; 86% did not keep records of internal quality assurance (QA) exercises; and 78% lacked slide boxes to preserve slides for later EQA. OTSS visits included two layers of EQA for MM: in addition to re-reading slides by laboratory supervisors during the OTSS visit, there was a second blind re-reading at INRSP. Data from 2054 EQA slides was entered into a database and analyzed with SPSS. OTSS visits provided on-the-job training to 471 microscopists and 439 clinicians. The % of laboratories in visits 1 and 4 without functioning microscopes was 6% and 0% respectively, the % experiencing stock-outs of essential diagnostic supplies in the same period was 20% and 3%; the % with MM in full agreement with national guidelines was 76% and 91%; the % using mRDTs in full agreement with national guidelines was 59% and 85%. Analysis of sensitivity (Se) and specificity (Sp) of microscopists (Mi) and their supervisors (Su) was, respectively: Se-Mi=82%, Sp-Mi=76%, Se-Su=81%, Sp-Su=90%. 80% of Su's false positives (FPs) overlapped with Mi's FPs. 80% of laboratories met a quota of 30 EQA slides per year, sufficient to calculate agreement at lab level. INRSP will compare the sustainability of an EQA scheme that sends only a sub-set of slides to INRSP for confirmation vs. proficiency testing of supervisors; Sus continue testing Mi's performance; retrain Mis and Sus not performing; and use blinding procedures to ensure that Sus are not influenced by Mi's results.

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INCREASED USE OF MALARIA DIAGNOSTIC TESTS IMPROVES TARGETING OF ANTI-MALARIAL TREATMENT IN RURAL TANZANIA: IMPLICATIONS FOR A NATIONAL ROLL OUT OF MALARIA RAPID DIAGNOSTIC TESTS

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The World Health Organization recommends diagnostic confirmation of all uncomplicated malaria cases. Tanzania is implementing a phased roll-out of malaria rapid diagnostic tests (mRDTs) in all levels of health care facilities as one strategy to increase parasitological malaria diagnosis. We evaluated artemisinin combination therapy (ACT) prescribing patterns in febrile patients with and without uncomplicated malaria in two areas with high and low levels of mRDT implementation (mRDT area and non-mRDT area, respectively). We conducted repeat cross sectional health facility surveys in two areas with health and demographic surveillance systems during both high and low malaria transmission seasons in 2010. We collected clinical information and a reference blood slide on all patients presenting for an initial illness consultation. Uncomplicated malaria was defined as fever and asexual *P. falciparum* parasitemia on a reference blood slide. We included 1,247 febrile patients in the analysis. In the mRDT area, 65% (95% confidence interval (95% CI): 52-76) of febrile patients received a diagnostic test compared to 50% (95% CI: 39-61) of patients in the non-mRDT area (p<0.001). In the mRDT area, 79% (95% CI: 66-88) of patients with uncomplicated malaria received recommended treatment with ACT compared to 67% (95% CI: 54-78) of patients in the non-mRDT area (p=0.11). Overtreatment with an ACT of patients without slide confirmed uncomplicated malaria was significantly less common in the mRDT area (23%; 95% CI: 17-30) compared to the non-mRDT area (35%; 95% CI: 29-43) (p=0.01). In conclusion, routine implementation of mRDTs resulted in increased diagnostic test use and reduced overtreatment with ACTs in one area in Tanzania. The national rollout of mRDTs will have to be monitored to assess whether these changes in case management practices will be replicated in other areas.

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OVER AND UNDER-USE OF ARTEMISININ BASED COMBINATION THERAPY AT PUBLIC HEALTH FACILITIES IN THREE REGIONS OF TANZANIA

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Artemisinin based combination therapy (ACT) is the first line drug in most malaria-endemic countries, but there are concerns that quality of care remains poor. Patients needing ACT often do not receive it, but there is also considerable over-treatment due to the lack of accurate diagnosis and inappropriate management. Tanzania is scaling up the use of rapid diagnostic tests (RDTs) to improve treatment of febrile illness. We conducted health facility surveys before RDT scale up to assess current treatment practices. We enrolled 1779 patients at 145 randomly selected health facilities in Mwanza, Mbeya, and Mtwara Regions between May and October 2010. Patients with fever in the previous 48 hours were enrolled on arrival and interviewed following their consultation. Data were collected on patient characteristics, previous treatment for fever, and care. Fingerprick blood samples were taken by study staff to test for

malaria parasitemia. Overall, 66.6% of patients attended a facility with any ACT in stock and 28.6% a facility with all 4 weight-specific doses of ACT available. 60.6% of patients had been seen by a health worker trained in ACTs. Only 6.3% of patients sought treatment at a facility that had RDTs in stock, and 5.7% saw a health worker who had been trained in RDTs. Overall, 9.8% of patients received a diagnostic test at the health facility; 82% of those tested received a blood smear and 18% an RDT. Of those tested, 54.8% were reported to have a positive test. ACTs were obtained by 58.5% of patients with a positive test, 11.4% of patients with a negative test, and 36.0% of patients who did not receive a diagnostic test during their consultation. Study RDTs conducted in all enrolled patients found that 24.5% of all patients had a positive RDT. ACTs had been obtained by 44.3% of patients with a positive RDT and 33.7% of patients with a negative RDT. Over-diagnosis of malaria remains common, with ACTs frequently prescribed to parasite-negative patients; it is anticipated that national scale up of RDTs should address this issue to some degree. However, under-treatment also remains a key problem, reflecting both ACT stockouts and inappropriate health worker practices.

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IMPROVED SEMI-NESTED MULTIPLEX PCR (SNM-PCR) FOR THE IDENTIFICATION OF THE FIVE HUMAN *PLASMODIUM* SPECIES

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Since the recognition of *Plasmodium knowlesi* as the fifth human *Plasmodium* species in Southeast Asia, cross-hybridization of the current most used PCR to identify *P. knowlesi* have been reported. Continuous attempts are made to improve the diagnosis through the development of new molecular techniques. In this report, a primer set for the identification of *P. knowlesi* in a semi-nested multiplex PCR was designed and validated against earlier published PCR based protocols. We aimed to take great advantages of the semi-multiplex PCR in detecting four popular human malarial species as published by Rubio et al, to develop a new nested PCR to identify *P. knowlesi*. Sequencing of both primary and nested PCR products were done with the view to confirming the primers amplified exactly genes of *P. knowlesi*. Human malaria species DNA, some of non-plasmodium DNA, field samples collected during malarial surveys in Ninh Thuan and Quang Nam provinces, Viet Nam were tested by three different protocols to make comparison of specificity and sensitivity. The new PCR protocol specifically amplified *P. knowlesi*, and PCR products had a band size of 500bp. No other monkey malaria strain or related was amplified by this PCR. Results were confirmed by sequencing confirming the PCR's specificity for *P. knowlesi*. that the new PCR was able to identify all *P. knowlesi* infections in our test. This protocol had a similar sensitivity and specificity with Imwong's protocol both performed better than the currently most used PCR to identify *P. knowlesi* as published by Singh et al 2004. This new protocol had as advantage to less labor intensive when analyzing for all 5 *Plasmodium* species infecting human was more economically with the Taq polymerase. Developments of new protocol starting from Rubio et al 2002 showed great number of advantages in identifying *P. knowlesi* in humans. It not only overcome cross- hybridization of the primers used with *P. vivax* - a well known problem of Singh's protocol, showed similar results with Imwong's protocol. Moreover, the success of new protocol contributes to reduce workload, time consuming and the cost of PCR technique in screening *Plasmodium* parasite in humans by sharing the same primary products for both SnM-PCR and nested PCR in detecting *P. knowlesi*.

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QUALITY ASSURANCE OF MALARIA DIAGNOSIS IN HAITI: PRELIMINARY RESULTS OF A PILOT IMPLEMENTATION IN THREE REGIONS, AUGUST 2010 - FEBRUARY 2011

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In Haiti, malaria diagnosis has relied on microscopy for many years. Following the January 2010 earthquake, rapid diagnostic tests (RDTs) were introduced in the emergency setting. The National Malaria Control Program and National Public Health Laboratory (LNSP) approved 3 *Plasmodium falciparum*-specific RDTs for use in Haiti and implemented a pilot malaria diagnostics quality assurance (QA) program in 3 regions to monitor field performance of RDTs and support microscopy. From August 2010 - February 2011, HRP2-based RDTs (CareStart™ Malaria HRP2) were introduced in 15 health facilities. Quality assurance teams from the LNSP conducted training on RDTs and introduced QA procedures. QA teams returned monthly to conduct on-site QA of microscopy and RDTs. For each patient tested by microscopy or RDT, QA personnel performed a separate blood smear for comparison. Data were reviewed and discrepancies between health facility and QA results prompted a second on-site reading of the QA blood smear. All QA smears were reread at the LNSP. 494 patients were tested for malaria during QA team visits from September 2010 - February 2011. Most sites received 3 QA visits, but activities were disrupted in October and November 2010 due to cholera response and security concerns around the Presidential election. Among the 290 blood smears performed, 55 were positive (18.9%) and 235 were negative. There were 3 false positive results and 4 false negative results, sensitivity= 92.9% and specificity= 98.7%. 465 RDTs were also performed and 105 (22.6%) were positive and 360 were negative. There were one false positive RDT result and 2 false negative results, sensitivity= 98.1% and specificity= 99.7%. QA team readings showed a high degree of internal consistency with just 1 discrepant reading. The pilot QA program demonstrated excellent performance of both RDTs and field microscopy and will be expanded to a total of 5 regions in 2011. The program was well-received by participating sites, but has high logistics and human resource demands which may be difficult to support during periods of crisis.

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NOVEL PCR-BASED ASSAYS FOR THE DETECTION OF *PLASMODIUM KNOWLESI*

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Recent studies in Southeast Asia have demonstrated zoonotic transmission of *Plasmodium knowlesi* to humans. This simian malaria parasite naturally infects long-tailed (*Macaca facsicularis*) and pig-tailed (*M. nemestrina*) macaque monkeys in much of Southeast Asia. It has a 24-hour asexual blood stage growth cycle that can lead to rapid increases in parasitemia, severe disease and, in a few human infections, has been fatal. As such, *P. knowlesi* infection requires immediate diagnosis and treatment. Microscopically, *P. knowlesi* exhibits stage-dependent morphological similarities to *P. malariae* and *P. falciparum*. These similarities have contributed to misdiagnosis of *P. knowlesi* as *P. malariae or P. falciparum*. PCR based molecular diagnostic tests were required to accurately detect

P. knowlesi in humans. The current PCR-based assay is based on the 18s ribosomal DNA sequences and can cross-react with *P. vivax* and other simian *Plasmodium* species (unpublished data). As such, we initiated the development of new PCR-based tests. In order to develop species-specific diagnostic tools for malaria, we have developed a bioinformatics approach to mine the available genome data and identify suitable DNA sequences that are highly specific to a given species of malaria parasite. Using this approach, we have identified highly specific, multicopy *P. knowlesi* sequences. We designed novel *P. knowlesi* primers for a single tube non nested PCR method. We show that this method has 100% specificity for the detection of *P. knowlesi* using three different strains of *P. knowlesi* (Nuri, H, and Hackeri) and one *P. knowlesi* infected patient specimen. In addition, no cross reactivity was observed with each of the four human malaria parasite species including 20 different strains of *P. vivax* and 5 simian malaria parasite species that were tested. This novel PCR assay is a suitable alternative for the accurate diagnosis of *P. knowlesi*. Additional laboratory and field-based testing of this assay will be necessary to validate its utility for clinical diagnosis of *P. knowlesi*.

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DEVELOPMENT OF A NOVEL CHEMICAL SERIES WITH ACTIVITY AGAINST BOTH BLOOD- AND LIVER-STAGES OF *PLASMODIUM FALCIPARUM*

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Recent progress toward the development of a novel compound series with promising *in vitro* efficacy against both blood- and liver-stage *Plasmodium falciparum* will be described. Following the failure of one such compound to cure malaria-infected mice, focus has been to enhance the pharmaceutical properties of the compound series. Newer analogs, incorporating structural changes expected to enhance these properties, have been prepared. The *in vitro* efficacies and pharmaceutical properties of these will be described. A thorough investigation of the pharmaceutical properties of representative members of the series has been undertaken, focusing on predicting their likely metabolic stability. These results, including those concerning the kinetics of *in vitro* microsomal degradation, and the subsequent identification of predicted metabolites, will be discussed.

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DEVELOPMENT OF A *PLASMODIUM FALCIPARUM* TRANSGENIC LINE FOR SCREENING DRUGS TARGETING GAMETOCYTE BY USING STRONG GAMETOCYTE SPECIFIC PROMOTER

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Malaria remains to be a devastating infectious disease, causing an estimated 500 million cases and 2 million deaths per year. Whereas the asexual stage is responsible for clinical disease, gametocytes are responsible for transmission from human host to vector. There is renewed acknowledgement that targeting gametocytes is essential for malaria control and elimination efforts. It is known that *Plasmodium falciparim* gametocytes (especially the mature stage) are relatively insensitive to many anti-malaria drugs; thus new drugs that can safely eliminate gametocytes or block transmission are needed urgently. We established a parasite cell line with the expression of the green fluorescent protein (GFP) under the gametocyte-specific promoter alpha-tubulin II. Our analysis showed GFP signals appeared in all gametocyte with relatively lower intensity in female gametocytes. The GFP signals increased from early gametocyte to mature stage (from stage I to V, even in stressed schizonts). The GFP expression was high enough to be detected by flow cytometry as early as stage 2. Based on this result, we are investigating the effect of several antimalarial drugs on gametocytes.

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N-ACETYLCYSTEINE CO-ADMINISTERED WITH PRIMAQUINE IN MALARIA TREATMENT IN ASSESSING PRIMAQUINE EFFICACY AND HEMOLYTIC TOXICITY

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Primaquine (PQ) is the only medication approved for a radical cure of malaria caused by *Plasmodium vivax or P. ovale* but toxic, hemolytic side effects in G6PD deficient individuals limits its usage. Because the toxicity of PQ may be due to oxidative stress induced by its reactive metabolites, we investigated whether N-Acetylcysteine (NAC), which has potent anti-oxidant activity, could attenuate the PQ-mediated oxidative stress without compromising efficacy. We co-administered NAC and PQ in the following animal models: the causal prophylaxis *P. berghei* efficacy model, utilizing *In vivo* Imaging Spectrum (IVIS), and the C3H G6PD deficient rodent screening model. Both are used to screen potential antimalarial drug candidates. In the efficacy model, 10 mice were given PQ 10mg/kg orally once daily and NAC 200mg/kg twice daily by intraperitoneal injection for 3 days. Luciferase expressing *P. berghei* sporozoites were inoculated on day 2. Images were obtained daily for 3 days and blood samples obtained for parasitemia every 2 days for a total of 2 weeks. In the G6PD deficient rodent model doses used were PQ 8.75 mg/kg given daily with or without NAC 400mg/kg administered twice daily both orally for 5 days. Hematologic parameters were tested at baseline and day 6 to detect hemolysis. In the efficacy model, no inhibition of activity was seen by, but 3/5 animals in the control and 2/5 in the experimental group died during the parasitemia follow up. These limited data did not show an effect of NAC on PQ efficacy. In the G6PD deficient model (n=8), there was no difference in hemolytic endpoints between groups (Mean change Mature RBC (M/ml), PQ only = -1.6, PQ+NAC = -1.8). Preliminary results suggest that low dose NAC produced no significant difference between the two groups in either model. Further testing of both drugs, at different doses in both models is on-going; results will be available for presentation.

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EFFICACY OF DIFFERENT NITRIC OXIDE-BASED STRATEGIES TO PREVENT EXPERIMENTAL CEREBRAL MALARIA

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The high case-fatality rate and morbidity of cerebral malaria despite parenteral antimalarial therapy incites investigators to develop preventive and adjunctive therapies for the disease. Preclinical studies using experimental models of cerebral malaria are useful to understand its pathophysiological processes and can be a first step to test therapies for the condition. Low nitric oxide (NO) bioavailability plays a role in the pathogenesis of experimental cerebral malaria (ECM) caused by *Plasmodium berghei* ANKA. The disease is prevented by the treatment with a high concentration of the NO donor dipropylenetriamine NONOate (DPTA-NO). However, it is not known how NO acts to prevent the development of ECM and if more physiologically and clinically relevant treatments aiming to improve its endogenous synthesis or effects through the generation of cyclic guanosine monophosphate (cGMP) would also show efficacy in the model. We studied the efficacy and safety of different strategies to improve NO bioavailability in preventing the development of ECM. Treatments with L-arginine, an arginase inhibitor (N-hydroxy-nor-Arginine) and tetrahydro-L-biopterin when given alone or in different combinations aiming to optimize the endogenous synthesis of NO through

the L-arginine-nitric oxide synthase-NO pathway were not efficient to prevent ECM. Prevention of ECM was neither achieved with sodium nitrite treatment, indicating that the phenomenon of nitrite reduction to NO may not play a significant role in the pathogenesis of the disease. Finally, treatment with low doses of DPTA-NO or the inhibition of the enzyme phosphodiesterase-5 with sildenafil did not prevent ECM, but a significant decrease in mortality was observed when both strategies were combined. The combined therapy did not cause anemia or hypotension, which are major side effects generated by the treatment with high doses of DPTA-NO. We conclude that therapies targeting the NO-sGC-cGMP pathway for ECM are feasible, but have to be optimized to decrease potential side effects caused by the administration of NO.

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AN *IN VIVO* GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6PD)-DEFICIENT MOUSE MODEL TO PREDICT HEMOLYTIC TOXICITY OF CANDIDATE 8-AMINOQUINOLINE (8-AQ) ANTIMALARIAL DRUGS

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Determination of hemolytic potential of 8AQ candidate compounds in animal models prior to commitment of resources for human clinical trials would be invaluable for the development of new 8AQ drugs with a higher therapeutic index. We developed a G6PD-deficient (G6PDD) mouse model that demonstrates a phenotype similar to human African type A^- population (10-15% of normal G6PD activity). We qualified this mouse model by testing three known hemolytic 8-AQs, *i.e.*, primaquine (PQ), pamaquine (PaQ) and tafenoquine (TQ), and two known non-hemolytic drugs, chloroquine (CQ) and mefloquine MQ). G6PDD mice given the hemolytic drugs consistently displayed all hemolytic parameters. The decreases in mature RBC counts (16 - 64%) in G6PDD mice in response to PQ (22 - 88 mg/kg) were dose- dependent. PaQ (70 mg/kg) and TQ (40 mg/kg) also decreased mature RBC counts (67% and 8%, respectively). Results indicated that the reticulocyte production and Heinz body formation were triggered in G6PDD mice by PQ, PaQ and TQ. Neither clinical signs of hemolysis nor obvious changes in hemolytic parameters were observed under the same experimental conditions by using CQ and MQ. We evaluated this mouse model with two regimens of dosing, one of 6 days duration and one of 10 days duration, with similar results. We also developed a hemolytic index (HI) to compare the hemolytic potential between 8-AQs, using PQ as the reference standard. In summary, our results demonstrate that the G6PDD mouse model appears to be useful in predicting the hemolytic toxicity of new 8-AQs and potentially other hemolytic drugs. This *in vivo* model has the attributes of: 1) employing a genetically altered, but otherwise normal G6PDD condition; 2) having a phenotype similar to the African type A^- G6PDD patients; 3) exhibiting a hemolytic toxicity response to 8-AQs; 4) showing no hemolytic response to two non-hemolytic antimalarials, and 5) displaying reproducible and statistically valid assay results. We now intend to utilize this new tool to identify safer 8-AQs.

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INHIBITION OF *PLASMODIUM FALCIPARUM* CALCIUM DEPENDENT PROTEIN KINASE 4 PREVENTS MOSQUITO INFECTION AND MALARIA TRANSMISSION

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Current antimalarial drugs allow continued transmission of malaria from infected individuals to mosquitoes after successful therapy. Effective control and eradication of malaria will require new tools to prevent transmission of these parasites. *Plasmodium* calcium dependent protein kinase 4 (CDPK4), shown to be essential for exflagellation of microgametes, sexual reproduction and infection of the mosquito host, is a promising target because orthologs are absent in mammalian genomes. CDPK4 has a serine at the gatekeeper position of the ATP binding site which renders CDPK4 sensitive to bumped kinase inhibitors (BKIs). We describe BKIs that inhibit *Plasmodium falciparum* calcium dependent protein kinase 4 (PfCDPK4) and block the infection of mosquitoes with malaria-parasites from mammalian-hosts. A BKI that has activity against PfCDPK4 prevents the exflagellation of *P. berghei* mouse malaria-parasites that express PfCDPK4. Administration of the BKI compound to mice stops the transmission of malaria to mosquitoes. Finally, addition of the BKI compound to blood with *P. falciparum* gametocytes stops exflagellation of microgametocytes and blocks the sexual-stage in mosquitoes. These compounds have a low likelihood to select resistance as the selective pressure for selection of resistance is only manifest in the mosquito gut, where natural infection involves only 2 to 10 gametocytes. Our studies thus far indicate that this strategy leads to non-toxic, selective inhibitors that block malaria transmission to mosquitoes, have favorable oral pharmacokinetic (PK) properties, and are thus excellent leads for further drug development. This series of BKI compounds will be further optimized, through structure based drug development and PK measurements, to achieve transmission blocking exposure during the life of the gametocyte. These compounds could be valuable in malaria control and eradication programs.

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POTASSIUM CHANNELS AS DRUG TARGETS IN *PLASMODIUM* PARASITES

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Potassium channels are integral membrane proteins devoted to regulation of membrane potential and cell volume. Malaria parasites encode two K^+ channel homologues (Kch1 and Kch2), which are well-conserved among members of the *Plasmodium* genus. In the rodent malaria parasite *P. berghei*, the two K^+ channel homologues PbKch1 and PbKch2 were studied using targeted gene knock-out. First, the transgenic parasites were characterized in a mouse model in terms of growth-kinetics and transmission potential. Second, using a tracer-uptake technique and $^{86}Rb^+$ as a K^+ congener, the K^+ transporting properties of the transgenic parasites were assessed. Third, the impact on parasite membrane potential of the two K^+ channels was investigated using a potential-dependent fluorophore DiBAC4 bis-oxinol. Results: *i)* Knock-out of either K^+ channel did not grossly affect the phenotypes in terms of asexual replication and

pathogenicity in mice. Though, *P. berghei* parasites deficient in PbKch1 (PbKch1-null parasites), but not PbKch2-null parasites, were unable to form oocysts in female *Anopheles stephensi* mosquitoes. *ii)* PbKch1-null parasites, but not PbKch2-null parasites, had a low $^{86}Rb^+$ uptake, when compared to wild-type (WT) parasites. The Kch1-mediated $^{86}Rb^+$ uptake was inhibited by K^+ channel blockers; the residual, non-Kch1-mediated, $^{86}Rb^+$ uptake was not sensitive to further inhibition by K^+ channel blockers. *iii)* Kch1, but not Kch2, apparently influenced the membrane potential of the parasites. In conclusion, our studies suggest unequivocally that the *Plasmodium* K^+ channel 1 is a functioning K^+ channel, which contributes to the K^+ permeability of the parasites plasma membrane. The channel is, for yet unknown reasons, necessary for sexual replication of *P. bergehi parasites* in the mosquito midgut. These studies provide a rationale for pharmacological inhibition of the Kch1 orthologue in human parasites as a novel strategy to disrupt malaria transmission.

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EFFECTS OF DHA-PIPERAQUINE ON THE QTC INTERVAL IN NORTHERN CAMBODIA

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To date there is not good evidence of clinically significant QT prolongation with piperaquine at therapeutic doses. Effects of DHA-piperaquine treatment on the EKG were assessed as part of an active observational cohort study of malaria epidemiology in healthy volunteers taking either 2 or 3 days of DHA-piperaquine in Northern Cambodia in 2010-2011. A total of 80 subjects were randomized to open label DHA-piperaquine using the same cumulative treatment dose currently recommended by WHO (360mg/2880mg) with either 2 or 3 days of dosing. 12 lead EKGs were obtained at screening, pre-dose, daily for 3 days, and then weekly for 4 weeks if QT prolongations >10ms were seen during the dosing period. A high proportion of subjects (8%) were excluded at screening from the cohort study based on QTc Bazett (QTcB) prolongations greater than 500ms. The mean increase in QTc interval following dosing was 5-6% over baseline by QTcB, and 6-8% by QTc Fridericia (QTcF). Only 2 of 80 volunteers had a prolonged QTcF greater than 20% over baseline and, in both cases, this was observed on a single day during the 6 week follow-up period. By QTcF, there were 7 AEs for QTc prolongation (17.5%) in the 3 day group by CTC AE v4.0 (4 grade 1 and 3 grade 2) vs. 8 in the 2 day group (20%) (7 grade 1 and 1 grade 3). There were not significant differences in QT prolongation between treatment groups. In many cases, prolongation was present at baseline or clearly influenced by the confounding effects of fever, malaria and increased heart rate. Piperaquine as part of a DHA-piperaquine combination caused modest QTc prolongation at treatment doses over 2 or 3 days, and the effect was similar to what has been reported in other studies. Further evaluation of the potential for QTc prolongation, particularly with repeated dosing is needed, as is the epidemiology of acquired and congenital long-QT syndromes in this population, given the high rate observed in this cohort. PK-PD analysis will be presented once PK analysis has been completed.

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TRIAGE OF HIGH THROUGHPUT SCREENING RESULTS AGAINST BLOOD STAGE *PLASMODIUM FALCIPARUM*

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A 500 member natural product library was assayed for activity against blood stage *Plasmodium falciparum* using a SYBR Green I-based fluorescence (MSF) assay, as reported previously. The screen was performed at a single concentration (10,000 ng/mL); from these initial results, IC_{50}s were determined against three plasmodium strains (D6, W2 and C235) for 40 compounds of interest. Nine compounds that met specific potency criteria against three *Plasmodium* strains were advanced to further studies in our malaria drug discovery testing strategy. Our testing strategy for discovery of new anti-malarial compounds, the results of our *in vitro* campaign and progression of several key compounds into an *in vivo* blood stage mouse malaria model will be presented.

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THE NONSELECTIVE CYTOCHROME P450 INHIBITOR, 1-AMINOBENZOTRIAZOLE, DOES NOT PREVENT PRIMAQUINE-INDUCED HEMOLYSIS IN A HUMANIZED NOD-SCID MOUSE MODEL OF G6PD DEFICIENCY

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The 8-aminoquinoline drugs primaquine (PQ) and tafenoquine are critical to malaria elimination efforts given their anti-relapse and gametocidal activity. Realizing the full potential of these drugs is impeded by drug-induced hemolytic toxicity in glucose-6-phosphate dehydrogenase deficient (G6PDd) individuals. We are exploring methods to improve the safety of this drug class.We previously showed that 1-aminobenzotriazole (ABT), a nonselective irreversible inhibitor of multiple cytochrome P450s (CYP450s), blocked the causal prophylaxis of PQ in a *Plasmodium berghei* sporozoite challenge mouse model. ABT inhibits CYP450 isoforms to varying degrees by *N*-alkylation of the heme moiety. We subsequentlyinvestigated the effects of ABT in PQ-induced hemolysis in a humanized severe combined immunodeficiency (SCID) mouse model of G6PDd. In this model, PQ and other hemolytic drugs cause a dose-dependent hemolysis in non-obese diabetic (NOD)-SCID mice engrafted with human G6PDd red blood cells (RBCs). Mice engrafted with human A minus-type G6PD RBCs were orally dosed with 150 mg/kg ABT two hours prior to oral dosing with 6.25 or 12.5 mg/kg PQ for 3 days. Comparator groups were treated with the same doses of PQ and ABT alone. After 7 days, PQ alone caused a dose-dependent loss of human RBCs and increase in mouse reticulocytes. Hemolysis did not occur in A minus G6PDd humanized mice treated with ABT alone and pre-treatment with ABT did not block PQ-induced hemolysis. These preliminary results suggest that CYP-mediated metabolites of PQ may not be responsible for inducing hemolysis in G6PDd. We are confirming the effect of ABT on PQ-induced hemolysis in NOD-SCID mice and a mutant G6PDd mouse model. Studies are also underway to confirm no effect of ABT on non-CYP pathways. The results attained thus far in SCID mice, along with our previous results indicating that ABT inhibits PQ's malaria prophylaxis activity in mice, suggest that we may be able to develop a strategy for improving the therapeutic index of 8-aminoquinolines.

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POPULATION PHARMACOKINETICS OF PYRONARIDINE FOLLOWING ORAL PYRONARIDINE/ARTESUNATE TREATMENT IN HEALTHY AND MALARIA INFECTED SUBJECTS

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Artemisinin-based combination therapies (ACTs) are currently recommended by the World Health Organization as a first-line treatment for uncomplicated *P. falciparum* malaria. Pyronaridine is a promising ACT partner drug as shown by its efficacy against antimalarial-resistant strains. This has led to the development of a new antimalarial drug, pyronaridine/artesunate (PA) fixed-dose combination. The population pharmacokinetics of pyronaridine are reported using data from healthy (166) and malaria infected (642) subjects participating in nine Phase I-III clinical trials. Pyronaridine blood concentrations were measured using validated HPLC and LC-MS/MS methods. Non-linear mixed effect modeling approach was performed to investigate the influence of nine covariates (age, weight, body mass index, malaria infection, creatinine clearance, alanine aminotransferase, aspartate aminotransferase, gender, and ritonavir administration) on the pharmacokinetics of pyronaridine. A two-compartment model with first-order absorption and elimination adequately described pyronaridine pharmacokinetics. After the inclusion of statistically significant covariates, the population parameter estimates of apparent clearance (CL/F), central volume of distribution (V2/F), peripheral volume of distribution (V3/F), apparent inter- compartmental clearance (Q/F) and absorption rate constant (Ka) were 434 L/day, 907 L, 4,430 L, 1,120 L/day and 16.7 day^{-1}, respectively. The corresponding inter-individual variability estimates for CL/F, V2/F, V3/F, Q/F and Ka were 53.6%, 103%, 29%, 28.8% and 67.5%, respectively. The elimination half-lives of pyronaridine in healthy adult subjects, adult and pediatric malaria subjects were estimated to be 11.3, 13.2, and 9.6 days, respectively. Malaria infection was a significant predictor for V2/F and CL/F and weight was a significant predictor for V3/F and CL/F. Model evaluation results showed that the final model is robust, predictive and stable as confirmed by non-parametric bootstrap, visual predictive check and condition number.

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ESTIMATION OF THE COMPARATIVE HEMOLYTIC POTENTIAL OF PRIMAQUINE AND ANALOGS IN A HUMANIZED NOD-SCID MOUSE MODEL OF G6PD DEFICIENCY

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8-aminoquinolines (8-AQs), of which primaquine (PQ) is the prototype, are critical to malaria elimination efforts, given their anti-relapse and gametocidal activity. Clinical use of 8-AQs is impeded by hemolysis in glucose-6-phosphate dehydrogenase deficient (G6PDd) subjects. A critical need is animal models in which human G6PDd sensitivity to PQ can be reproduced. We have previously reported a model in which 8-AQs cause hemolysis in NOD-SCID mice engrafted with human G6PDd RBCs. In this model, direct comparison of hemolytic potency of analogs with PQ is feasible, but fails to take account of the comparative efficacy. We therefore developed an approach which first estimates the effective causal prophylactic (CP) dose in an ICR mouse *Plasmodium berghei* (ANKA strain) sporozoite challenge model. In the CP model, drugs are dosed for 3 days (-1, 0, and 1) with sporozoite inoculation given on d 0; untreated mice succumb to infection after several days. 8-AQs are highly effective in this model with an effective dose (ED)100 for PQ at 25 mg/kg/d given orally for 3 d. In the NOD-SCID hemolytic model, this CP ED100 dose of PQ yields loss of about 75% of human G6PDd (A- genotype) RBCs by d 7. To generate a normalized PQ hemolytic dose-response curve to compare efficacy and toxicity, we did a hemolytic dose response curve at multiples of the CP ED 100. Thus, a new 8AQ analog's ED100 can be established in CP model, then the hemolytic potential of PQ and the analog can be directly compared in the huRBC SCID model at equi-effective CP doses. NPC1161B, an 8-AQ development candidate which is a pure (-) (*R*) enantiomer, has a 3-d ED100 in the CP model of 0.5 mg/kg, while at this dose elicits no hemolysis in the huRBC SCID model. In contrast, NPC1161A, the opposite (+) (*S*) enantiomer, has a 3-d ED100 of 8 mg/kg, and at this dose it is indistinguishable from PQ with regard to hemolysis. This result indicates that hemolytic potential can be reduced without compromising efficacy in 8-AQs.

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THE PHARMACOKINETICS AND PHARMACODYNAMICS OF (+) AND (-) PRIMAQUINE ENANTIOMERS IN HEALTHY RHESUS MONKEY MODEL

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Primaquine (PQ), the standard drug for radical cure of relapsing malaria for more than 60 years is administered as a racemate of (+) and (-) enantiomers. Differential toxicities of the enantiomers, particularly in hemolytic potential and methemoglobin (mtHb) formation could represent a pharmacodynamic advantage. While Schmidt reported in 1977 that (-) PQ caused increased liver toxicity twice that of (+) PQ, hematologic effects are less well understood. We administered oral primaquine enantiomers to healthy Rhesus macaques in dose-rising fashion at 1.3 (human-equivalent treatment dosage), 3.0 and 4.5 mg/kg/day (n = 3 per enantiomer, 1 control). Drug was administered for 7 days with a 2-week washout period between doses, and cross-over of enantiomers at the high dose. Pharmacokinetic samples were collected on day 7 of dosing at 3.0 and 4.5 mg/kg, and effects on blood, liver and kidneys were assessed. There was little appreciable hemolytic activity at any dose, and no effect on renal function. (+) PQ showed more consistent rises in mtHb, but only 1/6 animals had substantial mtHb formation (>10%), and this was observed in the same animal with both enantiomers. (-) PQ caused a reversible hepatotoxicity > 10x the upper limit of normal in 2 of 3 animals at 4.5 mg/kg. Effects of the enantiomers on both mtHb and liver function were plasma concentration-independent for the parent compound or the carboxyprimaquine metabolite which formed at 3-5x higher concentration with (-) compared to (+). There was no enantiomeric interconversion *in vivo*. In healthy Rhesus, primaquine enantiomers show divergent toxicity patterns, with hepatotoxicity associated with the (-) form, while the (+) form seems to exert more red cell oxidative stress, as reflected in mtHb generation. The relevance of the liver injury and mtHb formation for PQ clinical use are not clear, but the findings suggest that the two enantiomers have different patterns of metabolism and disposition.

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STANDARDIZED, HIGH-THROUGHPUT ANALYSIS OF *IN VITRO* ANTIMALARIAL SUSCEPTIBILITY DATA WITHIN THE WORLD WIDE ANTIMALARIAL RESISTANCE NETWORK (WWARN)

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The mission of the Worldwide Antimalarial Resistance Network (WWARN) is to provide the comprehensive, timely, quality-assured intelligence needed to track the emergence and spread of antimalarial drug resistance. *In vitro* testing remains a central pillar of antimalarial efficacy surveillance since drug susceptibility in parasites isolated directly from patients (termed ex vivo testing) is largely independent of clinical factors and hence provides current information that complements clinical assessment of drug efficacy. Moreover, isolates can be assessed to determine responses to each component in a combination therapy or to alternative drugs not in use in that location. WWARN's *In vitro* Module aims to enhance the quality, quantity and geographic extent of these ex vivo data available to the malaria community via a global data repository. In order to accommodate variations in analytical approach used by different centres, WWARN deals exclusively with primary (raw) data, allowing characteristics of methodology to be understood and analyses to be undertaken via a standardised approach. For ex vivo studies, the primary data are the output from assessment of drug effects on an individual isolate for a single drug. Here we describe the development, validation and application of data analysis tools that allow calculation of standard susceptibility parameters via non-linear regression. Data that meet prospective quality criteria can be displayed on the online WWARN Explorer map and entered into pooled analyses incorporating clinical, molecular and pharmacokinetic data.

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GENETIC DIVERSITY OF *PLASMODIUM FALCIPARUM* SARCO(ENDO)PLASMIC RETICULUM CA^{2+} ATPASE (PFSERCA) IN GREATER MEKONG SUBREGION: IMPLICATIONS FOR MALARIA CONTROL

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Recently, artemisinin combination therapies (ACT) to treat *Plasmodium falciparum* malaria have been widely used in endemic malaria endemic countries. Although the mechanism of artemisinin resistance was not clear, the *Plasmodium falciparum* Sarcoplasmic/Endoplasmic Reticulum Ca2+ ATPase (PfSERCA) has been speculated to be the target of artemisinins and thus a potential marker for resistance. Here we sequenced Pfserca gene (serca) in 213 samples from the Greater Mekong Subregion (GMS) and identified 23 SNPs, of which 13 were newly reported and 13 resulted in amino acid substitutions. Most SNPs (17/23) detected in our samples were clustered within the cytoplasmic domains of the protein. No isolates showed point mutations at codons S769N or L263E, which were reported to be associated with decreased sensitivity to artemether *in vitro*. We analyzed a worldwide sample collection of 862 *P. falciparum* isolates (19 populations from Asia, Africa, South America and Oceania) for the Pfserca gene (72 SNPs from around 3600 nucleotides per isolate). Of 110 nucleotides haplotypes observed, the ancestral haplotype (441 samples) was present in 16 populations and it was the predominant haplotype in samples from Africa, Asia and Oceania. The reference sequence haplotype (3D7) (54 samples) is the second most common haplotype, present in 9 populations from all four continents. The dN/dS ratios were below one, indicative of purifying selection. Molecular evolution studies did not detect significant departure for most of the populations. These results suggest further studies are needed to assess genetic diversity of serca in malaria endemic regions and to evaluate its usefulness in monitoring ACT sensitivity.

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MOLECULAR MARKERS OF ANTIFOLATE RESISTANCE IN *PLASMODIUM FALCIPARUM* ISOLATES FROM LUANDA, ANGOLA

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Plasmodium falciparum malaria remains a leading health problem in Africa and its control is seriously challenged by drug resistance. Although resistance to the sulfadoxine-pyrimethamine (SP) is widespread, this combination remains an important component of malaria control programs as intermittent preventive therapy (IPT) for pregnant women and children. In Angola, resistance patterns have been poorly characterized, and IPT has been employed for pregnant women since 2006. The aim of this study was to assess the prevalence of key antifolate resistance mediating polymorphisms in the *pfdhfr* and *pfdhps* genes in *P. falciparum* samples from Angola. Sixty-one *P. falciparum* samples collected in Luanda, in 2007, were genotyped by amplification and DNA sequencing of the *pfdhfr* and *pfdhps* genes. The most prevalent polymorphisms identified were *pfdhfr* 108N (100%), 51I (93%), 59R (57%) and *pfdhps* 437G (93%). Resistance-mediating polymorphisms in *pfdhps* less commonly observed in West Africa were also identified (540E in 10%, 581G in 7% of samples). This study documents a high prevalence of 4 *P. falciparum* polymorphisms that predict a significant level of antifolate resistance in Luanda. Further, a minority of samples contained additional mutations predicted to mediate high-level resistance. We concluded that the use of SP for IPT may no longer be warranted in Angola.

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WWARN MOLECULAR SURVEYOR: MAPPING THE GLOBAL DISTRIBUTION OF ANTIMALARIAL RESISTANCE MUTATIONS IN *PLASMODIUM FALCIPARUM*

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The spread of drug resistance has rendered sulfadoxine pyrimethamine (SP) ineffective for treatment of *Plasmodium falciparum* malaria in much of the malaria-endemic world. However, SP is still effective as first -line therapy in some regions, and has been shown to be efficacious as intermittent preventive therapy in pregnant women (IPTp), infants (IPTi) and children (IPTc) in many African regions with high malaria transmission intensities. SP-IPTp is being widely implemented in sub-Saharan Africa.

The World Health Organisation recently recommended SP-IPTi in African regions where SP resistance does occur but only within a specific resistance threshold. As patterns of SP use change with implementation of SP-IPT along with increased uptake of artemisinin combination therapies for curative treatment, patterns of resistance must be continuously monitored. Hundreds of studies have investigated the presence of SP resistance-conferring mutations in the dhfr and dhps genes of *P. falciparum*. In an effort to collate and display these published data freely, the WorldWide Antimalarial Resistance Network (WWARN) has created a collaboration to produce interactive web-based maps of global SP resistance mutations called Molecular Surveyor. Molecular Surveyor allows users to view the number of samples positive for a specific resistance marker along with the total number of samples tested, year of sample collection, location of survey and a web link to each original publication. Users may filter the map by drug, resistance marker, time span and sample size of surveys. Molecular Surveyor is currently driven by data from literature reviews conducted by three groups of investigators from four continents. The database has 2,460 data points from 525 sites around the globe. Future versions of Molecular Surveyor will map the distribution of other resistance markers such as pfcrt and pfmdr1.

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SURVEILLANCE OF MOLECULAR MARKERS OF SULPHADOXINE-PYRIMETHAMINE RESISTANCE IN GHANA AFTER THE CHANGE OF MALARIA TREATMENT POLICY

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In Ghana, sulphadoxine-pyrimethamine (SP) is used as intermittent preventive treatment in pregnant women (IPTp) since 2005. Before then, it was the second-line drug for the treatment of uncomplicated malaria. SP is an over the counter drug for IPTp and thus may be available for use by others. Drug pressure enhances the spread of resistant parasites and it is therefore imperative to monitor molecular markers of *Plasmodium falciparum* antimalarial drug resistance for early detection of the development of resistance. This information in addition to in-vivo and in-vitro assessment of drug resistance is crucial for treatment policy amendment and for non-immune travelers in their choice of prophylactic antimalarials. We therefore characterized mutations in *P. falciparum* dihydrofolate reductase (dhfr) and dihydropteroate synthase (dhps) for sulphadoxine- pyrimethamine (SP) resistance after the change in treatment policy in Ghana. 738 filter paper blood blots collected from 2005-2010 from children aged 6-59 months with uncomplicated malaria presenting to 9 existing sentinel sites for monitoring antimalarial drug resistance in Ghana were analyzed. PCR followed by restriction length polymorphism (RFLP) analysis was used to characterize mutations at codons 51, 59, 108, 164 of the dhfr gene and codons 436, 437 and 540 of the dhps gene. Data analysis included the determination of the prevalence of the mutations and the trend over the years. The overall trend showed an increase in the prevalence of the mutations from 2005-2010. The prevalence of the dhfr triple mutant (51, 59 and 108) was 29% for 2005-2006, 46% for 2007-2008 and 54% for 2010. No dhfr 164 mutant was observed in all the samples. The dhps double mutant (437 and 540) was 0.42%, 0.24% and 1.12% respectively for 2005-2006, 2007-2008 and 2010. For dhps double mutant (436 and 437) was 43%, 45% and 38% for 2005-2006, 2007-2008 and 2010 respectively. High prevalence of the 437 mutation (range: 59%-80%) was observed compared to low prevalence of the 540 mutant (0.24%-1,12%). The quintuple mutant (dhfr 51, 59, 108 and dhps 437, 540) was only observed in one of the 2010 samples. The study shows the effect of the continuous use of SP which enhances the selection and spread of resistant parasites in the country. Whether the use of SP alone for IPTp is justified will be further discussed.

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AMPLIFIED PFMDR1 COPY NUMBER IN *PLASMODIUM FALCIPARUM* FROM ARTEMETHER-LUMEFANTRINE TREATED PATIENTS IN EASTERN SUDAN

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Malaria remains a global health challenge being responsible for one million deaths annually world-wide. The dissemination of drug resistance from focal areas where it evolved to almost all endemic areas has fuelled increased disease burden and lead to recent changes in drug policies in sub-Saharan Africa. The pfmdr1 gene in *Plasmodium falciparum* is a candidate marker of drug resistance to antimalarial drugs with different mechanisms of action. Point mutations in this gene modulate susceptibility to 4-aminoquinolines (CQ), arylaminoalcohols (lumefantrine) and more recently artemisinins derivatives (artemether) both in clinical isolates and culture adapted laboratory clones. Increased copy number of pfmdr1 has been observed in clinical isolates from Thailand and Cambodia where mefloquine resistance is wide spread. However, there have been two reports of the occurrence of pfmdr1 amplification in Africa. The first report of a clinical isolate from Gabon collected in 1995 and the second more recent report of an isolate from Kenya collected in 2004. In this study, 74 pre-treatment and 14 post treatment isolates collected from eastern Sudan during an artemether-lumefantrine clinical trial in 2006 were investigated for amplification of the pfmdr1 gene employing a duplex hydrolysis probe qPCR assay. Fifty-seven pre-treatment isolates gave interpretable results. In these there was an increase in copy number in 10.5% of samples with a mean in this group of 1.89 copies range (1.63 to 2.33). Interestingly the isolate with the highest copy number 2.33 failed treatment on D14, but the recurrent isolate had only one copy of the gene. The second isolate with copy number of 2.07 successfully cleared parasitaemia by microscopy, however it harboured PCR detectable parasites on D14, and these also had one copy of the gene. Isolates with increased copy number were predominantly of the YFSND haplotype (N86Y, Y148F, S1034C, N1042D, D1246Y). It is clear that pfmdr1 amplification has spread to Africa in a different genetic background than that observed in South East Asia. This highlights the significance of monitoring genetic polymorphisms in pfmdr1 in the ACT era.

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INVESTIGATING THE POOR PARASITOLOGICAL PERFORMANCE OF ARTEMETHER-LUMEFANTRINE AGAINST MALARIA IN BUKOBA VILLAGE, MAYUGE DISTRICT, UGANDA

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Artemisinin-based combination therapies (ACTs) are the first-line treatments for home-based management of *falciparum* malaria in many countries. In Uganda, artemether-lumefantrine (AL) is the ACT promoted by the Ministry of Health. There are, however, reports of reduced ACT efficacy on the Cambodian-Thai border and we have encountered potential poor performance of AL in Uganda. To investigate the AL efficacy more carefully, we conducted a study in Bukoba village on Lake Victoria where prevalences of *Plasmodium falciparum*, *P. malariae* and *P.*

ovale sp. in children under six were 88%, 16% and 8% respectively. At baseline 188 children were screened using four malaria rapid diagnostic tests (RDTs), including HRPII and LDH targets. Children who were positive (n=176) by the HRPII test were treated with AL. On Day 7 children were retested with LDH tests and all positives were retreated (n=30) with AL. On Day 17 testing was repeated and RDT positives (n=20) were retreated with oral quinine. Of these, five still showed parasitaemia seven days later. Genotyping of the parasites to distinguish between recrudescences and new infections is ongoing. In addition, we are investigating mutations in the *P. falciparum* multidrug resistance 1 locus (*pfmdr1*) and chloroquine resistance transporter gene (*pfcrt*), among other genes, which could potentially be involved in the reduced drug response. Our results suggest that AL treatment is not entirely effective against malaria in this rural setting and we hope to gain an insight into the molecular basis for this.

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DRUG RESISTANCE IN *PLASMODIUM FALCIPARUM*: IDENTIFICATION OF A NOVEL ADDITIONAL PFCRT LOCUS WHICH LACKS INTRONS AND IS TRANSCRIBED *IN VIVO*

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The chloroquine resistance transporter located on the membrane of the parasite digestive vacuole is believed to influence access of chloroquine transport to its target. Data from transfection studies has proposed a role for polymorphisms in the pfcrt locus in mediating susceptibility to various antimalarial drugs, particularly the 4-aminoquinolines chloroquine and amodiaquine. Genetic studies have shown a considerable number of single nucleotide polymorphisms in this gene from various parts of the world that have been associated with susceptibility and resistance to chloroquine and more recently artemisinin combinations, particularly artemether-lumefantrine. In this study we sequenced amplified pfcrt cDNA from transcripts taken directly from clinical isolates collected during studies of artemether-lumefantrine efficacy in Eastern Sudan. Novel non-synonymous polymorphisms were observed in transcripts encoding PfCRT. Unexpectedly, from a handful of patients in one Sudanese site, splicing variants with 2 exons missing were observed. Further investigations of genomic DNA revealed complete removal of introns, and at least 2 exons, from the 3' end of a previously undescribed genomic pfcrt-like locus in these isolates. A similar phenomenon was not observed among *in vivo* cDNA sequences from 50 isolates collected from Tanzania in the same year, although novel SNP were also evident in this group of parasite isolates. A normal intron-interrupted pfcrt locus appears to also be present in the same isolates. The possible biological significance of our findings will be discussed in the context of a substantial shift in drug selection pressure away from aminoquinolines in east Africa.

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IMPACT OF SEASONAL INTERMITTENT PREVENTIVE TREATMENT IN CHILDREN: MOLECULAR MARKERS OF RESISTANCE IN THREE HEALTH DISTRICTS IN SENEGAL

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A study was conducted by the department of parasitology, IRD and the LSHTM in three health districts in central Senegal. Seasonal intermittent preventive treatment that three administrations of the combination sulfadoxine-pyrimethamine and amodiaquine is performed each month during the three month period of transmission of malaria in children under 5 years. So we evaluated mutations in pfdhfr and pfdhps genes resistance markers of *P. falciparum* to SP and pfmdr1 and pfcrt genes, markers of resistance to chloroquine and amodiaquine. This study is from January 2008 to January 2011, takes place in three sites with seasonal transmission (Mbour Bambey and Fatick). It was performed in a gradual manner with 9 areas in the first year, 27 in the second, 45 in the third year. So preliminary results showed a prevalence of mutations in codons 51, 59 and 108 gene pfdhfr turning around of 80%. Results obtained in this study showed that there was no significant difference between intervention areas and control areas. IPTc has no effect on mutations in the pfcrt, pfdhfr, pfdhps and Pfmdr genes. And as far as no mutations in codons 164 and 540 of the pfdhfr and pfdhps genes respectively. This could be a strong signal to the central question about the durability of the use of SP.

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USE OF MALARIA IMPORTED CASES IN NON-ENDEMIC COUNTRIES TO ASSESS THE RETURN OF CHLOROQUINE SUSCEPTIBILITY IN SENEGAL

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In compliance with WHO recommendations, African countries have discontinued chloroquine (CQ), due to widespread resistance, and now promote artemisinin-based combination therapy (ACT), as first-line treatment for uncomplicated malaria. Faced with an average CQ treatment failure of 25%, Senegal changed its national malaria policy in 2003 from CQ to amodiaquine (AQ) + sulfadoxine-pyrimethamine and in 2006, to AQ+artesunate. Studying travelers returning from a specific region and collectively infected by a wide variety of strains of *Plasmodium falciparum* (Pf), could be an effective tool for detecting the evolution of resistance onsite. The aim of the study is to describe the evolution of CQ resistance in Senegal after a decrease of drug pressure, through imported cases from the country. The study was conducted by the Malaria National Reference Centre in France in collaboration with the WorldWide Antimalarial Resistance Network (WWARN). We collated *in vitro* response of reference and clinical isolates for CQ with a standard 3H-hypoxanthine test and prevalence of pfcrt K76, the molecular marker for CQ susceptible Pf malaria. In total, 215 clinical isolates were tested from 1996 to 2004 and 348, from 2005 to 2010. The prevalence of the CQ susceptible pfcrt genotype increased from 35% (74/215) to 51% (177/348), respectively before and after 2004 (p<10-3). It tended to increase from 1996 to 2010 (Trend test, p=0.02). Mean estimated 50% inhibitory concentration (IC50) for CQ was 127nmol/L (95% confidence interval [CI], 105 to 150) in 1996-2004 versus 83nmol/L (95% CI, 71 to 94) in 2005-2010 (p<10-3) and the IC50 isolate/Pf3D7 ratio was 5.64 (95% CI, 4.49 to 6.81) (threshold for resistance in this laboratory = 3) versus 2.90 (95% CI, 2.38 to 3.43) (p<10-3), respectively. Thus, a reduction in resistance to CQ following the official withdrawal in 2003 was observed in imported cases from Senegal.

A return of the CQ susceptibility is consistent with observations in Malawi, even if the studied period after CQ withdrawal, was shorter in Senegal than in Malawi (7 years versus 12 years).

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CHLOROQUINE RESISTANCE IN HAITI: LESSONS LEARNED FROM IMPORTED CASES

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On 12 January 2010, an 7.0 magnitude earthquake placed many displaced residents and emergency responders at substantial risk for malaria In Haiti. In the following weeks, US military personal engaged in the relief operations were hospitalised with *P. falciparum* malaria resistant to chloroquine (CQ), first-line treatment for uncomplicated malaria on the island. We investigate if malarial drug resistance profiles (genotypic and phenotypic) of P.f strains detected in imported malaria cases from Haiti could have raised an early warning of chloroquine-resistance prior to this catastrophe. Retrospective data collected in 1988-2010 from malaria surveillance centres in France and in Toronto were studied. *In vitro* response of reference and clinical isolates to CQ and the pfcrt 76T molecular marker for CQ susceptibility were studied in patients with recent travel history in Haiti. In total, 40 P.f isolates were obtained from clinical cases imported from Haiti. Among 3 Canadian clinical isolates, all were pfCRT K76 (wild-type genotype) but after *in vitro* adaptation of two, mutant pfCRT 76T was found. The 50% inhibitory concentrations (IC50) were high for both, 506nM and 708nM. The ratio IC50 isolate/Pf3D7 (a CQ susceptible clone) was respectively 19.5 and 27.2. Among 37 French clinical isolates, all were pfCRT K76 and 29 were analysed ex vivo with a mean IC50 for CQ of 27nM (95% confidence interval [CI], 13.5 to 43.4) and a mean of 3D7 ratio of 1.05 (95% CI, 0.58 to 1.74). Three and 16 patients in Canada and France, respectively, were infected during and after the earthquake in Haiti. Although the few cases observed among travellers are probably not a representative sample, we did not detect early sign of resistance of CQ in Haiti but mixed population of parasites in 2 Canadian samples. It is likely that resistant parasites circulate within a majority of susceptible isolates and that the earthquake created the necessary epidemiological conditions, i.e. population movement, inappropriate shelters, which have contributed to evidence the resistance.

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PERCEPTION, KNOWLEDGE AND PRACTICES ON MALARIA OF THE CONGOLESE POPULATION AND ITS WILLINGNESS TO PARTICIPATE IN CLINICAL TRIALS

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In Republic of Congo, malaria remains a major public health problem. Interventions like bed nets and free treatment with artemisinin-based combination therapies (ACTs) have been introduced in the country without exploring the social dimensions. This is the first pilot study to investigate the perception, knowledge and practices of the Congolese population on malaria and to evaluate its willingness to participate in clinical trials. One hundred informants (65 men, 35 Female) aged 18 to 63 years in Brazzaville were randomly selected on the sampling frame of 2007 census. Sixty five percent and 82% of respondents reached the secondary school education and were economically active, respectively. About two thirds of informants (60%) identified the mosquito as the causative agent of malaria and 47% indicated cleaning the environment for a successful fight against malaria. More than 80% of respondents knew that the health center or hospital was the place for appropriate treatment of malaria while 21% were in favor of self-medication for sick children before consulting a clinician. Sixty-two percent (62%) gave a clear definition of a clinical trial. Willingness to participate in clinical trials was low for vaccines (37%) and higher (62%) for drugs. There was a positive relationship between level of education and "to have heard about clinical trial" and also between level of education and the trust in respect of the confidentiality of data collected by the investigators. In conclusion, the study shows that even though no clinical trial has been carried out in Congo-Brazzaville, the population of Brazzaville was willing to participate in malaria drug trial. Media like TV and radio may have played an important role to increase knowledge about malaria and clinical trial. Issues on risks to participate in clinical trials that needs to be carefully considered by investigators designing clinical trials were highlighted by the population.

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MALARIA ACTIVE CASE SURVEILLANCE: THE CASE FOR LUSAKA URBAN HEALTH FACILITY PROFILING

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Malaria parasite prevalence in Lusaka District, Zambia is extremely low, and confirmed cases are minimal. In response, Zambia's National Malaria Control Program (NMCP) is transitioning its Lusaka intervention strategy from universal IRS and ITN coverage, to more targeted, focal interventions. Among the interventions will be community-based, active infection detection, wherein response teams screen-and-treat neighborhoods of passively detected cases. This is a critical transition point and success will largely depend upon surveillance. Timely, accurate data are necessary to identify infection foci and to quantify the relative impact of this new focal strategy against traditional universal coverage. In order to prepare baseline data to monitor impact, the NMCP collected retrospective malaria data from all 26 urban health centers back to 2004. Surveillance teams visited each clinic and coordinated with the clinic in-charge and data personnel to review clinic and laboratory registers. These data complement the Health Management Information System which captures routine data ranging from disease to service delivery information for clinics and hospitals in all districts. Data elements collected include: total consultations, malaria cases, total tested by RDT and microscopy, total positive by method and the stocks of ACTs dispensed from the pharmacy. A preliminary review of these data has shown interesting trends: although there is very low level of malaria transmission in the district, there remains a relatively high usage of ACTs -- individuals with fevers are often presumptively

treated versus parasitologically confirmed. These data continue to be collected prospectively, on a monthly basis for consistent monitoring of intervention strategies. The profiling exercise provided reliable baseline data for monitoring the future impact of Lusaka District interventions. It also revealed some trends e.g. over-prescription of ACTs, that may warrant additional facility-based interventions. Both points underscore the critical need for accurate and timely surveillance data, particularly as Lusaka District continues towards malaria elimination. The facility profiling exercise has proven a useful method to monitor the successes of surveillance and intervention enhancements, as well as redirect efforts in a data-driven manner, especially during the rollout of malaria active infection detection.

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REACTIVE CASE DETECTION IN A RURAL AREA IN ZAMBIA - YEAR 2 OF TARGETING ASYMPTOMATIC RESERVOIRS OF *PLASMODIUM FALCIPARUM* MALARIA DURING THE LOW TRANSMISSION SEASON

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Asymptomatic foci of malaria may be important reservoirs of parasites and are difficult to detect. A targeted active case detection system using symptomatic cases that present at rural health centres during the low transmission season in the Choma and Namwala districts was conducted in 2009. Results of this pilot study indicated that this system appears to be able to identify more cases of asymptomatic asexual parasite and gametocytes carriers than controls. This study was repeated from July to November 2010 with an increased sample size to further validate the findings. The residence of all rapid diagnostic test (RDT) confirmed cases of malaria from participating rural health centres (RHC) during the low transmission season were located. Two controls per case were selected from the registries of the same RHC on the same day where the case presented. All consenting residents of the selected homesteads completed a questionnaire and were screened for malaria using microscopy, RDT, and nested-PCR. RT-PCR was conducted on all PCR positive samples to detect sexual stage parasites. In total, 218 and 408 participants residing in 40 case and 70 control homesteads, respectively, were screened. Unadjusted analysis resulted in case homesteads having 1.4% prevalence of malaria by RDT and 1.2% in control homesteads (Fisher's Exact p-value = 1.0; OR=1.11 95% CI=0.17-5.76). However, when data were stratified by proximity to permanent rivers, those living greater than 1km away from a river had 2.2 greater odds of being RDT positive for malaria compared to controls (95% CI: 0.29-16.74; Fisher's Exact p-value=0.38). The odds ratio for those living within 1km of rivers could not be calculated but the Fisher's Exact p-value was 0.51. The molecular analysis is ongoing.

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SYSTEMATIC REVIEW OF ANTIMALARIAL MASS DRUG ADMINISTRATION AS A TOOL TO REDUCE MALARIA BURDEN AND TRANSMISSION

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Mass drug administration (MDA), empiric administration of a therapeutic antimalarial regimen to an entire population or well-defined sub-population at the same time, has been an historic component of many malaria control and elimination programs, but is not currently recommended. This strategy is now being re-considered, but data on MDA effectiveness is lacking. We conducted a systematic literature review on the impact of MDA on the incidence or prevalence of asexual parasitemia, gametocytemia, anemia, clinical illness, and mortality. We identified MDA studies in Cochrane Infectious Disease Group Specialized Register, Cochrane Central Register of Controlled Trials (CENTRAL), MEDLINE+, EMBASE, CABS Abstracts, LILACS, and recent conference proceedings. Two authors independently assessed each study for inclusion and abstracted relevant data. Of 2,617 studies identified, 33 met inclusion criteria: 24 before-and-after observational studies, 3 cluster-randomized controlled studies, 3 non-randomized controlled studies, and 3 controlled before-and-after studies. Study dates ranged from 1935 to 2008, and study region ranged from the Americas (6), to Asia (8) and Africa (19). Few studies were conducted in settings with baseline asymptomatic asexual parasitemia rates (PR) ≤5% (5) compared to settings with PR >5% (28). MDA treatment regimens varied: 4 studies included an artemisinin derivative and 11 studies included an 8-aminoquinoline. Following MDA, 24 studies measured asymptomatic malaria parasitemia prevalence, 13 parasitemia incidence, 3 anemia prevalence, 11 gametocytemia prevalence, 1 gametocytemia incidence, 4 mortality, and 12 MDA-associated adverse events. MDA alone was used in 15 studies and combined with other malaria control interventions in 19 studies. Impact results are currently pending. Although we identified many MDA studies, few were well-designed and rigorously conducted. Heterogeneity in study setting, antimalarial regimen, malaria co-intervention use and outcome assessed, hinders making policy decisions based on the current database. Further MDA studies are needed to better understand this malaria control strategy especially with the use of artemisinin-based combination therapies and 8-aminoquinolines.

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APPROACHING MALARIA ELIMINATION IN SWAZILAND: USE OF A PCR-BASED POOLING STRATEGY AND SEROLOGY IN A NATIONAL CROSS-SECTIONAL SURVEY

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Global interest in malaria elimination is high. To guide efforts, countries need accurate assessments of transmission. In low transmission settings, use of pooled polymerase chain reaction (PCR) testing has potential to improve sensitivity and efficiency, and serological data may clarify temporal and spatial trends. Using a stratified two-stage cluster design, a cross-sectional household Malaria Indicator Survey was conducted in 2010 in the malaria endemic region of Swaziland. Blood was collected by finger prick for rapid diagnostic testing (RDT) and on filter paper for pooled PCR and ELISA detecting antibodies to merozoite surface protein-1 ($MSP\text{-}1_{42}$) and apical membrane antigen-1 (AMA-1). Three of 4330 participants tested positive by RDT but negative by PCR. By pooled PCR, one *Plasmodium falciparum* and one *P. malariae* infection were identified among 4031 RDT-negative participants. The *P. falciparum*-infected participant reported recent travel to Mozambique. Compared to performing individual testing, PCR pooling reduced labor and consumable costs by 95·5%. Compared to older participants, seroprevalence among subjects less than 20 years of age was significantly lower (1·9% *vs* 11·7%, p<0.001). Seropositivity was associated with recent travel to Mozambique (OR 4·4, 95% CI 1·0 to 19·0, p=0.048) and residence in southeast Swaziland (RR 3·78, p<0·001). Low overall parasite prevalence and low seroprevalence in younger participants

suggests that recent interventions have been effective and that elimination for this sub-Saharan African country may be feasible. Future efforts should aim to prevent imported malaria, and investigate and target interventions to limited foci of transmission. Pooled PCR and ELISA should be considered in other low transmission settings where accurate surveillance is needed to guide and measure progress of elimination efforts.

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A SPATIAL DECISION SUPPORT SYSTEM FOR MALARIA ELIMINATION INTERVENTION MANAGEMENT

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Regional partnerships have been established to conduct Geographical Reconnaissance (GR) to define the spatial distribution of target populations for malaria elimination in selected provinces of the Solomon Islands (Temotu and Isabel Provinces) and Vanuatu (Tafea Province). The aim was to support long lasting insecticidal net (LLIN) distribution and focal indoor residual spraying (IRS) interventions. GR surveys were carried out using integrated personal digital assistant (PDA) / global positioning system (GPS) handheld units to rapidly map and enumerate households, and collect associated population and household structure data to support long lasting insecticidal net (LLIN) distribution and focal indoor residual spraying (IRS) interventions. Data were uploaded and analysed in customized spatial decision support systems (SDSS) to produce household distribution maps and generate summary information. Subsequent LLIN distribution and focal IRS interventions were coordinated in the three elimination provinces using the SDSS. Following completion of field operations, group discussions were conducted to review and evaluate the SDSS. 16,869 households were geo-referenced and mapped, with a population of 73,664, and 43,714 household structures were recorded. Overall, IRS and LLIN household coverage in target areas were 91.7% and 97.5% respectively. The SDSS provided a strategic tool for coordinating follow-up operations to maximise intervention coverage. An overall high acceptability of the SDSS approach for management of malaria elimination activities was reported. Regional geo-spatial approaches developed for data collection and frontline intervention management have provided an effective operational tool to support the scaled-up demands of malaria elimination in resource-poor Pacific Islands.

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TACKLING MALARIA FROM THE MARGINS OF TRANSMISSION THROUGH A CROSS BORDER MALARIA INITIATIVE BETWEEN MOZAMBIQUE, ZIMBABWE, AND SOUTH AFRICA

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The MOZIZA Cross Border Malaria Initiative is a collaborative partnership between Mozambique, Zimbabwe and South Africa to reduce malaria transmission along shared border areas. The MOZIZA region comprises nine districts: four in Mozambique; four in Zimbabwe and one in South Africa with a total catchment population of 2.3 million people. The malaria incidence in the MOZIZA region ranges from 122-393/1000; 27-335/1000 and 1.69/1000 population at risk in the districts of Mozambique; Zimbabwe and South Africa respectively. As Mozambique strives to halt transmission by scaling up interventions to universal coverage, Southern Zimbabwe and South Africa are embarking on malaria elimination campaigns to achieve zero local transmission. Regional collaboration among these nations becomes important to progressively draw the margins of malaria transmission north by coordinating, harmonising and synchronising interventions across country boundaries. The MOZIZA Malaria Initiative is aligned to the Global Malaria Action Plan and key strategies of World Health Organization and the Southern African Development Community, essentially to scale-up intervention coverage for impact; ensure sustained control and progress towards malaria elimination. A baseline study to identify intervention coverage rates for surveillance, case management, vector control and health promotion has been undertaken to identify gaps and demographic information on the population in the MOZIZA region. This paper describes the current malaria epidemiological situation in the MOZIZA districts and presents data on each of the key coverage indicators for universal coverage and makes recommendations where gaps have been identified.

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SPATIAL STRATEGIES FOR MALARIA CONTROL AND ELIMINATION IN AFRICA

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The intrinsic potential for malaria transmission in the absence of interventions varies across spatial scales from local hotspots through to country and continental differences. To assess both the potential for current and future tools to facilitate local elimination and the degree of effort required to sustain elimination within this heterogeneous landscape, we developed a high resolution continental-scale simulation model for malaria transmission in Africa. The model simulates *P. falciparum* transmission between *Anopheles* vectors and human hosts. Seasonality is incorporated via rainfall dependent mosquito dynamics and temperature effects on sporogony and adult mosquito survival. Spatial and seasonal variation in transmission was modelled at a 1x1km resolution by a carrying-capacity for larval habitats determined by climate, topography, land-use, population density and health service spending (as an indicator of development). Parameter estimates were obtained by fitting the model to age- and season-specific MARA prevalence data (~21,000 observations) from 3597 locations prior to wide-scale intervention (1975-2000). A high correlation (>75%) between observed and predicted PfPr2-10 was achieved, with the correlation rising to >85% at spatial scales over 50km. Scaling-up LLIN distribution across the continent from2000 onwards to the RBM goal of 80% coverage resulted in mean PfPr2-10 being approximately halved across Africa, with much greater decreases in low transmission areas and a consequent "shrinking" of the map. Adding yearly IRS resulted in a 2/3 drop in mean PfPr2-10, with local elimination predicted in locations in Kenya, Ethiopia and Southern Africa. However, these interventions were insufficient to eliminate in areas of intense transmission including West and Central Africa. Two novel interventions - a transmission blocking vaccine with high efficacy and coverage and GM mosquitoes with a strong drive system (X-shredders which disrupt the male/female sex ratio) - were successful in driving transmission to near elimination when added to a combination of high LLIN/IRS coverage, although as a single tool neither was sufficient. Further spatial strategies will be discussed.

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OPTIMIZING STRATEGIES FOR MALARIA ELIMINATION BY MATHEMATICAL MODELLING

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Unprecedented efforts are now underway to eliminate malaria. If they are perceived as failing current high levels of funding will probably not be sustained. It is imperative that methods are developed to use the limited data available to design and optimize site-specific, cost-effective elimination programmes. Mathematical modelling can evaluate different strategies much more rapidly than is possible through trial and error in the field and is being used to guide and inform current elimination efforts. We have developed a range of mathematical models to help optimize the use of antimalarial drugs for malaria elimination. Although initially developed for Cambodia, many of the findings apply in a range of transmission settings and are broadly relevant to malaria control programmes worldwide. In particular these models are being used to predict the relative impact of treatment and mass drug administration (MDA). Antimalarials considered include artemisinin combination therapies (ACT), atovaquone-proguanil and primaquine. The spread and impact of artemisinin and atovaquone resistance with different strategies are examined in detail. A variety of modeling approaches is used to maximize the robustness of findings. The various models will be presented and major results to date summarized. Key findings: 1) high coverage with ACT treatment can produce a long-term reduction in malaria but the impact of a single round of MDA is generally only short-term; 2) primaquine added to ACT reduces time to elimination and slows the spread of artemisinin resistance; 3) atovaquone-proguanil has similar efficacy to ACT when used for MDA; 4) low levels of artemisinin and atovaquone resistance have negligible impact on the efficacy of MDA as the cumulative selective pressure is generally lower than that of treatment; 5) parasite prevalence is a better surveillance measure for elimination programmes than numbers of symptomatic cases; 6) combinations of interventions are more effective than high coverage single interventions; and 7) sustained efforts are crucial for successful elimination.

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PARADIGM SHIFT WITH THE PROGRAMMATIC TRANSITION FROM REDUCING MORBIDITY AND MORTALITY TO THE INTERRUPTION OF TRANSMISSION

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During the past 50+ years, malaria control has emphasized the reduction of morbidity and mortality, and has therefore focused on non-immune subjects such as children < 5 or 10 years of age. In contrast, with the transition to an emphasis on the interruption of transmission, the individuals of greatest interest for malaria control are now likely to be older persons with prolonged asymptomatic parasitemias because they have acquired the semi-immune state. Other challenges in this transition are likely to include a need for: greater caution in the interpretation of negative rapid diagnostic tests (RDTs), the development of novel surveillance strategies for infected individuals and changes in the timing of control interventions. False-negative RDTs based on the detection of histidine-rich protein 2 (e.g., ParaCheck) from spontaneous deletion of the subtelomeric *hrp2* gene are likely to become more frequent with the lower multiplicities of infection found with less intense transmission. Because standard surveillance strategies such as prevalence surveys require increasingly large numbers of subjects as prevalence rates decrease below 1-3%, novel alternative surveillance strategies are likely to be necessary as malaria control improves and prevalence decreases. Finally, the timing of most seasonal interventions is during the peak of the transmission season when the numbers of cases and intensity of transmission are greatest. However, in order to interrupt transmission, it will likely be necessary to focus on the nadir of transmission during the dry season rather than its peak during the transmission season. In summary, moving from the reduction of morbidity and mortality to the interruption of transmission will require different conceptual and practical approaches that have not yet been considered by the majority of investigators and malaria control programs.

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A GLOBAL MAP OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY (G6PDD)

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A major challenge to the ambition of malaria elimination is the relapsing liver stage of *Plasmodium vivax*. Primaquine is the only licenced drug that kills these dormant forms, whilst also causing a significant risk of haemolysis in G6PDd individuals. Knowledge of the prevalence of this genetic condition is therefore essential to help maximise safe deployment of this key component of the malaria elimination toolkit. G6PDd-triggered neonatal jaundice is a further clinical and public health burden. Here, we present the first modelled, continuous global map of G6PDd prevalence. Extensive literature searches were conducted to assemble a database of representative community surveys reporting phenotypically diagnosed G6PDd rates. Surveys meeting a set of standardised inclusion criteria, including being spatially identifiable, formed the evidence base informing the sex-specific Bayesian geostatistical model developed to predict the global prevalence of G6PDd. Combined with high-resolution population density maps, estimates of the affected populations were derived. Both prevalence predictions and population estimates are bounded by credible intervals quantifying uncertainty in the predictions. This map could become integrated into the evidence-base informing malaria control and enabling the tailoring of sub-national policy towards primaquine. Two key limitations highlight the direction of future work required in this field. First, inconsistency between surveys due to the range of diagnostics used may introduce variability to the designation of "deficiency". This variability strongly supports calls for a standardised, field-deployable diagnostic kit. Second, interpretation of the map is limited by major knowledge gaps in the relationship between G6PDd types and primaquine sensitivity. To address this and advance our understanding of G6PDd phenotypic spatial variability, this global prevalence map will be supplemented with distribution maps of the underlying common molecular variants.

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REVEALING A *PLASMODIUM* RESERVOIR AMONG ASYMPTOMATIC INHABITANTS ON ZANZIBAR

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Many countries have recently experienced dramatic reductions in the devastating malaria burden due to wide-scale implementation of successful control measures such as rapid diagnostic tests (RDT), artemisinin based combination therapy, impregnated bed-nets and indoor residual spraying. Zanzibar was among the first regions in sub-Saharan Africa to implement these measures on a wide scale and as such

P. falciparum prevalence decreased to below 1%. This information has raised the ambition from malaria control to elimination! However, this does not rule out the presence of a remaining low density reservoir in the community i.e. parasitaemias below the detection limit of microscopy and/or RDT, which might enable continued malaria transmission. Our aim was to possibly reveal and describe such *Plasmodium* reservoir in the Zanzibar community. PCR screening for the presence of *Plasmodium* parasites was performed on 450 randomly selected blood samples collected on filter papers from a community based cross-sectional survey conducted in the North A district on Unguja Island and Micheweni district on Pemba Island in 2009. In this survey only one of 2423 persons was microscopy positive. The samples were pooled 9-by-9 and after Chelex-DNA-extraction possible parasites were detected by a highly sensitive Cytochrome B nested PCR amplification. Positive pools were re-extracted and analyzed individually for species identification. *Plasmodium* DNA was detected in 26/450 samples (5.8%). 19 were *P. falciparum* (4.2%) and the remaining were *P. malariae.* The prevalence was lower in children < 5 years old (0.9%), while it was higher in older children 5 - 14 years old (12.4%). The prevalence was 1.5% and 8.9% in the North A and Micheweni district, respectively. In summary, we revealed a larger community parasite reservoir than expected, especially in older children and on Pemba Island, which may constitute an important source of transmission in Zanzibar. These findings may have critical implications for future attempts to persue malaria elimination.

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NEW TOOLS FOR MALARIA SURVEILLANCE IN CAMBODIA

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In common with many countries, passive reporting of cases presenting at health facilities forms the mainstay of malaria surveillance in Cambodia. Through the national health information system (HIS), malaria case data are compiled monthly and reported at district level. A parallel system of passive case detection through village malaria workers (VMWs) also provides monthly data, although currently these are not included in HIS reports and, as with the HIS, do not capture self- or privately-treated cases. In addition, since 2004, periodic national malaria surveys have provided data on a range of malariometric indicators at community level. Together, these surveillance activities provide relatively robust, nationally representative data to support strategic planning and monitoring and evaluation. However, two recent developments in Cambodia have highlighted the limitations of these systems in terms of providing timely, spatially specific data suitable for facilitating targeted response at the local level. Firstly, evidence of *Plasmodium falciparum* resistance to artemisinin-based drugs has emerged along the Cambodia-Thai border and containing it requires a surveillance system capable of rapidly identifying and responding to the presence of drug-resistant parasites. Secondly, in March 2011, Cambodia launched a new national strategy to eliminate malaria by 2025. The success of this strategy will in part depend on the availability of detailed spatial data for stratification and real-time information on incident cases. To address these new challenges the Cambodian national malaria programme and partners are developing and testing a variety of novel surveillance approaches, including piloting systems to detect and respond to artemisinin resistant cases and new platforms for VMWs and health facility staff to report data by SMS. Parallel activities are also ongoing to enhance existing HIS and VMW reporting systems to provide spatially specific data to support detailed risk stratification. In this paper we provide an overview of these initiatives and review lessons learned so far in their implementation.

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ZANZIBAR - TOWARDS ELIMINATION?

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The Zanzibar Malaria Control Program (ZMCP) has implemented comprehensive, well integrated combined malaria control interventions, free of charge and with high coverage starting 2003. The main components are long lasting insecticidal nets (LLIN) and indoor residual spraying (IRS) against the vectors, rapid diagnostic tests (RDT) and artemisinin-based combination therapies (ACT) in all public health facilities for malaria case management. The ZMCP initiative has become a unique case study for potential malaria elimination from a malaria endemic area in sub-Saharan Africa. We have studied the respective uptake and overall impact of these interventions more closely in two districts of Zanzibar, North A and Micheweni up to 2010. The impact is assessed with regards to different parameters such as incidence of confirmed malaria cases, child mortality as well as community parasite prevalence. Triangulation of data from community based cross-sectional surveys, health facility records and vital statistics provide evidence of sustainable malaria control in Zanzibar to a level equivalent with malaria pre-elimination.

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IS ACTIVE MALARIA CASE DETECTION IN THE COMMUNITY ABLE TO INHIBIT LOW-LEVEL FOCAL MALARIA TRANSMISSION IN ZANZIBAR?

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Intensive malaria control interventions in Zanzibar, including indoor residual spraying, long-lasting insecticidal nets and artemisinin -based combination therapy have resulted in malaria pre elimination phase (prevalence below 1% in 2011). However, a number of transmission foci have been identified after implementation of a clinic-based passive surveillance system to gather weekly malaria notifications. In 2011 Zanzibar introduced a proactive case detection (pACD) effort to screen the population in transmission foci with the aim to find and treat asymptomatic malaria cases and reduce transmission by decreasing the parasite prevalence in the population. Two small geographic areas were selected, Area-1 with high seasonal transmission (64 km2) and Area-2 with sustained perennial transmission (28 km2). In Area-1 the entire population was tested using a HRP2-based rapid diagnostic test (RDT) and assessed for current fever. In Area-2 all children ≤15 yrs were tested using the same procedures. Screening posts were positioned within the targeted villages over a 3-day period in mid-May 2011, just prior to the predicted increase in seasonal transmission. Confirmed malaria cases were treated with artesunate-amodiaquine according to national guidelines. A total of 6,276 (83%) of the targeted population was screened (83.4% in Area-1 and 83.3% in Area-2). Screening in Area-1and -2 yielded 13 and 64 RDT-positive cases, respectively, with a positivity rate of 0.1% among

residents <5 years of age in both areas. Residents older than five years had a positivity rate of 0.4% and 1.9% in Area-1 and -2, respectively. Variation in village-level positivity rates was detected (0.2-0.6% Area-1 and 1.0-2.7% Area-2). Data regarding clinical symptoms are being analyzed. A second screening session is planned in the first week of June 2011. The community participation in Zanzibar's first pACD effort was high and yielded 77 malaria cases outside of a clinic setting. Malaria cases with potential to perpetuate transmission were identified and treated successfully. Effects of the first and second screening activity on reducing malaria incidence in these communities will be carefully monitored through the existing weekly surveillance system.

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MODELING FOR MALARIA ELIMINATION IN A VARIETY OF TRANSMISSION SETTINGS

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A model is developed for planning malaria elimination in a variety of settings, including variation in baseline transmission intensity, seasonality, and vector population ecology and behavior. Rather than single interventions, the effects of combined interventions are studied for their effects alone and in combination with other interventions. Vector control measures such as insecticide-treated nets (ITNs) and indoor residual spraying (IRS) are studied, along with other forms of vector control such as larval control. These vector control measures are combined with potential pre-erythrocytic and transmission-blocking vaccines. In addition, we include the effects of drug treatments and distributions and study different modalities of distribution. Metrics include reductions in EIR, reductions in detected parasitemia, and reductions in true prevalence. Potential effects of vector control interventions depend strongly on vector ecology and behavior, and the distribution of local species can change in response to interventions. In moderate to high transmission settings, vaccine efficacy depends on the extent to which other interventions reduce baseline transmission. We also study the potential impact of improved diagnostics on test and treat drug administration results. Sensitivities of results to system parameters, vector model parameters, disease model parameters, campaign coverage, and basic model assumptions are explored and uncertainties are quantified. The Garki Project is then modeled retrospectively, as it actually happened, and redone with potentially available interventions.

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ELECTRONIC DATA CAPTURE AND REPORTING METHODS FOR INDOOR RESIDUAL SPRAYING (IRS) ACTIVITIES IN ZAMBIA

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Indoor residual spraying (IRS) along with long-lasting insecticide treated nets (LLIN) form the mainstay of vector and subsequently malaria control throughout sub-Saharan Africa. Zambia has invested heavily in IRS over the past decade and now boasts coverage levels in excess of 35% in urban/peri-urban settings contributing to a significant reduction in national malaria parasitemia from 22% in 2006 to 16% in 2010. Historically, IRS field operators recorded each sprayed house, along with a few limited data elements as a single line item on a paper form. Supervisors would then manually aggregate the data before entering it into a spreadsheet for reporting. Each individual spreadsheet would then be methodically cut-and-pasted into a master spreadsheet document. This slow, labor intensive and error-prone system was only able to collect a limited set of data. To address this issue, an electronic data capture solution has been developed and piloted for rapid collection and dissemination of IRS data. In our pilot study, IRS operators are individually equipped with a personal digital assistant (PDA) that guides them through collecting necessary data elements including GPS coordinates for every structure, spray application details, LLIN usage and previous spray history. Validation rules built into the software ensure that only valid data are entered. Supervisors can review these data at the end of each day, ensuring that data are accurate. Datasets are periodically exported for timely reporting to the district/provincial/central level(s). Importantly, there is no aggregation of data allowing field observations to be specifically analyzed. This feature allows rapid identification of areas of low spray coverage requiring additional IRS mop-up operations. This robust and expanded data collection method allows fine spatial mapping of spray activities to ensure that IRS applications are as effective and efficient as possible.

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ADAPTIVE CHANGES IN MALARIA TRANSMISSION DURING SCALED UP INTERVENTIONS IN SOUTHERN ZAMBIA

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As endemic countries scale up vector control and artemisinin-based combination therapy (ACT) interventions against malaria, widespread reductions in disease burden have been observed. A number of countries are now aiming for possible local or regional malaria elimination, including those in southern Africa, which are located towards the natural fringes of transmission. As intervention coverage is never total, and typically prioritizes vulnerable "non-immune" groups, we examined cross-sectional microscopic and sub-microscopic malaria parasite rates in the resident population of a 2000km^2 vicinity around Malaria Institute at Macha, from 2005-2009. Our data showed low-level microscopy-positive asymptomatic carriage spanning across all ages from less than 5 (3%) to over 65 years (2%). PCR screening showed even higher subclinical parasite rates, peaking in age-groups 10-14 (18%), 35-39 (21%) and 60-64 years old (15%). Furthermore, our data point to a temporal shift of patent gametocytaemia to older ages generally less protected by ITNs. Recent entomological surveys from Macha also suggest that the principal local vector, *Anopheles arabiensis*, is changing behaviour to bite during early evening hours, before bed-time. These changing epidemiological features, as the malaria parasite and vector adapt to interventions, will be key to guiding rational targeting for the next step to achieve malaria elimination. With the resilient scourge prevailing in significant segments of resident communities as asymptomatic and often low-grade, sub-microscopic infections, a spectre of possible resurgence may be looming. A detailed understanding of the epidemiological significance of asymptomatic, especially sub-microscopic infections and their role in transmission is imperative, to aid in policy decisions on targeting for intervention in areas approaching pre-elimination.

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FINANCING THE MOVE TOWARDS MALARIA ELIMINATION THROUGH DOMESTIC RESOURCES IN SOUTH AFRICA

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Financing malaria elimination is a recurring commitment that requires long-term sustainability. As South Africa embarks on a malaria elimination campaign, mobilising resources has become a key priority to operationalise

scale-up of new activities and strengthening of existing interventions. After a comprehensive malaria programme review in 2009, followed by a 10 year retrospective epidemiological analysis of all available malaria information, South Africa's National Department of Health determined that the nation was ready to embark on a national malaria elimination campaign. Subsequently, a strategic plan for malaria elimination (2011-2018) was developed and appropriate interventions were determined over the eight-year timeline. Currently, domestic (government) funding accounts for nearly 99% of all malaria programmatic funding. To ensure sustainability of the malaria elimination campaign, additional government resources will be needed to bridge the funding gap between current control programmes and future elimination programmes. A comprehensive costing exercise of the malaria elimination strategic plan was undertaken and determined that South Africa will need to invest approximately 16 million USD annually above its current budget to completely fund the campaign. In addition to costing the malaria elimination strategic plan, large-scale expenditure reviews of current spending were conducted to determine if any money in current budgets could be re-programmed for elimination activities. A funding gap of approximately 10 million USD annually still persists and exploration of domestic resources to fill the funding gap is underway. This paper presents data on past and present expenditure within malaria control programmes and discusses and makes recommendations on how South Africa can domestically fund its malaria elimination campaign.

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COMPARATIVE ACQUISITION OF ANTIBODIES TO *PLASMODIUM FALCIPARUM* AMA 1 AND MSP 1-19 AMONG CHILDREN LIVING IN TANGA, NORTHEASTERN TANZANIA

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Despite the presently declining trend in most parts of the world, malaria still ranks high among causes of morbidity and mortality in the developing world especially sub-Saharan Africa. Naturally acquired immunity develops as a function of age and exposure to *Plasmodium falciparum* antigens and is in part, antibody mediated. Studies for comparative antibody acquisition to validate putative vaccine candidates are necessary and usually precede longitudinal studies and clinical trials of selected and further developed malaria vaccine candidate molecules. A cohort study was conducted in children aged between 6 months and 10 years in Tanga, Tanzania. A baseline cross-sectional survey was conducted at the beginning of the study. Indirect Enzyme-Linked Immunosorbent assay (ELISA) was performed on collected blood samples to determine comparative natural acquisition of antibodies against malaria-specific antigens AMA-1 versus MSP1-19. Anti-AMA1 IgG, IgG1, and IgG2 levels were significantly higher compared to anti MSP 1-19 levels ($p<0.001$) and the proportion of responders to AMA-1 was higher than anti MSP 1-19 ($p < 0.05$ for total IgG, IgG2, IgG3 and IgG4). Generally, antibody responses were found to increase with age. Natural acquisition of antibodies to malaria antigens increase with age and the levels are dependent on the antigenic stimulation. Low response to MSP 1-19 could be due to declining malaria transmission in the area. Further analysis of the cohort follow-up will be done later to establish how naturally acquired antibodies may protect against malaria-related morbidity. In this era of declining malaria, further research is needed to address malaria morbidity and immunity aspects and more efforts should be directed towards elimination of malaria through integrated approach to malaria management.

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LONG-TERM PROTECTION AND SUSTAINABILITY OF IMMUNOLOGICAL MEMORY INDUCED BY EARLY AND LATE ARRESTING *PLASMODIUM BERGHEI* SPOROZOITES

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To date, inoculation of whole live parasite with differential cycle stage arrest are used in malaria immunization studies. The concept of radiation attenuated sporozoites (RAS) is based on broad disruption of genes with premature arrest of liver stage development. Alternatively, sporozoites administered concomitantly with chloroquine chemoprophylaxis (CPS) undergo full liver stage and develop into blood-stage parasites that are killed by chloroquine. Despite differences in degree of pre-erythrocytic development, both RAS and CPS immunization strategies induced complete protection when applied in mouse and man. Initial studies in the *P. berghei* murine model showed approximately a month after immunization similar protective efficacy and an important role of CD8+ effector memory T-cell response in both protocols. We therefore aimed in this study to investigate the sustainability of the protective immune responses by RAS or CPS. For this purpose, C57BL/6j mice are immunized and challenged after three or six months. At various time-points around challenge, cellular and humoral responses are to be assessed in blood, liver and spleen. Results to be presented comprise: (i) CD4+ and CD8+ T-cell memory, (ii) dynamics of regulatory T cells, (iii) antibody levels and (iv) functionality of T-cell memory response assessed by means of ex vivo assays. Presented data will be discussed in the context of long term protection as one of the main goal in malaria vaccine development.

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HIGH AFFINITY ANTIBODIES TO *PLASMODIUM FALCIPARUM* MEROZOITE ANTIGENS ARE ASSOCIATED WITH PROTECTION FROM MALARIA

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Antibodies are known to be of importance in protection against malaria. In this study, Surface Plasmon Resonance was used to evaluate the affinity of naturally acquired antibodies against *Plasmodium falciparum* merozoite antigens. The antibodies in serum samples from residents of Tanzania, Papua New Guinea, and Uganda bound with different affinities to different antigens. Monoclonal antibodies were also examined. The antibodies to AMA1 were of consistently higher affinity than antibodies to MSP2 antigens. High affinity antibodies correlated with reduced risk of febrile malaria during follow up, and the individuals with the highest affinities had a prolonged time to new infection. We also found indications that different parasites might vary in their ability to induce protective immune responses depending on the specific allele of polymorphic antigen they express. This is important information for understanding how immunity against malaria arises, and for evaluation of malaria vaccine formulations.

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A NANOPARTICLE VACCINE TARGETING *PLASMODIUM FALCIPARUM* CIRCUMSPOROZOITE PROTEIN CONFERS PROTECTIVE HUMORAL AND CELLULAR IMMUNITY

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An ideal vaccine against malaria would be a single platform that could induce long lasting cellular and humoral immune responses without the addition of adjuvant. We have developed a protective vaccine platform for the delivery of *Plasmodium falciparum* circumsporozoite protein (PfCSP) epitopes using an ordered array of NANP peptides displayed on the surface of a self-adjuvanting, self-assembling polypeptide nanoparticle (SAPN). In addition to this B-lymphocyte peptide, there are three different HLA (A2.1, B7 and B35) haplotype selected PfCSP CD8+ T-cell epitopes and a universal CD4+ T-helper epitope, PADRE, included in the SAPN construct. The nanoparticle vaccine can be given in saline either i.p., i.m. or i.v. Here we present our findings of immune modulation and protection against the human malaria *P. falciparum* specific protein in a murine model using a transgenic *P. berghei* sporozoite that expresses the complete *P. falciparum* CSP protein on its surface. Our *P. falciparum* SAPN are able to induce a > 95% protective humoral response for more than 9 months post vaccination with antibodies destroying sporozoites by the classical pathway of complement mediated lysis. Moreover, we are able to induce epitope-specific long-lived memory CD8+ T-lymphocytes which home to and reside in the liver and spleen and, independent of antibody, induce sterile protection against challenge. Our findings present, for the first time, a single platform pre-erythrocytic vaccine capable of inducing long lasting epitope specific protective humoral and cellular immune responses against the human malaria parasite *P. falciparum* CS protein.

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LEVELS OF ANTIBODIES AGAINST *PLASMODIUM FALCIPARUM* MALARIA IN MALIAN CHILDREN ARE MAINTAINED DURING DRY SEASON REGARDLESS OF HEMOGLOBINOPATHY

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Previous studies have shown that children with sickle-cell trait (HbAS heterozygotes) experience fewer *P. falciparum* malaria episodes than children with normal hemoglobin (HbAA homozygotes). To uncover the immunologic differences between these two groups, we initiated a 5-year longitudinal cohort study in Mali where malaria transmission is seasonal and intense. We collected plasmas from children aged 3-12 years in May 2009 (end of the dry season), December 2009 (end of the transmission season) and May, 2010. From 64 HbAS and 61 HbAA children, we collected plasma samples at all three points and determined IgG titers against 4 erythrocytic-stage antigens (AMA1, MSP1, EBA175, and MSP2) by ELISA. HbAS children experienced significantly fewer malaria episodes than HbAA children during the 2009 transmission season, despite showing significantly lower titers than HbAA children in May 2009. These titers increased in both groups of children during the 2009 transmission season and similar differences in titers were found in December 2009. There were no differences in the increase in titers between HbAS and HbAA children and the number of episodes during the transmission season did not correlate with the increase. To explain the lower titers in HbAS children, we determined whether levels of antibodies in HbAS children decay more rapidly compared to HbAA children during the 2010 dry season. Surprisingly, neither HbAA nor HbAS children showed significant reductions in titers from December 2009 to May 2010, despite virtual lack of malaria episodes during this time. In addition, for each of 4 antigens, IgG levels measured in May 2009, December 2009 and May 2010 correlated significantly: i.e., children with higher titers in May 2009 showed higher titers in December 2009 that were maintained until May 2010. These results indicate that IgG titers are maintained in Malian children for 5 months through the dry season, and suggest that IgG levels may be determined mainly by host and/or environmental factor(s) that do not change over relatively short periods of time.

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EVOLUTION OF MULTI-DOMAIN STRUCTURES IN MALARIA PARASITE ANTIGENS

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The *Plasmodium falciparum* var gene family encodes the surface expressed virulence factors PfEMP1, which mediate cytoadherence of infected red blood cells to a variety of host cell receptors. These diverse antigens are also one of the main immune targets, and protection against clinical infection has been shown to correlate with the acquisition of a repertoire of antibodies specific to different PfEMP1 variants. Var genes are characterized by a modular structure, and encode between two and nine different binding domains exhibiting varying binding specificities. In traditional theoretical models of genetically diverse pathogen populations, strong cross-immunity is expected to select against pathogen strains expressing antigens with multiple immunogenic epitopes. Given their highly immunogenic properties and the general abundance of target receptors it is not clear, therefore, why var genes encode multiple binding sites at once. Here, we show that models incorporating antibodies that function to prevent binding rather than preventing infection per se can lead to the evolution of antigens with multiple domains. Under this framework, the acquisition of an additional binding domain can relax the functional constraints upon the first domain, leading to antigenic diversification at that locus without compromising cell adhesion. Under these circumstances we predict that var genes would evolve towards multi-domain structures in which high affinity binding domains would associate with other low-affinity but antigenically diverse domains. We test this prediction by analyzing the domain structures of var genes from published *P. falciparum* genomes, and show that groups of var genes previously classified according to promoter regions A, B, and C, have distinct structures in this regard, with domain diversity varying significantly across the length of long var genes. Our approach represents the first attempt to explain the evolution of multi-domain structures among these antigens, and we show how domain-specific recognition is expected to develop with age in endemic regions of varying transmission intensity.

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ASSESSING THE REACTIVITY OF ANTIBODIES IN MALIAN CHILDREN AGAINST VARIABLE SURFACE ANTIGENS ON *PLASMODIUM FALCIPARUM* INFECTED RED BLOOD CELLS USING A FLOW CYTOMETRY-BASED ASSAY

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To profile the development of immune responses to *Plasmodium falciparum* antigens, we have conducted a longitudinal cohort study of children in Mali where malaria transmission is seasonal. As part of this study, we collected plasma from Malian children aged 3-11 years (total of 175 age- and hemoglobinopathies-matched) at the start (May) and end (December) of the 2009 transmission season. As reported in other studies, children with sickle cell trait (HbAS) experienced significantly fewer malaria episodes compared to children with normal HbAA. Previously, we evaluated plasma IgG titers against merozoite antigens by ELISA and found that HbAS children had significantly lower IgG titers compared to HbAA children. Using the same plasma samples, we compared the levels of antibodies to surface antigens on *P. falciparum* trophozoite-infected RBCs (iRBCs). We devised a novel high-throughput flow cytometry assay, based in part on an immunofluorescence microscopy assay used by Blythe et al. (Infect. Immun. 2008) to assess the reactivity of antibodies to surface antigens on iRBC. First, we wanted to evaluate whether age, sex, and hemoglobinopathies influence acquisition of antibodies to the iRBC surface. We found that some children produced antibodies to FCR3-iRBC. Older children tended to have higher titers to FCR3-iRBC, but these results were not statistically significant. Across all age groups, there was significantly higher titers to FCR3-iRBC at the end versus the start of the transmission season ($P<0.003$), suggesting that children were acquiring new and/or boosting existing IgG responses to iRBCs. Notably, although HbAS is associated with reduced levels of PfEMP-1 (a major variant surface antigen) on the surface of iRBCs, there was no significant difference in IgG titer to FCR3-iRBCs between HbAA and HbAS children at the start or end of the transmission season. We are also determining whether antibodies from Malian children recognize parasites from different geographic regions so that responses to other *P. falciparum* isolates will be gauged.

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ANTIBODY RESPONSES TO SELECT MALARIA ANTIGENS DIFFERENTIALLY DEVELOP AND WANE BY MALARIA TRANSMISSION INTENSITY

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The development of anti-malarial antibodies that mediate protective immunity to *Plasmodium falciparum* (Pf) infection depend on malaria transmission intensity. However, more information is needed on the heterogeneity and kinetics of this multi-antigen response, particularly in areas of unstable malaria transmission. A cohort of 236 children aged 10 months - 15 years, living in areas of stable (Kisumu) and unstable (Nandi) Pf-malaria transmission in Kenya, were surveyed at baseline and six-months later. Determinants of IgG responses to five *P. falciparum* antigens (AMA1 3D7, AMA1 FVO, MSP1 3D7, MSP1 FVO, and LSA1) were contrasted between the two areas. Comparisons in the relative change of antibody responses between the 6-month interval were also conducted. The proportion of positive IgG responses for all age groups was higher in Kisumu than Nandi; these were significant ($P<.05$) for AMA-1 3D7, AMA-1 FVO, and LSA-1. Antibody responses increased with age in Nandi but varied in Kisumu. Children 0-4 years old in Nandi had a two-fold difference ($P<.05$) in the median relative change in IgG responses to AMA-1 3D7, AMA-1 FVO and MSP-1 3D7 over a six-month period than similarly aged children in Kisumu. Antibody responses to AMA-1 3D7, AMA-1 FVO, MSP1-3D7, and LSA-1 among aparasitemic children were higher ($P<.05$) in Kisumu than Nandi. There were differences ($P<.05$) in antibody responses by parasitemia status in Nandi but few in Kisumu. Males and females in Kisumu had higher ($P<.05$) antibody responses to AMA-1 3D7, AMA-1 FVO, MSP1-3D7, and LSA-1 than those in Nandi. All measured antibodies correlated strongly with one another in Nandi ($P<.001$) but few correlated in Kisumu. Important differences in the dynamics and duration of naturally acquired immunity to *P. falciparum* exist by age, parasitemia status, and sex between areas of stable and unstable malaria transmission. These findings highlight the need to consider multiple factors beyond simply which antigens to target for vaccine development.

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CHARACTERIZATION OF THE HUMAN CD4 T CELL RESPONSE TO *PLASMODIUM FALCIPARUM* MALARIA

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In endemic areas clinical immunity to malaria can be acquired but only after repeated *Plasmodium falciparum* infections. Antibodies are known to play a major role in acquired immunity to malaria. Recent studies suggest that the gradual acquisition of protective humoral immunity to malaria may be due in part to the relatively inefficient acquisition of *P. falciparum*-specific memory B cells and long-lived plasmas cells. CD4 T helper cells play a critical role in orchestrating effective B cell responses in the germinal center - from B cell activation, affinity maturation and Ig class switching to memory B cell and plasma cell differentiation. However, little is known about the magnitude, quality, and antigen specificity of the CD4 T cell response to *P. falciparum* infection. In this study we stimulated PBMCs from malaria-experienced donors from Mali with lysates of different blood stages of *P. falciparum* (strain 3D7). After 18 hours of co-culture the intracellular production of IFNγ, TNFα, IL-2, IL-4 and IL-10 was measured in T cell subsets by flow cytometry. Importantly, given their role in supporting B cell responses in the germinal center reaction, we focused in particular on the phenotypic and functional analysis of follicular T helper cells (T_{FH}), identified by the expression of the transcription factor Bcl-6 and several chemokine receptors and activation markers. Comparing the CD4 T cell response, and the T_{FH} cell compartment in particular, of malaria-naïve and experienced persons of different ages and to different stages of the *Pf* life cycle may provide valuable insights into the mechanisms underlying the delayed acquisition of immunity to malaria.

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QUANTIFYING THE IMPORTANCE OF ANTIBODIES TO PFEMP1 AND OTHER SURFACE ANTIGENS OF *PLASMODIUM FALCIPARUM*-INFECTED ERYTHROCYTES, AND THEIR ROLE AS TARGETS OF PROTECTIVE IMMUNITY

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Effective clinical immunity that protects against symptomatic malaria in humans develops gradually after repeated exposure to *Plasmodium falciparum*. However, the primary targets and mechanisms of immunity are not well understood. Upon invasion of host erythrocytes, *P. falciparum* dramatically remodels the host cell for its own survival advantage via the export of novel parasite proteins. These modifications include the expression of variant surface antigens (VSA) on infected erythrocytes (IEs), which include PfEMP1 (*P. falciparum* erythrocyte membrane protein 1), Rifin, STEVOR and SURFIN proteins encoded by multigene families. Antibodies to VSAs are variant-specific and are associated with protection from symptomatic and severe malaria. However, the significance of each of these VSAs as targets of acquired immunity remains unclear due to a lack of tools to quantify antigen-specific responses. In this study, we used novel assays to dissect the importance of PfEMP1 and other VSAs as antibody targets using mutant parasite lines in which surface expression of PfEMP1 was inhibited with transgenic approaches. Comparisons between antibody reactivity to IEs of parental versus mutant parasites allowed us to quantify the importance of PfEMP1 and other VSAs as immune targets. This approach was applied to longitudinal cohorts of adults and children in Kenya and Papua New Guinea (PNG). Results from both populations indicate that PfEMP1 is the major target of naturally acquired antibodies to surface antigens of IEs, and that antibodies specific to PfEMP1 are associated with protective immunity. Antibodies to VSAs are thought to act by promoting phagocytic clearance of IEs and we further demonstrated that PfEMP1 is the major target of antibodies that mediate opsonic phagocytosis. A subset of individuals had prominent antibodies to VSAs other than PfEMP1, suggesting that other VSAs may still have an important role as immune targets. These findings are invaluable to understanding acquired immunity to malaria and advancing vaccine development.

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APICAL MEMBRANE ANTIGEN 1 IS A MAJOR TARGET OF HUMAN INVASION-INHIBITORY ANTIBODIES AGAINST *PLASMODIUM FALCIPARUM* MEROZOITES

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Acquired human immunity to *Plasmodium falciparum* malaria is thought to be mediated in part by antibodies to merozoite antigens that act by inhibiting erythrocyte invasion and blood-stage. Knowledge of the key targets of these antibodies and significance of polymorphisms in antigens will greatly benefit vaccine development. However, the major targets of protective and invasion-inhibitory antibodies in humans remain unclear. Apical membrane antigen 1 (AMA1) is an essential erythrocyte invasion ligand and leading vaccine candidate. Although studies have shown that antibodies to AMA1 are associated with protective immunity, there is little data on the functional activity of antibodies. We investigated the importance of AMA1 as a target of acquired inhibitory antibodies and the significance of AMA1 polymorphisms in a cohort of children and adults in Papua New Guinea. We generated transgenic *P. falciparum* lines expressing six different AMA1 alleles on the same genetic background and used these transgenic parasites in novel assays to quantify AMA1-specific invasion-inhibitory antibodies. This approach enabled the detection of AMA1-specific inhibitory antibodies separately from other inhibitory antibodies. We found that AMA1 is a major target of human invasion-inhibitory antibodies in both children and adults. Measuring AMA1 antibodies by standard ELISA and competition ELISA suggests that antibodies to different AMA1 alleles have a similar prevalence in the population. However, the prevalence of allele-specific invasion-inhibitory antibodies varied substantially for different alleles; there was a high prevalence of inhibitory antibodies for some alleles, but a very low prevalence for other alleles. These results have important implications for understanding the targets and acquisition of human immunity and vaccine development, suggesting that specific AMA1 alleles would be preferred over others in a multi-allele AMA1 vaccine.

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ACTIVELY-INDUCED ANTIGEN-SPECIFIC CD8$^+$ T CELLS BY EPITOPE-BEARING PARASITE PRE-INFECTION BUT NOT PRIME/BOOST VIRUS VECTOR VACCINATION COULD AMELIORATE THE COURSE OF *PLASMODIUM YOELII* BLOOD STAGE INFECTION

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Although malaria parasite is an obligatory intracellular microorganism, the lack of MHC molecules on red blood cells had questioned the immunological function of CD8$^+$ T cells against malarial blood stage (MBS) infection. Several recent reports, however, contradicting with this notion, suggested their influential function on the course of MBS infection. In the present study, we generated genetically-engineered murine malaria, *Plasmodium yoelii*, which expresses a well-defined *Trypanosoma cruzi*-derived, H-2K^b-restricted CD8$^+$ T cell epitope, ANYNFTLV. Prime / boost vaccination by the use of recombinant adenovirus and recombinant MVA, which induced enhanced number of antigen-specific CD8$^+$ T cells, failed to prevent pathological outcome to occur in the course of ANYNFTLV-expressing murine MBS infection. In contrast, the pre-infection of mice with *T. cruzi* which intrinsically bears the same CD8$^+$ T cell epitope significantly improved the survival of ANYNFTLV-expressing malaria-infected mice but not that of control malaria-infected ones. We conclude that the actively-induced antigen-specific CD8$^+$ T cells could ameliorate the pathologies caused by the MBS. Although the protection was observed only in certain situation, our study indicated an important clue that the CD8$^+$ T cell-mediated vaccine against MBS would be feasible.

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INDUCTION OF MALARIA IN VOLUNTEERS BY INTRADERMAL INJECTION OF CRYOPRESERVED *PLASMODIUM FALCIPARUM* SPOROZOITES

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Vaccines and new drugs are needed to prevent the nearly one million deaths and hundreds of millions of cases caused by malaria annually. To develop immunization strategies and to assess experimental anti-malarial vaccines and drugs, volunteers are immunized by the bites of laboratory-reared, malaria sporozoite (SPZ)-infected mosquitoes, or challenged by such mosquitoes after being immunized with experimental vaccines or treated with experimental drugs. Such studies have been critical for development of the most promising experimental vaccines and vaccine strategies, and of anti-malarial drugs. Because it is technically and logistically challenging to produce infectious, SPZ-infected mosquitoes, only a few laboratories in the world perform such experiments. In this trial we assessed the capacity to infect volunteers by intradermal (ID) injection of aseptic, purified, vialed *Plasmodium falciparum* (Pf) SPZ that had been cryopreserved for more than a year. Eighteen healthy Dutch adult volunteers received ID injections of PfSPZ in an open-label, dose-escalation study. Volunteers (N=6/group) received 2,500, 10,000, or 25,000 PfSPZ. The primary outcome variable was detection of blood-stage parasites by microscopy. Kinetics of blood stage parasitemia were assessed by quantitative PCR. Fifteen of eighteen volunteers (84%) developed Pf parasitemia, 5/6 volunteers from each dose group. There were no differences between groups in time until detectable parasitemia, parasite kinetics, clinical symptoms and signs, or laboratory values. We have demonstrated that aseptic, purified, cryopreserved PfSPZ manufactured in compliance with regulatory standards and administered ID infect volunteers. These PfSPZ can be used to assess the efficacy of new antimalarial vaccines and drugs in any clinical trial center in the world. It was recently demonstrated that immunization of volunteers taking chloroquine by exposure three times to PfSPZ-infected mosquitoes induced 100% protection against Pf infection. The PfSPZ used in our current study can translate this artificial immunization protocol into an implementable vaccine.

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THE USE OF FUNCTIONAL IMMUNOASSAYS (ISI AND ILSDA) BASED ON CRYOPRESERVED PRIMARY HUMAN HEPATOCYTES FOR SCREENING PRE-ERYTHROCYTIC *PLASMODIUM FALCIPARUM* VACCINE CANDIDATE ANTIGENS

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The inhibition of sporozoites invasion (ISI) assay and inhibition of liver stage development assay (ILSDA) were developed to functionally assess the effect of humoral immune response on sporozoite invasion and liver stage development *in vitro*. Previously we reported that cryoperserved primary human hepatocytes (CPHH) provided superior invasion rates for *Plasmodium falciparum* compared to the traditional cell lines (HepG2 and HCO4, respectively) used for these two assays. We also reported the development of a real time PCR (RT-PCR) procedure to detect the malaria parasite infection load. Here we demonstrate the correlation of RT-PCR with fluorescent microscopy results as assay read-outs. In addition, we present data evaluating the ability of polyclonal sera induced in mice and/or rabbits against novel pre-erythrocytic *P. falciparum* candidate vaccine antigens to functionally inhibit sporozoite development using the optimized ISI and ILSDA assays. Our results indicate the value of these assays for use as antigen discovery down-selection tools.

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CAN ANTIGENIC DIVERSITY OF APICAL MEMBRANE ANTIGEN-1 BE OVERCOME? RATIONALE FOR THE DEVELOPMENT OF A SECOND GENERATION AMA1 VACCINE

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Apical Membrane Antigen-1 (AMA1) vaccination induces invasion inhibitory antibodies in both naïve and malaria immune populations, however, its antigenic diversity continues to pose a considerable hurdle in Phase 2 efficacy trials. Analysis of a large number of field isolate sequences show a continuum of variability is present within AMA1 with no clear allele families like those of MSP1 and MSP2. Mapping of antigenic escape residues within AMA1 showed that a small group of polymorphic residues on the C1L loop were the primary determinants of strain-specificity. Confining the diversity analysis to only the C1L genotype made it possible to divide AMA1 alleles into a small number of allelic groups. C1L based grouping was also useful for assessing allele specific protection in humans. We have now expressed and purified AMA1 from seven major C1L groups in the *E. coli* expression system. Using anti-sera generated in rabbits against these seven monovalent vaccines, we selected a set of strains to be included in a future second generation AMA1 vaccine. As a proof of concept for a polyvalent approach to overcoming antigenic diversity, we immunized a group of rabbits with a Quadrivalent AMA1 vaccine composed of 3D7, FVO, HB3 and W2mef strain (Quadvax). The C1L sequence analysis showed that Quadvax was able to cover C1L polymorphisms present in ~95% of the field isolates. Cross-strain GIAs also showed a varying degree of susceptibility of all the *P. falciparum* tested, in particular strains that were not present in the Quadvax. Further analysis of the mechanisms of cross-strain invasion inhibition showed that mixing more than two AMA1 alleles in a vaccine can have synergistic effect on the quality of the induced antibodies. Formulating a polyvalent vaccine such as the Quadvax allows us to increase the molar concentration of conserved epitopes in an AMA1 vaccine, which in turn results in refocusing the antibody response to more conserved regions of the protein outside domain-1. All the evidence so far suggests the need for continued development of AMA1 as a component of a multi-stage malaria vaccine.

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HIGH THROUGHPUT GENOMICS SCREENING FOR MALARIA ANTIGEN DISCOVERY

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Malaria is the most burdensome parasitic disease of man, exacting an estimated toll of 863,000 deaths and 243 million clinical cases per year. It is important to develop a vaccine that can effectively prevent the disease. Up to now, there are few identified malaria antigens, representing less than 0.3% of the 5,300 proteins encoded by the *Plasmodium* parasite, and those have limited efficacy in vaccine clinical development. Here, we report a new approach employing adeno-array technology for high-

throughput discovery of pre-erythrocytic *P. falciparum* antigens using orthologues identified in the *P. yoelii* mouse model.To identify highly expressed *P. yoelii* pre-erythrocytic antigens for the adeno-array, we performed bioinformatics data mining using publicly available genomic and proteomic databases. Based on expression abundance data from microarray and protein mass spectrometry analysis by several research groups, we prioritized 300 sporozoite stage and liver stage candidate *P. yoelii* genes with identifiable *P. falciparum* orthologues for generation of the adeno-array. These genes were cloned into a high-level expression cassette located in the E1 region of an E1/E3-deleted adenovirus type 5 genome using high-throughput methodologies. In the antigen discovery screen, we infected antigen presenting cells (APC) with individual adenovectors from the adeno-array. The infected APC were then incubated with splenocytes from mice immunized with known protective regimens of Radiation Attenuated Sporozoites (RAS), and antigen-specific $CD8^+$ T cell responses were measured by Intracellular Cytokine Staining. So far, we have identified 37 new antigens that recalled antigen-specific CD8+ T cell responses from RAS-immunized mice. We are currently testing these adenovirus vectors from the array for their capacity to protect mice from a *P. yoelii* sporozoite challenge. The most highly protective antigens will be prioritized for malaria vaccine development and their *P. falciparum* orthologues will be cloned into adenovectors and advanced to preclinical testing.

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INDUCING POTENT IMMUNE RESPONSES TO MULTIPLE LIVER-STAGE MALARIA ANTIGENS USING DNA VACCINES

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Despite intense research efforts, the currently most advanced malaria vaccine candidate, RTS,S, an adjuvanted recombinant protein, confers only partial protection against clinical disease. Thus, the development of a vaccine that induces long-term protection against malaria remains an important global goal. We have developed a DNA-based vaccine candidate targeting 4 important *Plasmodium falciparum* (P.f.) liver-stage antigens: circumsporozoite protein (CS), liver stage antigen 1 (LSA1), thrombospondin-related-anonymous-protein (TRAP) and cell-traversal protein for ookinetes and sporozoites (CelTOS). Consensus antigens were designed for each vaccine target with several modifications to improve expression. Immunogenicity of the vaccines and a multi-antigen vaccine cocktail (containing all 4 vaccines), delivered with electroporation (EP), was initially evaluated in mice. The vaccines elicited strong antigen-specific T cell responses that were similar to, or surpassed, those induced by other vector systems. Specifically, the vaccine induced an IFNγ response as measured by ELISpot (SFU): CS (1607±391), LSA1 (1908±821), TRAP (929±255) and CelTOS (477±160). Flow cytometry indicated vaccine-specific CD4+ T cell production of IL-2 (2.5%) and TNFα (1.4%) and CD8+ T cell production of IFNγ (0.9%), IL-2 (3.1%) and TNFα (1.5%). LSA1-specific IFNγ production was also detected in 1.5% of hepatic CD8+ T cells. Both vaccine approaches induced robust antigen-specific serotype conversion (IgG endpoint titers >100,000). Inclusion of IL-28B in the vaccine cocktail increased the total IFNγ response and decreased the regulatory T cell population. An on-going non-human primate (NHP) study is evaluating the immune responses elicited by this promising P.f. vaccine candidate. Results from a pilot study indicate this vaccine, delivered intradermally with IL-28B by EP, induces robust immune responses in NHPs. After the 2nd immunization, endpoint titers for CS and LSA1 were >400,000 and >75,000 for TRAP and CelTOS. The vaccine induced 328.3±129.0 IFNγ SFU following the 2nd vaccination and this response increased 4.6-fold with the 3rd vaccination (1512.3±321.6 SFU). In summary, we have developed a novel DNA-based malaria vaccine that elicits potent immune responses, which exceed or are equivalent to the levels induced by other vaccine platforms, indicating this promising vaccine candidate merits further study in human clinical trials.

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POST-PHASE III EVALUATION OF THE RTS,S MALARIA VACCINE CANDIDATE - THE WAY FORWARD

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The RTS,S malaria vaccine candidate has the potential to play an important role as part of future integrated control programs to further reduce the burden of malaria. It is several years ahead of any other malaria vaccine in terms of assessment of clinical efficacy. Following the Phase II results from Mozambique (30% protection against clinical malaria, and close to 50% protection against severe malaria) Kenya and Tanzania (children 5-17 months old; vaccine efficacy against 1st or only episode was 39% over a 12 months follow-up), a large Phase III pre-licensure trial was initiated; initial results will be available in the last quarter of 2011. If the results from the Phase III trials are similar to those from the Phase II trial, we will have a vaccine that is capable of reducing severe malaria by half. The public health relevance of this vaccine, when used to complement existing malaria control and elimination efforts could be immense. There will be a need for effectiveness studies to rapidly assess the full programmatic impact of the vaccine. The clinical trials investigators and associated partners have started to address this need. 4 main axes of development are discussed. 1) continue clinical trials in special populations such as malnourished and/or HIV infected individuals, 2) careful analysis and in-depth evaluation of the protective immune responses and related modeling to better understand the vaccine's potential mode of action, 3) modeling of the potential effect of vaccination in different endemic settings and as part of different integrated intervention strategies/ intervention mixes including economic appraisals, and 4) contribute to the policy dialogue and process that will guide recommendations for use. Most malaria endemic countries in Africa will be faced with decisions on the integration and routine use of the candidate malaria vaccine, and donors will have to decide on financing its introduction into endemic countries, since the expectation in endemic countries will be that children should be vaccinated at no cost to the family. The malaria community will be faced with the ambitious goal of achieving malaria elimination through an expanded malaria control program that includes vaccination and is tailored to the different socio-ecological endemic settings. The clinical trials investigators and associated partners are willing to actively contribute to this endeavor.

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PHASE III SAFETY EVALUATION OF THE RTS, S/AS01 MALARIA VACCINE CANDIDATE: REACTOGENICITY, UNSOLICITED ADVERSE EVENTS AND FATALITIES

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The RTS, S/AS01E malaria candidate vaccine is currently being evaluated in a multicenter Phase 3 randomized controlled double blind trial, in children aged 5 to 17 months and 6 to 12 weeks old at first vaccination, across 11 African sites in 7 African countries. Acceptable safety profile will be paramount in determining whether the candidate vaccine is suitable for implementation in sub-Saharan Africa future vaccination programs. The following safety results will be presented: solicited local and general reactogenicity within 7 days post vaccination and unsolicited Adverse Events occurring within 30 days post vaccination in a subset of 200 subjects of the 5-17 months old age category from each study centers (N=2200) and Serious Adverse Events (all, fatal, related) in all children (N = 15460) from dose 1 up to the 31 May 2011. Seizures occurring within 7 days post vaccination will be presented as per Brighton collaboration guidelines. All results will be tabulated and presented with 95%

Confidence Interval per treatment arm. All unsolicited Adverse Events will be reported classified by MedDRA preferred term. Safety analyses will be performed on the Intention-To-Treat population. Unsolicited events were captured by passive detection. Access to clinical evaluation and care was facilitated in all study centers.

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DESORPTION OF CONJUGATED PFS25-REPA FROM ALHYDROGEL BY A HIGH PH METHOD

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Pfs25 is an ookinete surface protein of the *Plasmodium falciparum* malaria parasite that elicits transmission-blocking antibodies in animals and humans. Pfs25 has been chemically conjugated to the recombinant non-toxic carrier protein *Pseudomonas aeruginosa* ExoProtein A (rEPA) to increase immunogenicity, and a Pfs25-rEPA malaria vaccine candidate was prepared using Alhydrogel, a commercially available aluminum hydroxide adjuvant. In order to satisfy the regulatory requirements for identity and integrity testing of this formulation, we have developed a method to extract a sufficient amount of Pfs25-rEPA from Alhydrogel for further analysis while maintaining epitope functionality. Two previously developed extraction methods relied on buffers containing high salt concentrations, notably citrate and phosphate, to lower the adsorptive capacity of alum during incubation for extended periods of time, ranging from 2.5 to 5 hours. Testing revealed these extraction buffers removed a less than desirable amount of the Pfs25-rEPA conjugate from this formulation. A third method was developed which involved raising the pH of the formulation above the isoelectric points of Alhydrogel (pI ≈ 11) and the Pfs25-rEPA conjugate (pI ≈ 5.57), and this method eluted the maximal amount of Pfs25-rEPA conjugate from alum. Desorption of the Pfs25-rEPA conjugate was immediate, which may be due to the negatively charged antigen and adjuvant in the formulation. Accordingly, the incubation time was minimal and the entire procedure was completed within minutes. Furthermore, the retention of epitope functionality was demonstrated by Western blot using conformational antibody 4B7. In conclusion, the high pH method allowed the greatest desorption of Pfs25-rEPA conjugate from alum and facilitated identity and integrity evaluations of the Pfs25-rEPA on Alhydrogel malaria vaccine candidate.

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ACCELERATED STABILITY STUDY OF PFS25-EPA CONJUGATES FOR A TRANSMISSION-BLOCKING VACCINE

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Pfs25, the major surface protein of *Plasmodium falciparum* zygotes, is a leading malaria transmission-blocking vaccine candidate. To enhance the immunogenicity of Pfs25, we have conjugated the protein to recombinant nontoxic *Pseudomonas aeruginosa* ExoProtein A (rEPA), and the stability of the conjugate was evaluated in this study. Pfs25-EPA conjugate was subjected to three freeze/thaw cycles, and was also evaluated for thermal stability after storage at temperatures of 4°C or 37°C for 7, 14 and 56 days, and at -80°C for 56 days. The forced freeze/thaw and thermal stability samples were analyzed for identity by Western blot with monoclonal antibodies 4B7 and penta-His, and polyclonal anti-EPA antibodies, and for integrity by Tris-Acetate SDS-PAGE with silver staining, capillary gel electrophoresis (CGE) using pluronic F-127 as resin, and size-exclusion chromatography with multi-angle light scattering (SEC-MALS). Our results showed that the Pfs25-EPA conjugate was stable following three freeze/thaw cycles. The conjugate was also stable for up to 56 days at 4°C or -80°C. However, change was observed in samples stored at 37°C for 7 days or longer, as evidenced by the release of low molecular weight proteins and a decrease in average molecular mass from 558 kDa on day 0 to 367 kDa on day 56. These results indicate that the stability of the Pfs25-EPA conjugate is suitable for human testing.

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TRANSMISSION BLOCKING ASSAYS FOR CLINICAL DEVELOPMENT OF VACCINES TO INTERRUPT MALARIA TRANSMISSION

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Transmission blocking vaccines work by inducing antibodies in vaccinated individuals that inhibit the development of malaria parasites in the midgut of the mosquito, thus interrupting the cycle of transmission to the next human host. A standard membrane feeding assay has been used to evaluate the ex vivo transmission blocking activity of antibodies induced by vaccine candidates, but the assay needs to be qualified to determine to what extent it is predictive of transmission blocking activities in the field. A study is currently under way to compare results of mosquito feeding assays in malaria exposed adults and children in Bancoumana, Mali. Insectary-raised progeny of field-caught mosquitoes are directly fed on gametocytemic individuals and age-matched gametocyte negative individuals. Infectivity in these mosquitoes is then compared against those of mosquitoes fed in direct membrane feeding assays in Mali and standard membrane feeding assays in the USA. Data will also be generated on the dynamics of gametocyte carriage rates through the year. Results to date of feeding experiments will be presented, and potential clinical development paths for transmission blocking vaccines using these data will be discussed.

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PRE-CLINICAL DEVELOPMENT OF A POTENT TRANSMISSION BLOCKING PLANT-PRODUCED *PLASMODIUM FALCIPARUM* SEXUAL STAGE PFS25 VACCINE CANDIDATE

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Malaria is a serious and sometimes fatal mosquito-borne disease caused by a protozoan parasite. Each year it is estimated that over one million people are killed by malaria and yet the disease is preventable and treatable. Developing vaccines against the parasite is a critical component in the fight against malaria and these vaccines can target different stages of the pathogen's life cycle. We are targeting sexual stage proteins of *Plasmodium falciparum* which are found on the surface of the parasite's reproductive cells present in the mosquito gut. Antibodies against these proteins block the progression of the parasite's life cycle in the mosquito, and thus block transmission to the next human host. Transmission blocking vaccines are essential to the malaria eradication program to ease the disease burden at the population level. In the work presented here, we focus on the process development, formulation, scale-up and pre-clinical evaluation of a potent Pfs25 recombinant antigen that shows

effective transmission blocking activity. The antigen was successfully expressed in our plant-based launch-vector system and purified to a high level of homogeneity. The resulting Pfs25 antigen has undergone high throughput screening formulation development, extensive biochemical characterization and dose ranging studies to determine the minimal effective dose in pre-clinical animal studies. The purification process has been successfully scaled through pre-clinical production levels in preparation for large-scale production in our pilot GMP facility. These data demonstrate the feasibility of expressing *Plasmodium* antigens in a plant-based system for the economic production of a transmission blocking vaccine against malaria.

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QUANTITATIVE ASSESSMENT OF *PLASMODIUM FALCIPARUM* SEXUAL DEVELOPMENT REVEALS POTENT TRANSMISSION-BLOCKING ACTIVITY OF THE SYNTHETIC DYE METHYLENE BLUE

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Drugs that block the transmission of *Plasmodium falciparum* sexual stage parasites to mosquito vectors could play a key role in eliminating malaria. However, efforts to measure the activity of existing antimalarials on sexual stage gametocytes and to identify transmission-blocking agents have been hindered by a lack of quantitative assays. Here, we describe experimental approaches using *P. falciparum* GFP-luciferase reporter lines that enable the assessment of dose- and time-dependent drug action on gametocyte maturation and transmission. Our studies reveal activity of the first-line antimalarial dihydroartemisinin and the partner drugs lumefantrine and pyronaridine on immature gametocytes, along with appreciable inhibition of mature gametocyte transmission to *Anopheles* mosquitoes. Prophylactic 8-aminoquinolines had broad gametocytocidal activity at elevated concentrations. In contrast, methylene blue potently inhibited all gametocyte stages and almost fully abolished transmission to mosquitoes at concentrations achievable in humans, highlighting its potential to reduce the spread of malaria.

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UNDERSTANDING MALARIA PREVENTION AMONG SEASONAL MIGRANT WORKERS FROM HIGHLAND ETHIOPIA

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To address the information gap in understanding malaria prevention among seasonally migrating workers to malaria endemic lowlands of Ethiopia during main crop harvesting season that coincides with major malaria transmission season, we have conducted knowledge, attitudes, and practices (KAP) study in two sugarcane plantations in the rift valley, central-east Ethiopia. This survey provides essential background information to understand situation of malaria in non-immune migrant workers and possible preventive strategies. Standardized questionnaires were distributed to seasonal workers from highland Ethiopia presenting for employment at recruitment centers at the Metehara and Wonji sugarcane plantations. Standard interview questionnaires were completed by 876 new recruits; 445 (50.8%) were returnees to the plantation for second or more seasons. Three hundred eighty seven (44.2%) of the interviewee had one or more attack of historical malaria in their life time. Of those with history of malaria 335 (86.6%) were returning migrant workers who had the attack during their previous stay or within 4 weeks after leaving these plantations. Among all migrant workers 546 (62.3%) had information about risk of acquiring malaria at these plantations. Only 112 (12.8%) migrant workers knew one or more of malaria prevention measures although only 34 of them (3.9% of all migrant workers) sought pre-travel professional medical advice. Sixty six (7.5%) of all study subjects had knowledge about personal protection measures against mosquito bites, but only 7 (0.8%) carried mosquito repellents or insecticides. Only 15 (1.7%) of them had already taken prophylactic drugs up on arrival at the plantation. In conclusion, risk of malarial attack is extremely high for migrant workers. The KAP of migrant workers at malaria endemic plantation about malaria is alarmingly low. To reduce the rate of malaria attack to non-immune migrants and avoid morbidity and mortality from severe form of malaria, targeted health education and preventive interventions should be routine provisions to non-immune migrant seasonal workers upon arrival at malarious plantations.

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METABOLIC PROFILES AND MALARIA TRANSMISSION

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Plasmodium vivax malaria is a serious public health concern in the Amazonian city of Iquitos, Peru. One malaria control strategy that goes beyond vector control is blocking malaria transmission by trying to stop infection at a critical stage in the parasites life cycle. Since parasite infectivity, competence of the vector, and host factors all play a role in malaria transmission, understanding each of these mechanisms will help in the development of anti-malarial vaccines. This study looks at one host factor: the nutritional environment for the parasite inside the human host. The nutritional environment is the composition of macromolecules and nutrients in the human host and this study examines how variations of the nutritional environment affect malaria transmission. Because the parasite is dependent on the host for nutrition, the nutritional status of the host may affect parasite biology. For example, differences in diets influence lipid serum levels, and the malaria parasite must scavenge lipids entirely from its host to survive. Since parasite biology is most often studied *in vitro,* cultured parasites often cannot account for the slight biological variations of the human host, such as nutritional environment. Previous results show that the *P. falciparum* parasite exists in the human host in at least three distinct physiological states, apparently related to glycolytic growth, a starvation response and a general stress response. These metabolic states were due to the different nutritional environments in which the parasites lived. Therefore, the metabolic state of the malaria parasite *in vivo* likely plays an essential role in parasite transmission. Examining an existing cohort of 100 persons living in Iquitos with previous *P. vivax* malaria episodes, we used a questionnaire and food log to assess type of food eaten, amount of calories consumed, and amount of oil used. We also measured physical characteristics such as height, weight, and Body Mass Index. We will use this dietary data to study how trends in diet relate to gametocyte density and gametocyte infectivity for mosquitoes. Studying *ex vivo* parasite biology can give insight about disease outcome and transmissibility, host-pathogen interactions, and potential discoveries of new therapeutic targets.

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UTILIZATION OF MOBILE PHONES FOR SAFETY REPORTING ASSOCIATED WITH SYSTEMATIC SCREENING AND TREATMENT OF *PLASMODIUM FALCIPARUM* ASYMPTOMATIC CARRIERS WITH ARTEMETHER-LUMEFANTRINE IN A COMMUNITY SETTING IN AFRICA

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Malaria pharmacovigilance in developing countries is essential, but it is challenging. The use of mobile phones for safety monitoring during a two-arm, community-based study for Coartem (artemether-lumefantrine [AL]) administered for asymptomatic (following systematic screening by rapid diagnostic test [RDT]) or symptomatic *falciparum* malaria in a rural district

of Burkina Faso is described. Pharmacovigilance procedures required close collaboration among study personnel, local health care facility (LHF) team, and district hospital staff involved with the study. Mobile teams were periodically deployed to screen half of the population (intervention arm) by RDT. Asymptomatic carriers were treated with AL or alternative medication, with a follow-up at Day 7. Subsequently every fever case was to be assessed by RDT at the LHF for diagnosis of a symptomatic malaria episode, and if positive treated similarly with a follow-up at Day 7. Severe cases were transferred to the district hospital. Every serious adverse event (SAE) was notified to the Principal Investigator team by the LHF staff (usually a head nurse) using a mobile phone. Subsequently a study physician was dispatched to the LHF to collect data and transcribe them onto a SAE form in English within the required timeframe. Original training on GCP and safety reporting performed at the onset of the study emphasized the requirement to report SAEs in all subjects throughout the study duration, as well as AEs within 7 days following treatment with AL. Despite that, the quality of SAE reporting was inconsistent, diagnosis of severe malaria was suboptimal, and no pregnancies were reported over 3 months. In order to improve reporting, 5 training sessions tailored to different study personnel, as well as LHF and district hospital staff, were conducted in March 2011. Channels for detection of SAEs were identified and implemented. Retraining proved to be necessary to increase awareness of study procedures at the district hospital, improve fever case management at the LHF, and ensure pregnancy reporting.

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MALARIA CONTROL IN PAST AND IMPACT OF GLOBAL FUND ON REDUCING RATES IN BANGLADESH

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Following the Malaria Eradication Programme (MEP) in early 1960s, malaria incidence dropped from 10.8 per 100,000 in 1968 to 4.22 per 100,000 people in 1971. The MEP relied upon indoor residual spraying using DDT, active surveillance for new cases, and effective drug treatment. After independence of the country in 1971, the MEP declined with a rise of malaria to 60.44 per 100,000 people in 1976. The MEP, reconverted to the Malaria Control Programme (MCP) in 1977 focused on vector control in limited susceptible areas without active surveillance. The malaria trend remained static until the early 1990s when malaria cases further increased following the official ban and cessation of DDT-use. The Bangladesh Govt. and BRAC recently received global fund money near US$ 80 million in 2006 and 2009. The goal of the global fund proposals were to reduce malaria specific morbidity and mortality by 50% with provision of early quality diagnosis, effective treatment and to expand use of LLIN and IRS to achieve 100% coverage in three Chittagong Hill tracts that reports >80% of the malaria cases and >90% of the deaths each year. The present control efforts rely on passive case-detection, effective treatment, and provision of indoor insecticide-treated nets. The global fund project has resulted in reducing reported mortality from 501 in 2005 to 37 in 2010; malaria incidence initially escalated from 48,121 in 2005 to 84,690 in 2008-due to improved detection at the community level but has started dropping consistently in the past 2 years 63,873 in 2009 and 55,873 cases in 2010. It is not clear if the decline in number of malaria cases is solely result of the Global Fund activities, the result of climate variability, or is even real. In addition to the passive reporting of the government our early findings from active malaria surveillance site amongst a population of 20,000 indicate that over 60% of the cases infections were asymptomatic. Strategies that identify and treat this large asymptomatic malaria positive population may be necessary to reduce transmission and sustain gains in malaria reduction.

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SYSTEMATIC SCREENING AND TREATMENT WITH ARTEMETHER-LUMEFANTRINE OF *PLASMODIUM FALCIPARUM* ASYMPTOMATIC CARRIERS IN A COMMUNITY SETTING IN AFRICA: IMPLEMENTATION PLAN

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Despite nationwide adoption of artemisinin-based combination therapy, and associated decline in malaria-related deaths, complementary interventions are still required to further reduce the disease burden. This 18-cluster (9 intervention clusters in villages; 9 control), randomized, single-center, controlled, parallel, prospective study will evaluate the impact of systematic treatment of asymptomatic carriers (ACs) of asexual forms of *Plasmodium falciparum* with artemether 20 mg-lumefantrine 120 mg (AL, Coartem/Coartem Dispersible, BID for 3 consecutive days) in approximately 9000-14000 subjects (male/female adults, children, and infants) from a community setting in Africa. The primary objectives are to evaluate whether treatment of *P. falciparum* ACs is associated with a lower number of symptomatic malaria episodes, RDT confirmed per person-year over a 12-month follow-up period and an improvement in hemoglobin levels after 28 days. Subjects will be excluded from receiving AL if they have severe malaria, known disturbances of electrolyte balance, history of congenital QTc prolongation or sudden death, body weight <5 kg, hypersensitivity to AL, or if they are in the first trimester of pregnancy. Those subjects will be treated with alternative drugs per current national guidelines. Responsibilities of the investigator's central site include microscopy, data entry, source data archiving, and supervision of the Demographic Surveillance System (DSS). DSS will monitor each cluster population every 2 months during the study for births, deaths, and in/out migrations; and provide an up-to-date demographic status of the study population. A mobile team supervised by the principal investigator will be supported by community healthcare workers (CHWs), a lead CHW, and a local healthcare facility for different study procedures. A unique permanent identification number will be assigned to each inhabitant. If the reduction of ACs and disease burden is confirmed, policymakers may consider this approach in the surveillance strategies being implemented by malaria control programs across Africa.

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NATURAL ENVIRONMENTAL AND HUMAN SOCIAL FACTORS DETERMINE PATTERNS OF CHILDHOOD MALARIA RISK IN MALAWI

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Plasmodium infection and malaria disease result from a combination of physiological, behavioral and environmental factors in the context of human adaptation. We studied patterns of infection in Malawian children to characterize disease patterns and devise predictive risk models using health and GIS datasets. A household-level, geolocated malaria indicator survey of ~7,200 households was performed during 2007. Locations of Malawi MOH-supported health facilities were obtained. Other GIS layers for roads, waterways, elevation and landcover were obtained from DIVA-GIS (www.diva-gis.com). Distances from residences to nearest health facilities, roads and water were calculated, and bivariate associations of all covariates and malaria-positive households were evaluated. From the survey data, we selected an optimal model through Akaike's Information Criterion (AIC) to predict probability of *Plasmodium* infection for all locations. Comparisons suggested that infected vs. non-infected children resided further away from health services (7.55 vs 6.05 km) and roads

(1.25 vs 0.9 km), at lower elevations (717 vs. 888 m) and nearer to water bodies (1.09 vs 1.26 km). An optimal logistic regression model included distance to health facilities (OR 8.73 [4.96,15.35]), roads (OR 4.48 [2.19,9.16]), water (OR 0.27 [0.15,0.50]), elevation (OR:0.998 [0.997,0.999]) and landcover categories, including cultivated land (ref), forest (OR: 1.04 [1.01,1.07]), and artificial surfaces (0.96 [0.92,1.00]). Our model indicates that malaria infections are best predicted by a combination of vector-based and human factors. Conditions favorable to vector reproduction increased infection risk, but health facilities and infrastructure modified this relationship, likely through a combination of prompt treatment, vector management and preventative interventions (e.g. ITNs). The resulting comprehensive map of malaria risk in Malawi showed highest risk among households along Lake Malawi, the Lower Shire basin and those proximal to game parks. Further efforts to create predictive models of malaria risk should not ignore important human-vector-environmental interactions.

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DIFFERENTIAL PROTEOMIC STUDY OF *PLASMODIUM FALCIPARUM*-INFECTED AND NON-INFECTED SALIVARY GLANDS OF *ANOPHELES GAMBIAE*: WHAT CONSEQUENCES FOR THE MALARIA TRANSMISSION?

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Malaria is a disease caused by parasites *Plasmodium* genus transmit by *Anopheles* mosquitoes during the blood feeding. During this blood meal, saliva and parasites are injected in the vertebrate host skin. Salivary molecules possess a pharmacologic role and proprieties of immunomodulation allowing a correct blood feeding and to fight the acquired immune response of host to bites. This saliva is also involved in the human-vector relationships. When the salivary glands are infected, the parasite modifies the vector biology. In the literature it has been demonstrated that the expression of salivary proteins of *Anopheles gambiae* infected by *Plasmodium berghei* are modified. Here we investigate what modifications in salivary glands of *An. gambiae* are occurred during the infection by *P. falciparum*. To assess this question we are comparing the salivary extracts of *An. gambiae* infected and not by proteomic approach. Experimental infections of *An. gambiae* by *P. falciparum* are carried out in Cameroon and salivary glands are dissected 14 days post-infection. The quantitative-PCR allowed to quantify the *P. falciparum* infection into the salivary glands. Then two-dimensional electrophoresis are underway with different pools of non-infected versus infected salivary glands. The differential analysis could show that several components are over- or down-expressed during the infection. Mass spectrometry analysis could know what proteins have a modified expression. These results could show that the parasite induces surely modifications of salivary proteins, certainly favoring the transmission. This study allows to have more knowledge about the relationships between the vector and his host and to develop new tools to evaluate the risk of malaria like a immuno-epidemiologic biomarker. These observations represent important new applications for the vector control strategies.

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TERRAIN DIFFERENCES AFFECT MALARIA VECTOR DISTRIBUTION IN WESTERN KENYA HIGHLANDS

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One of the key drivers to malaria in African highlands is the terrain characteristics because topography affects climate, hydrological characteristics and thus larval habitat availability and stability and micro-climate of the highland. This study examined the impact of three different terrains (U-shaped, V-shaped valleys and plateaus) on malaria transmission. A model based on the terrains of each terrain and the precipitation thresholds was established. Two parameters that define the terrains are the size of the area at the bottom of the valley with a zero or close to zero side slope and slope of the river flow direction. These numerical values were determined by geospatial analysis with GIS techniques as the major parameter that affects drainage, habitat stability, vector productivity and rate of malaria transmission. The primary results indicate that the U-shape valley has 3-fold more *Anopheles gambiae* female mosquitoes, the principal malaria vector in western Kenyan highlands, than the V-shape valley system. These support our hypothesis that the greater stability and productivity of the breeding habitats in the U-shape valleys compared to the V-shape valley.

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THE TANZANIAN NATIONAL VOUCHER SCHEME: IMPROVING TAKE-UP BY REDUCING THE TOP-UP PRICE PAID BY VOUCHER BENEFICIARIES

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Since 2004, the Tanzanian National Voucher Scheme (TNVS) has made insecticide treated nets widely available and accessible to pregnant women and infants through a donor supported voucher system that subsidizes the cost of nets purchased in commercial retail outlets. Between 2005 and 2008 voucher nets became less affordable as the top-up amount paid by beneficiaries rose in-line with increasing net prices, reaching the equivalent of nearly 2 USD by 2008. As a result, the proportion of vouchers redeemed for a net fell from 88% in 2005 to 54% in early 2009. 20% of voucher recipients in 2008 cited lack of money as the reason for not using the voucher and significant inequity in redemption existed. In late 2009, this inequity was addressed by increasing the value of the voucher and fixing the cash top-up amount to ~ 0.35 USD. Quarterly voucher redemption rates were tracked from July 2004 to March 2011. Redemption rates were calculated by dividing the number of redeemed vouchers with matching returned stubs by the total number of voucher stubs returned in a given time period. Between the January 2010 and March 2011, Pregnant Women and Infant Voucher redemption rates returned to their initial levels by rising from 54% to 82% and 51% to 84%, respectively. Compared to the 15-month period prior to implementation of the voucher upgrade, the overall number of redeemed vouchers rose by 29% from 1.3 to 1.7 million. The number of vouchers redeemed in rural areas increased by 46% and for urban areas the increase was 28%. These improvements occurred during a period when more than 20 million free long lasting insecticidal nets were issued in two mass campaigns, allaying earlier concerns that free net distribution would weaken the TNVS, which relies on a small co-payment. Thus, the current

TNVS approach may contribute towards a keep-up mechanism to maintain high net ownership among targeted risk groups across socioeconomic groups after free net mass distributions are completed.

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POSITIVE DEVIANCE: AN INNOVATIVE APPROACH TO IMPROVE MALARIA PREVENTION AND TREATMENT PRACTICES AMONG MOBILE AND MIGRANT WORKERS IN CAMBODIA

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Reaching mobile and migrant populations is one of the key strategies in the containment and elimination of artemisinin resistance in the Greater Mekong Subregion (GMS). Positive Deviance (PD) is an asset-based behaviour change approach with the underlying notion that every community has certain individuals (*positive deviants* or champions) whose malaria prevention and treatment practices result in better health outcomes than their neighbours. Malaria Consortium (MC) supported Cambodia's National Malaria Programme to pilot PD among residents and migrants in three villages in Sampov Loun district. The PD pilot aims to identify and promote good health seeking practices in both communities. The baseline survey conducted in Aug 2010 (n=309), suggested that knowledge about malaria and prevention were high in both communities but health-seeking behaviour for fever could be improved (residents 44.4%; migrant 33.3%), The PD process included 6 steps: pre-orientation meeting, community orientation, situation analysis, PD inquiry, participatory analysis, and community feedback. During the process, 13 in-depth interviews and 6 group discussions were conducted to identify the champions. For example, we identified a female migrant worker who never gets malaria by always sleeping under a bed net, wearing long sleeved clothes, covering her legs with a scarf while watching TV, and immediately going to the health centre when ill. All PD practices were shared with other community members through a 6 month PD-informed intervention which included training of volunteers, interactive health education sessions, role plays, art competitions and an advocacy seminar. A one-year follow up survey will be conducted in Aug 2011 to better evaluate this intervention, but preliminary results suggest that PD can serve as 1) a malaria intervention targeting migrants; 2) an alternative or supplementary method to deliver existing BCC/IEC interventions; and 3) an innovative model to promote community-based, bottom-up approaches.

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IDENTIFICATION OF NOVEL BINDING PARTNERS OF ANTI-*PLASMODIUM* IMMUNE FACTORS

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In *Anopheles gambiae* mosquitoes, defense against *Plasmodium* parasites is controlled by the innate immune system. We have previously identified a number of *Plasmodium* effector molecules through the use of microarray analysis and RNAi mediated gene silencing assays. However, their exact mechanism of action remains largely unknown. As a first step to further elucidate the signaling cascades and protein complexes involved in anti-*Plasmodium* defenses, we utilized the yeast two hybrid system to identify novel binding partners of the selected anti-*Plasmodium* effectors AGMDL1, APL1A, APL1C, FBN9, FBN39 and LLRD7. Thus far, we have discovered a number of different interacting proteins some of whose functions in immunity have not been previously described. RNAi studies showed that many of these *Plasmodium* effector interacting proteins also play a role in controlling *Plasmodium* development in the mosquito midgut. We are currently using biochemical methods to further characterize and investigate the role of these newly discovered protein-protein interactions in the innate immune response against *Plasmodium* infection.

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TRANSCRIPTOMIC AND FUNCTIONAL ANALYSIS OF DENGUE VIRUS INFECTION IN THE *AEDES AEGYPTI* SALIVARY GLAND

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The *Aedes aegypti* salivary gland plays a crucial role in dengue virus (DENV) transmission. Infection of the salivary gland is essential for horizontal DENV transmission to occur, and the gland also produces numerous immune-modulatory, vasodilatory, and anti-coagulant molecules that facilitate bloodmeal acquisition. To characterize the anti-DENV immune response in this organ, we performed a genome-wide microarray analysis of the naïve and DENV-responsive *A. aegypti* salivary gland transcriptomes. Two candidate genes identified from this analysis had the ability to modulate mosquito midgut DENV titers: RNAi-mediated knockdown of MD6 (an MD2-like gene family member) significantly decreased DENV titers, while knockdown of LAP4 (a gene encoding a leucine-rich repeat-containing protein) significantly increased DENV titers. Similar assays are currently being performed to evaluate their effect on salivary gland DENV replication. Our microarray analysis also identified two salivary gland-enriched odorant-binding proteins (OBPs) that were also induced by DENV infection in this organ. Knockdown of these OBPs in the salivary gland decreased the percentage of mosquitoes that probed on an anesthetized mouse. In addition, mosquitoes that did probe took longer to initiate the probe, and spent more time probing before successfully acquiring a bloodmeal. Thus, DENV infection in the salivary gland not only regulates genes that modulate virus replication, but also genes that potentially affect bloodmeal acquisition (and hence DENV transmission) by modifying mosquito host-seeking or probing behavior. Further characterization of these genes will yield a clearer picture of host-pathogen interactions in this poorly-studied organ.

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THE SIGNIFICANCE OF A MOSQUITO HYPER-VARIABLE PATTERN RECOGNITION RECEPTOR, *AGDSCAM*, IN THE ANTI-*PLASMODIUM* DEFENSE

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The innate immune system of the mosquito, unlike that of vertebrates, appears to lack the adaptive immunity and immunological memory, which relies on the limited numbers of germ line-encoded pattern recognition receptors to generate the specificity towards the pathogen recognition. The studies of the molecular mechanisms that determine the recognition of the pathogens are of the biggest interest. AgDscam, *Anopheles gambiae* down syndrome cell adhesion molecule, which have the potential to generate 31,920 alternative splice forms with different interaction specificities. We have shown that AgDscam is an essential hypervariable receptor of the *Anopheles gambiae* immune surveillance system, which produces splice form repertoires that are pathogen challenge-specific. In this study, we have used siRNA gene silencing approach to target the specific isoforms of AgDscam and are able to show that AgDscam's anti-*Plasmodium* responses are splice-form specific. We furthermore show this defense specificity by using transgenic *A. stephensi* mosquitoes which are overexpressing either *Plasmodium falciparum* or *P. berghei* specific spliceforms upon a blood meal. Similarly, transgenic mosquitoes also showed specificities in controlling microbial proliferation in their midguts.

At cellular level, through confocal microscopy we show co-localization of AgDscam with both *P. falciparum* and *P. berghei* which suggests that AgDscam is directly associated with the parasites. Transgenic mosquitoes with overexpression of *P. falciparum* specific isoform has more abundant protein co-localized with the *P. falciparum*. Gene expression analyses suggests that the Toll and Imd immune pathways are involved in the regulation of alternative splicing of AgDscam. Preliminary experiments show that several putative immune responsive putative splicing factors are involved in the regulation of AgDscam splicing. Further detailed studies are undergoing to investigate the pathogen binding specificities of different spliceform through an *in vitro* recombinant protein strategy.

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APPLICATION OF TANDEM MASS SPECTROMETRY FOR ARGININE QUANTIFICATION IN *AEDES AEGYPTI* FEMALES, THE MAIN VECTORS OF DENGUE AND YELLOW FEVER

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In order to gain insights into uricolysis and argininolysis, two metabolic pathways involved in the urea synthesis in *Aedes aegypti*, it is necessary to develop an efficient method to identify and quantify arginine (Arg) in mosquitoes. In our laboratory, a procedure used for the identification of Arg in urea disorders in newborn babies was adapted to identifying and quantifying Arg in mosquito excreta by tandem mass spectrometry. We derivatized 14N-Arg and 15N2-Arg (labeled guanidine) as isobutyl esters and then the fragmentation patterns of both compounds were analyzed by electrospray ionization tandem mass spectrometry. When isobutyl esters of 14N-Arg (m/z=231) or labeled Arg (m/z=233) are fragmented, neutral losses of 161 Da, or 163 Da respectively, occur and produce fragments of m/z=70. Based on these studies, the mosquito excreta from blood fed females were collected and mixed with 15N2-Arg (an internal standard). The samples were then derivatized as isobutyl esters and sprayed into a Q-trap 4000 mass spectrometer. The levels of Arg in the excreta of a single mosquito, at different times after a blood meal, can be successfully monitored at 30 eV by multiple reaction monitoring scans. This method provides an efficient and rapid tool to quantify Arg in mosquitoes, as well as in other insects whose small size severely limits the use of more conventional biochemical methods of analysis.

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OVARY ECDYSTEROIDOGENIC HORMONE (OEH) STIMULATES BLOOD DIGESTION AND EGG MATURATION IN FEMALE *AEDES AEGYPTI* INDEPENDENT OF INSULIN SIGNALING

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The yellow fever mosquito *Aedes aegypti* is an important vector of human disease pathogens and is a model for invertebrate endocrinology. Blood ingestion by females stimulates the release of two types of neuropeptides, Ovary Ecdysteroidogenic Hormone (OEH) and an insulin-like peptide (ILP3), which activate the ovaries to produce ecdysteroid hormones. We recently reported that ILP3 directly binds to the mosquito insulin receptor (IR) and regulates vitellogenesis and blood digestion. Its activation of these processes is greatly amplified by amino acid sensing and activation of the target of rapamycin (TOR) pathway. We now show that OEH similarly regulates these processes and depends on amplification by amino acids and TOR signaling. However, OEH does not activate IR phosphorylation, and its activity is not affected by a specific IR inhibitor. Together these results indicate that OEH and ILPs both regulate egg maturation in this species, but their activity involves interactions with different receptors.

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TRANSLATION REGULATION IN RESPONSE TO *PLASMODIUM FALCIPARUM* INFECTION IN *ANOPHELES GAMBIAE*

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Midgut invasion is the greatest bottleneck among the mosquito host stages of the *Plasmodium* lifecycle as a rapidly responding immune system labels ookinetes and recruits killing factors from the midgut and surrounding tissues, dramatically reducing the population of invading ookinetes before they can successfully traverse the midgut epithelium. As it is so crucial to *Plasmodium* survival, the midgut bottleneck represents one of the best points in the *Plasmodium* lifecycle to combat malaria. A better understanding of genetic regulation of the vector in response to parasitization at this point may provide critical targets and enhance strategies for impacting parasites at one of their weakest links, yet little is known at the level of translation. We isolated *An. gambiae* midguts at 24 hours after a bloodmeal with *P. falciparum* gametocytes, followed by sucrose gradient fractionation to separate transcripts based upon association with polysomes. Transcriptome sequencing has provided over 32 million reads representing over 10,000 different transcripts. We have found 577 genes where transcriptional change is insignificant (2fold) between infected and uninfected samples, with a $p<0.05$. This led to the identification of 11 different transcripts involved in immune response that underwent significantly higher polysomal association and therefore more active translation during infection. REL-2, a NF-kB-like transcription factor, was more actively translated in response to infection while insignificant change occurred at the steady state mRNA level, which supports previous findings that REL-2 plays important roles in the immune response to *Plasmodium*. We are currently examining the extent to which the different isoforms of REL-2 are involved. IKK2, an Anopheline ortholog of a Drosophila Imd-pathway component linked to REL-2, was also upregulated at the translational level. Our findings support the hypothesis that the antimalarial response of *Anopheles* occurs at both the transcriptional and translational level.

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HUMAN DENGUE-RESISTANT GENE, FKBP1 IS FUNCTIONALLY CONSERVED IN *AEDES AEGYPTI*, YELLOW FEVER MOSQUITO

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There are 22 human genes that encode resistant factors to both West Nile virus (WNV) and dengue virus (DV). These genes were identified by genome-wide RNAi screening using HeLa cells, in which RNAi silencing of those 22 individual genes caused DV titers to increase. We investigated orthology and functional conservation of the 22 human dengue virus resistant (DVR) genes in the yellow fever mosquito, *Aedes aegypti*. Homology searches of the 22 human DVR genes identified 12 orthologs in *Ae. aegypti*. Functional conservation of the 12 *Ae. aegypti* DVR orthologs was examined by siRNA silencing and co-infection of dengue virus in an *Ae. aegypti* cell line (Aag2). After Aag2 cells were transfected with siRNAs individually targeting each of 12 DVR orthologs and subsequently infected with DV (NGC Type 2, m.o.i.= 0.05), the cell culture media were harvested to determine the DV titers by a standard plaque assay at 3 days post infection. Among the 12 DVR orthologs tested, the knockdown of only one candidate, fk506-binding protein 1 (FKBP1), promoted dengue replication by 24 and 51% compared to the control group in two independent experiments, each with six replicates (*t* test, $P<0.01$). This result suggests that Aag2 cells knocked down for FKBP1 became more susceptible to dengue virus infection than the control. Apparently, the human DVR gene, FKBP1B, is functionally conserved in *Ae. aegypti*. Humans and mosquitoes may share a common mechanism(s) of dengue resistance mediated via FKBP. FKBP1s are immunophilins that bind to

immune suppressive drugs such as FK506 or rapamycin to suppress T-cell activation and proliferation via deactivation of NF-AT or NF-κB in mammals. Both human and *Ae. aegypti* FKBP1 proteins consist of 108 amino acids and share 67% amino acid identity between them. High sequence homology and functional conservation of FKBP1 as a DVR in humans and *Ae. aegypti* should warrant further studies to elucidate the inhibitory mechanism of FKBP1 against DV in humans and mosquitoes. These studies may provide a novel paradigm to control dengue transmission.

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JUVENILE HORMONE III SUPPRESSES FOXO IN THE FAT BODY AND REDUCES FAT ACCUMULATION IN THE DIAPAUSING MOSQUITO, *CULEX PIPIENS*

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Juvenile hormone III (JH) controls a variety of physiological and developmental events including diapause and nutrient metabolism. The focal point of endocrine regulation in adult reproductive diapause is initiated by a halt of JH synthesis. The other key molecular event is the signaling pathway from insulin to forkhead transcription factor (FOXO) in diapause females. We hypothesized that halt of JH synthesis is related to activation of FOXO, which results in increasing lipid reserves in the fat body at the onset of the diapause program. In this study, the full length sequence of the foxo gene was characterized, and the protein abundance pattern of the foxo gene product was analyzed by immunoblotting and immunohistochemistry. FOXO was predominantly present in the fat body of diapausing females and to a less extent in the fat body of nondiapausing females; it was either absent or very weakly present in other tissues one week after adult eclosion. Interestingly, when we topically applied JH to diapause-destined females after adult eclosion, FOXO was suppressed and fat accumulation was reduced. In contrast, the activity of nucleoli, observed by confocal microscopy with DAPI-staining, was higher in fat body cells of diapausing females that received a topical application of JH, as compared to those not receiving JH.

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BROAD SCREENING OF *ANOPHELES*` MICROBIOTA WITH ANTI-*PLASMODIUM* EFFECT

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Malaria is disease caused by parasites of the *Plasmodium* genus that are transmitted by anopheline mosquitoes. For successful malaria transmission the parasite needs to complete a complex life cycle in their vectors. *Plasmodium* spends its firsts 24 hours in the mosquito midgut lumen. During this period the parasites pass through different developmental stages and are exposed to the mosquito's immune responses, digestive enzymes and the resident microbial flora before they can invade the midgut epithelial cells. We have identified a variety of bacterial strains from wild caught mosquito populations and showed that an Enterobacter species has a potent anti-*Plasmodium* activity. Here we present a comprehensive analysis of anti-*Plasmodium* activity of seven different field isolates of bacteria: *Comamonas,* two *Pseudomonas, Acinetobacter, Serratia, Chryseobacterium* and *Pantoea*. *In vitro* and *in vivo* experiments showed that different strains of bacteria are capable of inhibiting *Plasmodium* development at different stages and through different mechanisms. Some of these bacteria species may be used for the development of powerful biocontrol strategies to block malaria transmission.

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THE EFFECT OF CHOLESTEROL ON *WOLBACHIA*-MEDIATED VIRAL INTERFERENCE AND HOST FECUNDITY IN THE DENGUE MOSQUITO *AEDES AEGYPTI*

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Infection of the dengue mosquito *Aedes aegypti* with the bacterial endosymbiont *Wolbachia pipientis* induces a number of physiological manipulations in the host. These include interference against arboviruses, including dengue virus (DENV) and also reduced fecundity and egg viability after bloodfeeding on non-human hosts. These manipulations are potentially the product of competition with the host for scarce nutritional resources. One such resource is cholesterol, which is only available to adult mosquitoes after a bloodmeal. Cholesterol is critical to cellular signalling and membrane organisation, to egg and larval development in mosquitoes, vacuole formation in endosymbiotic bacteria, and viral entry and replication in certain arboviruses. To observe if cholesterol was involved in viral interference and fecundity in *Wolbachia*-infected *A. aegypti* we fed groups of female LEWIS rats on four different cholesterol-enriched diets for 10 weeks, and then obtained their blood via cardiac puncture. Blood cholesterol levels of rat and human (control) blood were estimated using the Amplex Red Cholesterol Quantification Kit (Molecular Probes, Invitrogen). These bloods were then fed to *Wolb*+ and *Wolb*- mosquitoes and fecundity and egg viability were observed over four replicate experiments. Results indicated that cholesterol had no significant, repeatable effect on either fecundity or egg viability, suggesting it is not involved in the reduced fecundity manipulation. To examine the effect on viral interference, aliquots of rat blood were dosed with DENV and fed to *Wolb*+ and *Wolb*- mosquitoes over three replicate experiments. At 14 days post-feeding, DENV copy numbers were quantified using qPCR. Results indicated that for *Wolb*- mosquitoes, cholesterol levels had no effect on DENV load. The majority of *Wolb*+ mosquitoes were refractory to DENV regardless of cholesterol levels, however a small subset showed DENV breakthrough with a strong negative correlation between cholesterol levels and viral load suggesting a possible role for cholesterol in *Wolbachia*-mediated viral interference.

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THE CHARACTERIZATION OF GLUTAMATE-GATED CHLORIDE CHANNELS FROM *ANOPHELES GAMBIAE* AS A POTENTIAL INSECTICIDAL TARGET

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Malaria is one of the most devastating mosquito-borne diseases, causing millions of deaths annually. Current malaria parasite transmission control methods are heavily reliant on insecticide treatment of walls and bednets. This has fostered insecticide resistance, and focuses control primarily against endophagic mosquitoes. There is a need to develop insecticides with new modes of actions and the ability to control the spread of malaria parasites from exophagic mosquitoes. We have recently demonstrated the potential of mass drug administrations of the anthelmintic drug ivermectin to control malaria parasite transmission. Glutamate-gated chloride channels (GluCls) are the target of ivermectin, and other macrocyclic lactones. These drugs agonize GluCls located on muscle tissue causing paralysis and death. The purpose of this study is to assess GluCls as a target of mosquitocidal drugs and vaccines. We have cloned GluCl from *Anopheles gambiae*, the primary vector of malaria in sub-Saharan Africa. To characterize channel activity we have expressed the channel in the *Xenopus laevis* oocyte. Using whole-cell electrophysiological analysis we obtained initial evidence of channel function in response to glutamate and

ivermectin. To more precisely measure channel activity we have expressed the channel in the C6/36 mosquito cell line. Using outside-out voltage clamp technique and the piezo liquid switch perfusion system we have obtained initial evidence of channel activity in response to glutamate. To test AgGluCl as a potential target for a mosquitocidal vaccine, we fed *A. gambiae* blood meals spiked with increasing titers of a polyclonal antibody against the extracellular N-terminal domain of GluCl. Anti-GluCl antibodies caused rapid paralysis and death of nearly all of mosquitoes that blood fed on the highest concentration (3 mg/ml), and more than 50% of the mosquitoes that blood fed on the 1.5 mg/ml antibody concentration. In conclusion, we have initial *in vitro* and *in vivo* evidence that *An. gambiae* GluCls are a potential target for mosquitocidal drugs and vaccines.

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THE EFFECT OF EXPRESSING APOPTOSIS-REGULATING GENES ON SINDBIS VIRUS REPLICATION IN THE MOSQUITO VECTOR *AEDES AEGYPTI*

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Apoptosis is known to be a defense against some viruses in insects and mammals. The role of apoptosis in mosquito immunity against arboviruses is largely unexplored; although some studies have suggested a correlation between apoptosis and resistance to infection, no direct evidence exists supporting a causal relationship. The mosquito *Aedes aegypti* is an important vector for yellow fever and dengue. Because of its ability to be engineered to express foreign genes, Sindbis virus (SINV; Togaviridae) was used to study the possible role of apoptosis in *A. aegypti* immunity against arboviruses. A series of infectious SINV clones based on the construct p5'dsMRE16ic was engineered to express pro-apoptotic or anti-apoptotic genes from a duplicated viral subgenomic promoter. Control virus clones were also constructed containing the same inserts but in antisense orientation. A previous study demonstrated that the clones expressing apoptosis-regulating genes either induced or inhibited apoptosis as expected in cultured *A. albopictus* cells. In this study, adult female *A. aegypti* were infected by artificial blood meal containing the recombinant SINV clones, and virus infection was analyzed at various times post-infection in midguts by immunofluorescence (IFA) against the viral E2 protein. Viral replication was also monitored by titering the amount of infectious virus in individual mosquitoes. Virus clones expressing pro-apoptotic factors caused increased caspase activity and TUNEL staining in midgut compared to controls, indicating that apoptosis was stimulated by these virus clones. IFA and viral titer results indicated that infection with SINV clones expressing pro-apoptotic genes decreased the rate and spread of virus infection in the mosquito compared to controls. The results suggest that if apoptosis occurs in infected cells, it may be able to play a role in defense against arbovirus infection in mosquitoes.

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XANTHURENIC ACID RECEPTOR STUDIES IN GAMETOCYTES OF *PLASMODIUM FALCIPARUM*

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Xanthurenic acid (XA) had been identified as the gametocyte activating factor (GAF), which may play a major role in *Plasmodium* gametogenesis in the gut of the mosquito. There is evidence that XA may stimulate this process via a G-protein mechanism. XA may bind to a G-protein receptor, and this complex in turn may stimulate a GTP dependent kinase activity begining a cascade of events for signal transduction. It is our hypothesis that XA ligand binds to a 'XA binding G-protein associated receptor'. *P. falciparum* strain 3D7, is known to be responsive to XA stimulation and was used to obtain the sexual forms (gametocytes). Gametocytes/RBC preparations were produced and tested in XA radio-ligand binding assay along with RBC controls. It was found that XA was binding significantly to the control RBCs and hence we started examining the XA binding to RBC-membrane free gametocytes. Initial experiments showed a large difference in binding of XA to the naked gametocytes *vs.* RBCs. Cold XA did abolish ^{3}H-XA binding at the concentration tested (100:1 cold to hot) indicating the binding was specific to a XA receptor. Saturation experiments with RBC membrane-free gametocytes showed that concentrations of XA at 5 µM did not achieve saturation with 0.2 x 10^{5} gametocytes. With 200,000 gametocytes at the highest concentration tested (5 µM) this would indicate either a very high concentration of receptor (6x10^{6}/gametocyte) or one with a low binding affinity. The binding of XA was temperature dependent indicating that some energy requiring step may be necessary to support the receptor binding. If identified the XA receptor can potentially be targeted for vaccine and drug development.

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SALIVARY GLAND SURFACE (SGS) PROTEINS FORM A MAJOR COMPONENT AND IMMUNOGEN OF MOSQUITO SALIVA

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The SGSs (salivary gland surface) are a family of large proteins, some of which are expressed in the salivary glands of female mosquitoes. Although little else is known about them, mosquito SGSs appear to have been horizontally transferred into the mosquito genome from *Wolbachia* proteobacteria and have been implicated in salivary gland invasion by *Plasmodium* parasites. Using proteomic and immunologic analyses, we show that the expression of *Anopheles gambiae* SGS4 and SGS5 is associated with blood feeding and that SGSs form a major component of the saliva of both *An. gambiae* and *Aedes aegypti*. Western blots and RT-PCR showed that *Anopheles* SGS4 and SGS5 expression is salivary gland and female specific, increases with age, increases after blood feeding, and SGS4 and SGS5 levels fluctuate in a circadian manner. Immunohistochemistry and Western blots showed that *An. gambiae* SGS4 and SGS5 localize primarily in the distal lateral lobes of the salivary glands, with lower levels also found in the secretory duct of the proximal region. Additional Western blot and bioinformatic analyses suggest that SGSs are secreted in a nonclassical pathway which includes proteolysis, yielding a ≈300 kDa secreted N-terminal fragment. SDS-PAGE, Western blots, "bite blots", and immunization via mosquito bites revealed that SGSs form a major protein component and immunogen in *Anopheles* saliva and that they are likely the most prevalent proteins (by mass) in *Ae. aegypti* saliva. SGS's incriminating mass, in combination with data from others, suggest that SGSs might play an immunomodulatory role during mosquito blood feeding.

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AEDES AEGYPTI IMMUNE SYSTEM ACTIVATION BY THE INTRACELLULAR BACTERIUM *WOLBACHIA PIPIENTIS* AND INTERFERENCE WITH RNA VIRUSES REPLICATION

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The intracellular bacterium *Wolbachia pipientis* naturally infects up to 70% of all insect species. Its success can be attributed to the ability of *Wolbachia* to manipulate the reproduction of its hosts to enhance its own transmission. This transmission advantage might also be enhanced by its ability to confer protection to insect hosts agains a range of pathogens. The mosquito *Aedes aegypti* is not naturally infected by *Wolbachia* but in recent years it has been artificially infected with a number of different *Wolbachia* strains which block transmission of both dengue and chikungunya viruses (*w*Mel and *w*MelPop). The molecular origin of "*Wolbachia*-mediated-protection" in its natural host *D. melanogaster* as well as in its heterologous host *A. aegypti* is unclear. Some studies suggest that *Wolbachia* primes the insect innate immune system and others

that the bacterium competes directly with viruses for limiting subcellular resources. To further understand this issue we undertook a comparative analysis of the *A. aegypti* transcriptome in response to *w*MelPop and *w*Mel infections. 210 gene transcripts were affected by both *Wolbachia* strains. Among them an elevated number of immune genes were activated. Because *w*MelPop and *w*Mel also protect their natural host *Drosophila melanogaster* against RNA viruses, a subset of immune gene transcripts were then analyzed in flies in response to the two *Wolbachia* strains. No consistent immune activation was detected. This comparison in both native and heterologous hosts suggests that immune priming by *Wolbachia* is an artefact due to its recent introduction into *A. aegypti*. This immune response might contribute to the pathogen protection effect, but our data indicate that the fundamental mechanism is more likely to be related to competition for limiting resources between *Wolbachia* and RNA viruses.

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QUANTITATIVE PROTEOMIC ANALYSIS OF O'NYONG-NYONG VIRUS INFECTION IN THE *ANOPHELES GAMBIAE* MIDGUT

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Multiplex quantitative proteomics has become an invaluable tool in assessing genome-wide protein modulations of organisms in response to pathogens. The protein expression patterns of mosquitoes stemming from arbovirus infection are particularly intriguing. These studies not only offer insight into host-virus interactions, but may also reveal pathogenesis factors significant to human disease; for example, differences in human and mosquito-vector responses to the same alphavirus, may illuminate how apoptosis is induced in the former, but a persistent, controlled infection in the latter. The current study investigated protein expression profiles of *Anopheles gambiae* midgut tissue resulting from an alphavirus infection (o'nyong nyong virus, ONNV, Togaviridae). Three pools of 50 midguts were harvested from uninfected (control) and infected mosquitoes at six-days post infection. Total protein extracts were then comparatively quantified using 6-plex tandem mass tagging (TMT). As a result, 22 peptides were found to have been significantly modulated, either positively or negatively, in the ONNV-infected midguts compared to the control (Bonferroni adjusted $P<0.1$). Intriguingly, among those peptides identified was an ortholog of an FKBP-type peptide that was overexpressed in the ONNV-infected mosquito midgut. More interestingly, FKBP has recently been identified as a human resistance factor to both dengue and West Nile viruses, suggesting possible functional conservation of antiviral activity mediated via this protein. Also of interest were peptides associated with pathways known to be targeted by alphavirus nonstructural peptides, and those involved in modifying ion transport, which in mammalian cell culture is an early indicator of cytopathic effect. These and other peptide responses will be discussed in the context of mosquito-pathogen interactions, as well as their implications for future studies.

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EFFECTS OF HUMAN IGF1 ON LIFESPAN, IMMUNE SIGNALING AND PARASITE SURVIVAL IN THE MALARIA VECTOR *ANOPHELES STEPHENSI*

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The highly conserved insulin/IGF-like signaling (IIS) pathway regulates metabolism, development, lifespan, and immunity across a wide range of organisms. Previous studies in our laboratory show that human insulin ingested in the blood meal activates mosquito IIS, resulting in attenuated lifespan and increased malaria parasite infection. Since human IGF1 (hIGF1) is present at higher concentrations in blood than insulin and is closely tied to lifespan and immune processes, we predicted that hIGF1 could affect lifespan and parasite infection in *Anopheles stephensi*. Preliminary results showed that hIGF1 in the blood meal induced activation of FOXO and p70S6K, proteins of the PI3K branch of the IIS, in the *An. stephensi* midgut. This activation was dose dependent, with low levels of hIGF1 inducing greater levels of IIS activation than higher levels. In addition, low concentrations of hIGF1 extended mosquito lifespan by 5 days or 11.8% relative to controls, while higher levels of hIGF1 did not differ from the control treatment. Effects of hIGF1 treatment on asexual parasite growth and infectivity were determined with a standardized *in vitro* assay and with *An. stephensi* infection, respectively. Our data showed that while hIGF1 did not alter growth of asexual *P. falciparum*, it did alter malaria parasite development in the mosquito, presumably via IIS activation. We have demonstrated that the IIS pathway is a promising target to genetically alter mosquito lifespan or to block malaria parasite transmission. However, a more complete understanding of the naturally occurring ligands in blood that activate IIS will be necessary to optimize strategies for transgenesis.

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LEVEL OF ANTIBODIES ANTI- *AEDES AEGYPTI* SALIVA AND CLINICAL PRESENTATION OF DENGUE FEVER IN NORTE DE SANTANDER COLOMBIA

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Dengue is endemic in Colombia and it is highly prevalent in Norte de Santander where its main vector is *Aedes aegypti*. We evaluate the association between the levels of anti-*Ae. aegypti* saliva IgG and IgM antibodies in people with dengue fever and healthy individuals. We found that the level of IgG antibodies where significantly higher in people with confirmed dengue fever than in people with febrile syndrome and/or healthy individuals, in contrast to the level of IgM antibodies in which we did not found any significant difference among the study groups. Additionally, we found differences in the salivary proteins recognized by the pool of serum from these three groups. This results suggest that antibodies against *Ae. aegypti* saliva are useful markers for both mosquito bite exposure and risk for clinical dengue fever.

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HABITAT SUITABILITY AND SPATIAL DISTRIBUTION OF FIVE *ANOPHELES* SPECIES IN AMAZONIAN BRAZIL

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Availability of suitable larval aquatic habitat may strongly influence the distribution and population dynamics of anopheline mosquito malaria vectors (Dipera: Culicidae. Mosquitoes breed in a wide variety of terrestrial water accumulations, but individual species prefer particular types of habitats, with many environmental variables correlated with the presence or development quality of *Anopheles* larvae. Data on presence or absence of five species, *An. oryzalimnetes*, *An. marajoara*, *An. janconnae*, *An. triannulatus* and *An. nuneztovari* were used for analysis of associations based on physiochemical factors of the water, canopy coverage, shade and available resources. 54 aquatic habitats containing mosquito larvae were characterized in Pará and Roraima states. *An. triannulatus* and *An. nuneztovari* had the greatest overall occurrence across the distribution; however *An. marajoara* had the greatest overall abundance. A principal

components analysis was conducted to examine multifactor environmental effects on larval density. The most important factors associated with larval abundances were water quality, available resources, and to a lesser extent canopy protection, temperature and water movement account for 73% of the variance among habitats. Four of the five species appear to be more specialized, suggesting possible adaptive niches. Negative correlations of abundances and environmental variables were found for: available resources for *An. oryzalimnetes*; temperature and water movement for *An. marajoara;* canopy protection and water movement for *An. nuneztovari*; and water quality and canopy coverage for *An. janconnae*. *An. janconnae* also showed a positive correlation of abundance with available resources and water movement. *An. triannulatus* had no significant correlations suggesting it is a habitat generalist. Increasingly warm and variable climate is likely to increase the range and abundance of many insect vectors. Comparison and characterization of larval habitats is critical for understanding the spatial and temporal distribution patterns of anopheline species and for implementation of control strategies.

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MARK- RELEASE-RECAPTURE STUDY TO MEASURE DISPERSAL OF *AEDES ALBOPICTUS* IN CHIANG MAI PROVINCE, NORTHERN THAILAND

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Aedes albopictus is widely distributed throughout Thailand and plays an important role in transmitting viral diseases i.e dengue fever and chikungunya. Adult control is targeted within 100 meters around a patient house. To ascertain whether this limit is appropriate we conducted a mark-release-recapture study to measure dispersal of *Ae. albopictus* in the rural area of Chiang Mai northern Thailand, where this mosquito species is found in abundance. Male and female mosquitoes reared from wild-collected immature stages were marked with fluorescent dust. A total of 3,000 female and 1,000 male were replicated releases during dry (March 2010) and wet season (July 2010). Recapture sites were set at 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275 and 300 meters respectively. Collections were made 6 days post-release. In each recapture site one pair of collectors collecting the mosquito every 10 minutes by using the sweeping hand net and aspirator. Collection started from 6.00 am to 6.00 pm. during 6 day post release. As a total of 177 and 36 female (5.9%, 1.2%), 36 and 11 male (3.6 %, 1.1 %) were recaptured in wet and dry season respectively. In wet season the furthest being caught was 275 meters from release point and the mean distance traveled was 153 m. In dry season, mosquitoes fly shorter of 150 meters furthest from the release point. There was a significant tendency for dispersal in wet season, and the recapture dates lasted till 6 days while it lasted 4 days post release in dry season. The result suggests that adulticiding may have to extend beyond 100 meters if at least 6 days has elapsed since *Aedes* mosquito could have fed upon viremic dengue cases.

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PREVALENCE OF *ANOPHELES* SPECIES AND THEIR INFECTION STATUS IN A MALARIA HYPOENDEMIC AREA OF RURAL BANDARBAN, BANGLADESH

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Bandarban, a district in the Chittagong Hill Tracts of Bangladesh, is hypoendemic for malaria. In 2009 an entomological study was begun to identify the malaria vectors, their population and infection dynamics year round during which a large scale malaria epidemiological study on more than 4,500 households with 20,000 people is ongoing. More than 95% of these households have at least one treated bednet. *Anopheles* mosquitoes were collected indoors with CDC miniature light-traps every month from selected houses in the study site. Each trap was deployed for at least 12 hours (6 pm to 6 am). After collection, mosquitoes were identified to species level using standard keys. ELISA was performed to detect *Plasmodium falciparum*, *P. vivax*-210, and *P. vivax*-247 circumsporozoite proteins (CSP). In total, 8,169 female *Anopheles* mosquitoes belonging to 22 species were collected from 1,619 trap-nights extending through December 2010. *An. philippinensis/nivipes* complex was the predominant species (25.3%), followed by *An. jeyporiensis* (16.8%) and *An. vagus* (14%). Seasonal variation existed in abundance of mosquito species. Ninety mosquitoes belonging to 13 species tested positive for *Plasmodium* infection, with an overall infection rate of 1.1% (90 of 8,061). The highest infection rate was found in *An. nigerrimus* complex (2.9%) followed by *An. maculatus* (2.7%) and *An. umbrosus* (2.4%). For the first time infections in *An. jeyporiensis* and *An. kochi* were documented in Bangladesh. Other important infected species were *An. baimaii*, *An. minimus s.l.*, *An. philippinensis/ nivipes* and *An. karwari*. In terms of density and incrimination, the *An. philippinensis/nivipes* complex*, An. jeyporiensis*, *An. vagus, An. nigerrimus* complex and *An. maculatus* seemed to play vital roles in malaria transmission in rural Bandarban. This study suggests that even in presence of insecticide impregnated bed-nets, a number of *Anopheles* species can still play a role in the transmission of malaria in Bangladesh.

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TOWARDS THE CONTROL OF CHIKUNGUNYA VECTOR IN LA REUNION USING THE STERILE INSECT TECHNIQUE: SEXUAL COMPETITIVENESS AND MATING SUCCESS OF STERILIZED MALES *AEDES ALBOPICTUS*

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The sterile insect technique (SIT) is widely used as part of area-wide integrated pest management programs for insects such as fruit flies, screw-worm flies and tsetse flies. It is based on the releases of large numbers of sterile males, which transfer their sterile sperm to wild females for the fertilization of the eggs, resulting in embryonic death. The lack of offspring could then lead to a decrease of the wild population density. The SIT is currently being developed at the FAO/IAEA in Vienna and on La Reunion, France, for the potential control of *Aedes albopictus*, vector of Chikungunya and secondary vector of Dengue viruses. In this context, we investigated the impact of the sterilisation process on male mating success and sexual competitiveness in the laboratory and under semi-field conditions. Males from a young laboratory colony (F4) were irradiated as pupae (>20h old) with gamma-rays at 35 or 40 Gy. In the laboratory, the time for male sexual maturation was not affected by the irradiation, neither was the insemination rate of 4 days old males as compared to un-irradiated ones. The daily individual mating success of sterile males during 15 days was reduced as compared to un-irradiated males; however the differences were significant only during the second week. Sterility was maintained over successive matings and after a resting period. Under semi-field conditions, sterile males competed well against wild males, though a resting period of 5 days in the laboratory before release greatly improved their efficiency. A ratio of five sterile to one wild male resulted in a two-fold reduction of the wild females' mean fertility. Valuable information on the effects of the sterilization process was collected and it is suggested that an efficient reduction of a wild population might be achieved by releasing sterile males as adults. Moreover, the results indicate that a resting period allows a recovery from radiation-induced somatic damages which improved the biological quality of the males.

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IMPACT OF HIGH COVERAGE WITH INSECTICIDE TREATED NETS ON MALARIA VECTOR TRANSMISSION INDICES IN SOUTH COAST KENYA

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In studies conducted during 1994-1998 on the south coast of Kenya, *Anopheles funestus* and *An. gambiae* s.s. were the primary malaria vectors, with *An. arabiensis* and *An. merus* playing a secondary role. To evaluate the impact of a recent upsurge in household bednet use in this area, the present study collected indoor resting malaria vectors in the same region using pyrethrum spray catches over a 21-mo period in 2009-2010. Species distribution of the recovered malaria vectors was determined, and house densities (mosquitoes/house) and human-biting rates (HBR) were estimated and compared with those reported in the same study area between 1994-1998, when bed net coverage was minimal. Present day bednet coverage and use were also determined in the houses where mosquito collections were conducted. In 2009-2011, the predominant malaria vectors in the study area were *An. funestus* and *An. gambiae* s.l., both of which are highly anthropophilic. A significant decline in the relative proportion of *An. gambiae* s.s. was observed, coupled with a proportionate increase of *An. arabiensis*. After 3-4 years of 60-86% coverage with ITNs, the density and human biting rate of indoor resting mosquitoes was estimated to have been reduced by more than 97% and 90% for *An. funestus* and *An. gambiae* s.l. respectively. The host feeding choice of both vectors shifted to non-human vertebrates, with an increase of 15% for *An. gambiae* s.l. and 5% for *An. funestus.* HBR was higher in houses without nets compared to houses with nets. This difference was significant for *An. funestus* but not for *An. gambiae* s.l. These entomological indices indicate a diminishing role of *An. gambiae* s.s. in malaria transmission in the study area due the high bed net coverage. While increasing bed coverage beyond the current levels may not significantly reduce the transmission potential for *An. arabiensis*, it is anticipated that increasing and sustaining high bed net coverage may result in a diminished role for *An. funestus* in malaria transmission.

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STABLE ISOTOPES INDICATE RESOURCE PARTITIONING AND PRIMARY FOOD RESOURCES OF MOSQUITO LARVAE IN WESTERN KENYA

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Larval density and inter-/intra-specific competition have been shown to have a significant effect on the development and growth rates of mosquito larvae. Food resource acquisition and the degree of resource partitioning among larvae sharing the same habitats are poorly understood; however, these are fundamental determinants of aquatic habitat productivity of malaria vectors in western Kenya. The current study uses $\delta^{13}C$ and $\delta^{15}N$ stable isotope analysis to evaluate resource partitioning and primary resources used by naturally occurring mosquito larvae in Iguhu, Kenya. Laboratory reared *Culex quinquifaciatus* and *Anopheles gambiae* s.s. fed with the same diet did not differ significantly from each other or the lab food ($F = 3.7270$, $P = 0.0662$ for $\delta^{13}C$ and $F = 0.0086$, $P = 0.9915$ for $\delta^{15}N$). Preliminary results from three natural habitats indicate that carbon isotope ratios were able to distinguish anopheline and culicine larvae, however nitrogen isotope ratios did not distinguish any difference (Rank sums test, $P = 0.0005$ for $\delta^{13}C$ and $P = 0.46$ for $\delta^{15}N$). Preliminary data suggests that isotopic ratios can also be able to distinguish among anopheline species sharing the same habitat. This work may provide insights into ecological control methods, such as using controphic species as bio-control tools. Controlled microcosm experiments and identification of relative food resource importance using natural stable isotopes are ongoing.

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MULTIPLEX XENOMONITORING OF *WUCHERERIA BANCROFTI* INFECTION AND *ANOPHELES PUNCTULATUS* SPECIES IN PAPUA NEW GUINEA

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The filarial parasite, *Wuchereria bancrofti* (Wb), causes lymphatic filariasis (LF) and is endemic to Papua New Guinea (PNG) with an estimated prevalence of >1 million individuals; *Anopheles punctulatus* sibling species are vectors of Wb. Currently LF elimination campaigns are underway in PNG and it will be important to monitor the progress of these efforts. Here we describe a high throughput post-PCR xeno-monitoring approach coupling an ITS2-based *Punctulatus* group identification method with detection of Wb DNA. Ligation detection reaction-fluorescent microsphere assay (LDR-FMA) strategies specifically differentiated the *Punctulatus* group species known to transmit Wb (*An. punctulatus, An. koliensis*, *An. farauti* s.s., *An. hinesorum* and *An. farauti 4*) and Wb (sensitivity = single microfilaria). Individual microfilaria were isolated from patient samples known to be Wb-positive by conventional parasitological diagnosis. Field-captured mosquitoes (n=1056) from five East Sepik villages were analyzed by the multiplex LDR-FMA. *Anopheles* species PCR amplification and LDR-FMA identification was successful for >90% of the collection, with *An. punctulatus* (AP) as the predominant species collected (n=752), followed by *An. koliensis* (AK) (n=195) and *An. hinesorum* (AH) (n=8). Wb DNA was detected in 19.1% of the collected specimens. Wb DNA was detected in 22% of mosquitoes identified as AP, 6% of AK and 50% of AH. This assay represents a useful xeno-diagnostic strategy for detecting the presence of Wb in specific species of the *Punctulatus* group. Because monitoring prevalence of Wb in the human population requires nighttime blood collections this assay provides an important alternative for evaluating LF-elimination progress. As malaria is co-endemic in PNG and transmitted by members of the *Punctulatus* group, future expansion of this assay seeks to include human *Plasmodium* species.

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A SPATIO-TEMPORAL BAYESIAN MODEL TO IMPROVE SURVEILLANCE AND CONTROL OF WNV VECTORS IN PIEDMONT, NORTHERN ITALY

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West Nile virus (WNV) first emerged in Italy in 1998 in an equine outbreak near the swamps of Padule di Fucecchio, Tuscany. No other cases were detected during the following decade, but there was evidence of continued virus circulation in the country. Between 2008 and 2010 outbreaks with a total of 18 cases of WNV in humans (4 of them fatal) and 70 in horses (19 fatal) occurred in 6 regions, spreading from their initial Northern foci throughout Italy. These outbreaks resulted in increased attention by public health authorities to the role of migratory and residential birds, and local mosquito vectors in virus dispersal and amplification. In order to identify areas with environmental conditions conducive for WNV amplification and transmission, we analyzed

longitudinal (2000-2006) mosquito data from CO_2-baited traps covering the Piedmont Region of Northern Italy, where migratory bird routes and suitable habitats for the vectors overlap. Weather data (temperature, rainfall and relative humidity) were collected from weather stations within the study area. Remote sensing imagery was employed for landscape characterization. We applied spatial statistics and a Bayesian Generalized Linear Mixed Model (GLMM) to: (a) describe the patterns of abundance and distribution of three putative WNV vectors *Ochlerotatus caspius, Culex pipiens* and *Cx. modestus*, and (b) predict the environmental conditions associated with their occurrence and spatial distribution. *Oc. caspius* and *Oc.caspius* were most abundant in rural areas, while *Cx. pipiens* near urban areas. Based on the best models we developed a prediction map of each vector species for the entire Piedmont Region, and predicted areas with the highest risk for WNV introduction and amplification. Our models show the importance of weather and environmental factors in predicting the abundance of mosquito abundance. More generally, our findings provide public health authorities with an useful surveillance tool that can be included in planning for vector in Piedmont and other regions of Northern Italy.

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CHANGES IN HETEROGENEITY AND INEQUALITY OF MALARIA RISK AFTER THE INTRODUCTION OF INSECTICIDE-TREATED BED NETS IN MACHA, ZAMBIA

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In 2007, insecticide-treated bed nets (ITNs) were introduced in Southern Zambia as a malaria control measure. To determine the effect of ITNs on heterogeneity in mosquito host choice, human microsatellite genotypes from blood fed mosquitoes were used to determine the proportion of multiple blood meals taken in a single gonotrophic cycle by *Anopheles arabiensis*, the primary malaria vector in the region. Compared to pre-ITN data, the multiple feeding rate dropped from 18.9% to 9.1%, suggesting that mosquito biting may have focused onto a smaller fraction of the population. To validate this hypothesis, unique human genotypes were identified from blood meals taken by mosquitoes in eight households before and after the introduction of ITNs. Pre-ITN, 20% of individuals in a household provided 40% of blood meals, which increased to 59% post-ITN. To measure heterogeneity over a larger scale, weekly mosquito collections were conducted in 90 households in two village areas over two months. In these collections, the top 20% of households contributed 73.9% of *An. arabiensis* mosquitoes, and 88.7% of blood fed *An. arabiensis*. Statistical analysis showed evidence that these high-risk households were spatially clustered. These data indicate that there is substantial heterogeneity in malaria risk both at the local and household level. This suggests that with additional tools, high risk areas can be identified and targeted for malaria control in order to more efficiently decrease transmission in an effort towards local elimination.

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SEASONAL VARIATION IN METABOLIC RATE AND BODY SIZE IN RELATION TO DRY-SEASON SURVIVAL OF *ANOPHELES GAMBIAE* IN THE SAHEL

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The African malaria mosquito, *Anopheles gambiae*, is distributed throughout sub-Saharan Africa, with much environmental variation across its range. Some populations inhabit areas without surface water for 3-8 months, yet whether populations persist over the long dry season by aestivation of adults or by migration from areas with permanent water soon after the first rains remains unknown. Recent studies suggest that M-form *An. gambiae* aestivate, however, the physiological and behavioral mechanisms by which they survive the dry season are still unknown. We undertook a year-long (October 2009-August 2010) study to measure seasonal variation in metabolism rate (MR) and body size, a key determinant of MR. Measurements were made prior to the dry season, throughout the dry season, and in the next wet season. Further, to assess specific differences between Sahelian populations and those near permanent water, similar measurements were made at another village (130 km away) near the Niger River. Significant and highly-seasonal variation in body size and MR was found in the Sahelian population, but not that located near the river. Body size of the M form increased from the wet season (Aug) to the mid dry season (Feb), but surprisingly fell before the end of the dry season (Apr-May). Notably, body size of the putative founder mosquitoes (appearing 1-2 weeks after the first rains, before new larvae could complete development) remained small. These seasonal differences in body size were observed in both females and males, suggesting two subpopulations of M form mosquitoes with distinct activity patterns exist during the dry season. Contrary to our *a priori* expectations, MR of the M form was higher in the dry season as compared with the wet (after accounting for assay temperature, flight activity, and body size). These results suggest that aestivating adults do not reduce, and possibly increase, their MR. However, we cannot rule out that these measurements pertain only to active, blood-seeking females collected indoors rather than to those hidden in their as-yet unknown shelters.

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DIVERSION EFFECTS FROM A SPATIAL REPELLENT APPROACH FOR DENGUE VECTOR CONTROL

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Aedes aegypti transmits dengue, an important viral disease of public health significance worldwide. Currently, there are no treatments for dengue and disease control relies on reducing vector populations. Control of *Ae. aegypti* has focused on source reduction of larval breeding sites or residual spraying of chemicals to kill adults. However, increasing rates and distribution of dengue cases world-wide indicate that other approaches to vector control are warranted. A proof-of-concept study is currently underway to evaluate the role of spatial repellency to reduce the density of host-seeking *Ae. aegypti* inside homes. The concept includes pushing the vector away from a house prior to entry in order to break man-vector contact using sub-lethal chemical doses. One debate surrounding this approach is whether or not repelled vectors will divert to untreated homes in the area at a greater rate than under conditions in which a spatial repellent is not used. If so, this may pose a greater risk of virus transmission to individuals in unprotected houses. Quantifying this behavior will provide insight into expected disease impact and necessary coverage rates of spatial repellent interventions. We report on *Ae. aegypti* diversion rates generated under field conditions using varying experimental hut mark-release-recapture study designs in both Thailand and Peru and provide comments on integrated approaches to mitigate risk to untreated spaces based on our results.

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OVERVIEW OF THE MALARIA TRANSMISSION CONSORTIUM RESEARCH PROJECT IN ZAMBIA

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Zambia is one of the six nations that the Malaria Transmission Consortium Project implementing various studies to develop tools for measuring malaria transmission intensity, assessing the effectiveness of combinations of malaria vector control interventions, and evaluate the impact of vector behaviour and insecticide resistance on the effectiveness of the control program. Entomological studies were carried out to evaluate mosquito sampling techniques for measuring malaria transmission intensity, and evaluate the impact of vector control interventions on the behaviour of malaria vectors. Our results have shown that light traps set beside to occupied mosquito nets could serve as alternative for conventional human landing catches for sampling the malaria vectors and estimating transmission intensity. Both *Anopheles funestus* and *Anopheles gambiae s.l* were equally predisposed to bite indoor or outdoor ($P = 0.529$ and $P = 0.0524$, respectively) regardless of vector control interventions in the area, and hence the need for additional vector control interventions to prevent residual transmission occurring outdoor and reduce mosquito population at source. Furthermore, large-scale longitudinal community based epidemiological and entomological studies are going on in fourteen clusters each with about 1000 people to determine the impact of combined use of indoor residual spraying and insecticide treated nets on the incidence of malaria and entomological inoculation rate. We are measuring monthly incidence through active and passive case detections as the main outcome measure and entomological inoculation rate as secondary outcome measure. The community cohorts are central in the implementation of large-scale epidemiological and entomological studies in the area.

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A SIMPLE AND EFFICIENT TOOL FOR TRAPPING OVIPOSITION SEEKING *ANOPHELES*

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No effective tool currently exists for trapping ovipositing malaria vectors. This creates a gap in our ability to investigate the behavior and ecology of gravid *Anopheles*. Here we describe a simple trap that collects ovipositing Anopheline and Culicine mosquitoes. It consists of an acetate sheet coated in glue that floats on the water surface. Ten breeding sites were selected in rural Tanzania and 10 sticky traps set in each. These caught a total of 74 gravid *Anopheles* (54 *An. arabiensis*, 1 *An. gambiae* s.s. and 16 unamplified) and 1333 gravid Culicines, in just two trap nights. This simple sampling tool provides an opportunity to further our understanding of the behavior and ecology of gravid female Anophelines. It strongly implies that at least two of the major vectors of malaria in Africa land on the water surface during the oviposition process, and demonstrates that Anophelines and Culicines often share the same breeding sites. This method has clear potential for the study of oviposition site choice and productivity, gravid dispersal, and vector control techniques which use oviposition behavior as a means of disseminating larvicides.

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COMPARING THE HUMORAL RESPONSE TO SAND FLY AND MOSQUITO SALIVARY PROTEINS IN INDIVIDUALS FROM AN AREA IN CENTRAL MALI

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Vector-borne diseases like malaria and cutaneous leishmaniasis (CL) are public health problems in several tropical and subtropical countries. Several reports have linked the presence of antibodies to arthropods salivary proteins and risk of vector-borne diseases. However, cross-reactivity between the antibodies to salivary proteins of blood-sucking arthropods has not been properly scrutinized. Here, we compared the humoral immune response to salivary proteins from blood-sucking arthropods in individuals living in Kemena and Sougoula, two villages located in central Mali, West Africa. Sand flies, mosquitoes and other blood-sucking arthropods are abundant in these villages. Therefore, individuals living in these dwellings are prone to bites of different blood-sucking arthropods during their lifetime. Firstly, we tested if individuals living in these villages had anti-saliva antibodies to *Phlebotomus duboscqi, Anopheles gambiae* and *Culex quinquifasciatus* (vectors of CL, malaria and filariasis, respectively). We measured IgG levels in the sera of 117 individuals between 2 to 92 years old by ELISA. We determined that most of these individuals produced antibodies to all the three species tested. To evaluate if antibodies specific to *P. duboscqi* would react against mosquito saliva, we compared the anti-mosquito saliva IgG levels before and after sera incubation with *P. duboscqi* saliva. Incubation of sera with *P. duboscqi* saliva had no effect on the levels of anti-mosquitoes saliva IgG. Moreover, we tested if incubation with *P. duboscqi* saliva would change the recognition pattern of mosquito salivary proteins by Western blot technique. We found that there was no alteration in the number of mosquito salivary proteins revealed before or after incubation with *P. duboscqi* saliva. Here, we suggest that there was no major cross-reactivity among *P. duboscqi and* mosquito saliva antibodies.

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ASSOCIATIONS BETWEEN LEVEL OF ANTI-*AEDES* SALIVA ANTIBODIES, PRESENCE OF MOSQUITO LARVA IN HOUSES AND SEVERITY OF DENGUE FEVER

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In November 2010 we performed a survey including 57 houses of three main cities in Norte de Santander (Colombia): Cucuta, Los Patios and Pamplona. Houses were examined for the presence of breeding sites positive for mosquito larvae, and blood was collected on filter paper from volunteers (n=97) after informed consent was given. Our survey found that the main breeding site was water tanks inside houses and that the main mosquito species was *Ae. aegypti*. In addition, following serological testing we found an association between the level of anti-*Ae. aegypti* saliva IgG antibodies and people living in houses positive for mosquito larvae. These levels were also higher in people with history of symptoms consistent with dengue fever than in people that did not report the disease. Interestingly, people with history of severe dengue presented significantly lower IgG antibody levels than people that have suffered dengue fever alone; although these levels were significantly higher than

those in people with no history of dengue. Our results suggest that the level of antibodies can be used as a proxy for mosquito bite exposure and a measure of dengue fever risk.

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EVALUATION OF PREDICTIVE MAPS FOR *AEDES AEGYPTI* LARVAL HABITATS IN TWO URBAN AREAS OF COSTA RICA

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The abundance of *Aedes aegypti* can be associated with urban structure and tree cover, which conceals and protects containers. The purpose of this study was to create and evaluate predictive maps for *Ae. aegypti* larval habitats in Puntarenas and Carpio, two very different urban environments in Costa Rica. Linear regression models for number of mosquito larval habitats had been developed for Puntarenas, and they showed a significant association with tree cover when corrected by the number of locations evaluated (R^2 = 0.650, p<0.001). Land cover maps were created from Quickbird satellite imagery of both sites. Data was extracted from 50 by 50 m cells, and parameters from the model were used to create predictive maps by determining the expected number of *Ae. aegypti* positive larval habitats in all cells that cover the urban areas. To evaluate maps, cells were randomly selected, and entomological evaluations were performed. Four categories were created for the number of larval habitats per cell: low (0-1), medium (2-3), high (4-5), and very high (6 or more). For both sites, the expected number of wet containers in sample cells fell within the 95% confidence interval of predicted values. In Puntarenas, 382 wet containers were identified, container index was 22.5% and Breteau index 43.7. Expected and observed categories of *Ae. aegypti* larval habitats per cell in Greater Puntarenas were significantly correlated (p=0,037). Only 32.5% of cells harbored the exact number of expected habitats, 74% contained the expected number +/- 2 habitats, and only 16% underestimated total larval habitats. In Carpio, 693 wet containers were identified, container index was 11.4% and Breteau Index 24.7. Expected and observed categories of *Ae. aegypti* positive habitats per cell were not significantly correlated in Carpio. Only 50% of cells contained the expected number +/- 2 habitats, and 29% underestimated the total observed. The most frequent *Ae. aegypti* larval habitats in Puntarenas included outdoor containers and miscellaneous objects, while larval habitats in Carpio were commonly human-filled, such as drums and buckets. These maps and models may be considered adequate for areas like Puntarenas, whereas they do not seem to apply for Carpio. Tree cover may provide useful information in sites where *Ae. aegypti* larval habitats include mostly outdoor rain-filled containers, as opposed to sites where containers are greatly affected by the need for water storage.

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CLIMATIC VARIABILITY AND LANDSCAPE HETEROGENEITY IMPACT URBAN MOSQUITO DIVERSITY AND VECTOR ABUNDANCE AND INFECTION

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Urban habitat heterogeneity can modify patterns of interactions across species and lead to spatially fine grained differences in β-diversity patterns and their associated ecosystem services. Here, we study the impacts of landscape heterogeneity and climatic variability on: (i) the richness and diversity patterns of mosquitoes (Diptera: Culicidae) and (ii) the abundance and West Nile virus infection rate of the house mosquito, Culex pipiens, in Chicago, USA. We conducted a four year long study (2005-2008) in 8 sites that captured a gradient of urban heterogeneities. We found a total of 19 mosquito species, a representative sample of mosquito species richness in the area, according to both model estimation (Chao2 ± S.E. = 20.50 ± 2.29) and faunal records for Chicago. We found that heterogeneity in the landscape was the best predictor of both mosquito species richness and diversity, with the most heterogeneous landscapes harboring the largest number of species. In general there were no changes in species richness over the years that could be associated with weather patterns and climatic variability (WPCV). In contrast, changes in diversity evenness showed signatures of WPCV. Our results also showed that WPCV had major impacts on house mosquito abundance and West Nile virus mosquito infection rate (MIR) patterns. Although MIR was independent of mosquito diversity, it was associated with overall mosquito abundance, which had a convex association with species richness (i.e., abundance increases to a point after which it decreases as function of species richness). Finally, our results highlight the importance of considering dominant vector species as part of a community of vectors, whose biodiversity patterns can directly or indirectly impact the risk of infectious disease transmission.

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NON-LINEAR IMPACTS OF CLIMATIC VARIABILITY ON *AEDES AEGYPTI* POPULATION REGULATION

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Aedes aegypti is one of the most common urban tropical mosquito species and an important vector of dengue, chikungunya, and yellow fever viruses. It is also an organism with a complex life history where larval stages are aquatic and adults are terrestrial. This ontogenetic niche shift could shape the density dependent regulation of this and other mosquito species because events that occur during the larval stages impact adult densities. Here, we present results from simple density-dependence mathematical models fitted using maximum likelihood methods to weekly time series data from Puerto Rico and Thailand. Density dependent regulation was strong in both populations. Analysis of climatic forcing indicated that populations were more sensitive to climatic variables with low kurtosis (i.e., climatic factors highly variable around the median) rainfall in Puerto Rico and temperature in Thailand. Changes in environmental variability appear to drive sharp changes in the abundance of mosquitoes. The identification of exogenous factors forcing the sharp changes in disease vector populations using their statistical properties, such as kurtosis, could be useful to assess the impacts of changing climate patterns on the transmission of vector-borne diseases.

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THE ROLE OF SWINE IN THE ECOLOGY OF JAPANESE ENCEPHALITIS VIRUS TRANSMISSION OF SOUTHERN VIETNAM

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Japanese encephalitis virus (JEV) is a mosquito-borne flavivirus disease of major public health importance and is endemic to both north and south Vietnam. Swine populations play a role in JEV transmission as both a reservoir and amplifying host. In general, infected adult pigs support transient but high titer viremias and remain asymptomatic. In contrast, naïve piglets exhibit fever and experience weight loss, and gilts or sows who become infected from 40-80 days of gestation often abort or give birth to stillborn mummified fetuses. In some JEV-endemic countries, swine vaccination is performed within commercial livestock sector to prevent losses in reproductive performance of breeding herds. Here we review previous unpublished studies of JEV seroprevalence