but different for the human-baited tent, with a majority (41%) of *An. vagus*. Eight of the nine morphological species represented in this study were captured on each of the three hosts, suggesting a plasticity in host attraction behavior. Multiple host feeding and flexibility in feeding behavior could have important implications for malaria control.

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ASPECTS OF ECOLOGY OF POTENTIAL RIFT VALLEY FEVER VIRUS MOSQUITO VECTORS, KHARTOUM STATE, SUDAN

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Rift valley fever epidemics are disruptive and expensive to local and regional economies. After a devastating outbreak of Rift Valley Fever in khartoum state, Sudan 2007; ecological baseline surveys were conducted in Khartoum State, Sudan, during the rainy season (end of July to the beginning of September) 2008 in order to identify mosquito species present and evaluate their emergence and survivorship. Larval identification of species of Culicine and Anopheline mosquitoes present in Khartoum State taken from five study sites represents Khartoum state indicated that *Anopheles arabiensis* is the only species of the Anopheline mosquitoes found. Three species of culicine mosquitoes were found: *Culex quinquefasciatus*, *Cx univittatus* and *Cx arbeeni*, Species of *Aedes* were found in irrigated schemes at one study site and was absent from the other four study sites, these species were *Ae. vittatus* and *Ae. vexans*, whose presence was recorded after the onset of the rainy season.the same breeding site was first occupied by *Ae.vittatus* then *Ae. vexans,* with an interval of habitat drying. Daily emergent adults Culicine and Anopheline mosquitoes present were taken from randomly selected breeding sites in the five study sites, population measurements were performed. The absolute number of emergent adults was obtained by collecting mosquitoes under net-traps covering the breeding sites. Records were taken each day for seven constitutive days, synchronized emergence of males and females was observed at all the study sites, showing an overall marked predominance of females in emergence trap catches. Adult survival rate was the most important factor determining the stability of the population and total egg production. Females that become infected when taking a blood meal must survive throughout the incubation period of the pathogen. Under controlled laboratory environment, effect of food types (sucrose 10%, sucrose 10% and blood diet) on longevity of adult female mosquitoes was conducted, sugar-fed and blood-fed mosquitoes exhibited very high percentage of surviving rates beyond the 15 days (incubation period for RVFV),However these have varied among the five study areas. Also results indicated prolonged survival of sugar-fed female mosquitoes more than blood and sugar fed females, this served to increase survivorship of females until they find the appropriate host.

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MAIN MOSQUITO BREEDING SITES FOR *AEDES AEGYPTI* IN THE PAN-AMERICAN HIGHWAY: CUCUTA-PAMPLONA AREA (NORTE DE SANTANDER - COLOMBIA) IN 2010

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Aedes aegypti is the principal dengue vector in Colombia where dengue transmission is limited by the presence of the vector; unfortunately in this country, the presence of *Ae. aegypti* has been documented up to 2200 m.a.s.l. Norte de Santander is the second most endemic area for dengue in the country. Previous studies have associated travel and transport as key factors in the spread of diseases and vectors. With this pilot study, we investigated the main breeding sites and mosquito larva species on the highway from Cucuta (325 m.a.s.l) to Pamplona (2342 m.a.s.l) in 75km distance. We found that tires where the main breeding site followed by plastic containers and small pools along the way. The main specie collected was *Ae. aegypti* followed by *Culex quinquefasciatus*. *Anopheles* mosquitoes were not found in the highway area. Tire repair shops were the places with the highest number of infected tires; we also found abandoned tires infected with mosquito larva.

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A COMPARATIVE EVALUATION OF SIX DIFFERENT MALARIA VECTOR COLLECTION METHODS IN LOW-LYING MALARIA ENDEMIC REGIONS OF WESTERN KENYA

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Outdoor biting and other forms of behavioral adaptation by malaria vectors to domestic insecticide-based control measures may compromise the sensitivity of conventional sampling tools operating indoors such as light traps or indoor resting catches, thus preventing effective surveillance and management of vector populations. We evaluated six different vector collection methods to optimize a protocol for operational sampling of malaria vectors robust to variations in vector behavior, notably variations associated with the presence of important malaria control methods. Over 30 days, we replicated a Latin square design 10 times at sites in 4 districts in western Kenya: Kisumu, Bondo, Nyando and Rachuonyo. Each site consisted of 3 locally representative houses through which the six different sets of trapping methods were rotated every 3 nights in a random order of three possible arrangements: 1) Indoor human landing catch (HLC) and outdoor HLC, 2) CDC Light trap placed beside an occupied insecticide-treated net indoors combined with Ifakara tent traps outdoors, and 3) Window traps to catch exiting mosquitoes combined with both pot and box formats of resting traps placed both indoors and outdoors. At each site, a fourth house was selected for pyrethrum spray catch (PSC). The top collection methods with their corresponding number of *Anopheles* per collection effort were PSC (10.5), HLC indoor (3.0), Light trap (3.0) HLC Outdoor (2.8) and Ifakara tent traps (2.7). Resting Boxes and Pots positioned both indoors and outdoors caught less than 1 *Anopheles* per collection effort. HLC outdoor collected the highest amount of *Culex* at 77.4 per collection effort. Irrespective of the intensity or type of insecticide based vector control method in place and of biting behavior of the local malaria vectors, we conclude that pyrethrum spray catch is the most sensitive method for vector collection in low lying malaria endemic regions of western Kenya.

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HETEROGENEOUS FEEDING PATTERNS OF *AEDES AEGYPTI* IN HOUSEHOLDS IN IQUITOS, PERU

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Heterogeneous biting by female mosquitoes can significantly alter transmission of mosquito-borne pathogens. Previous studies show *Aedes aegypti*, the primary vector of dengue viruses (DENVs), more frequently bite individuals with higher body mass index (BMI). Because BMI increases with age, we expect positive linear relationship with age and biting. Studies show, however, that young adults receive more bites than older adults. Factors such as sex, mosquito exposure time and previous DENV infection should, therefore, be used to analyze heterogeneous feeding patterns. Between October 2009 and November 2010, 2,035 interviews with 280 participants were conducted in 19 households in Iquitos, Peru.

Interviews focused on anthropomorphic characteristics and time spent in houses. In the week following interviews adult mosquitoes were collected twice daily, yielding 1,878 engorged and partially engorged mosquitoes. Engorged abdomens were excised and participant DNA was obtained by cheek swab. All DNA was extracted using Qiagen extraction columns. Human DNA was amplified at 10 microsatellite loci, and allelic profiles identified using capillary electrophoresis. A computer program matched participant profiles to mosquito blood meals. To date, 99 of 115 identified blood meal profiles have been matched to participants. 29 young adults (ages 15 - 35) received 50 bites (1.72 bites/person). 14 children (<15) and 23 older adults (>35) received 154 and 34 bites, respectively (1.07 and 1.48 bites/person). In one household of 12 residents ranging in age from 5 to 70 years with BMI of 13 to 32 kg/m^2, 2 young adults ages 27 and 31 with BMI's of 23 and 21.2 contributed to 46% of the 26 identified blood meals, consistent with the idea that young adults are bitten most often, and indicating that age better predicts biting frequency than BMI. Analysis of the remaining 1,763 mosquitoes and interview data will be completed in the next 4 months. Results will be used to model virus transmission and to compare various vaccine delivery strategies.

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WHOLE GENOME SEQUENCING OF *ANOPHELES PUNCTULATUS* SIBLING SPECIES OF PAPUA NEW GUINEA

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The *Anopheles punctulatus* (AP) group in Papua New Guinea and Southwest Pacific consists of at least 13 sibling species that include the vectors of malaria and lymphatic filariasis. Understanding the population organization of the mosquitoes as well as the molecular basis for the phenotypic variability related to vector competence or control is complicated by limited data on the genetic diversity of these mosquitoes. We present here data generated by whole genome sequencing from individual AP mosquitoes and show that this approach provides extensive catalogues of genetic polymorphisms and can significantly contribute to better understand the biology of these mosquitoes. We extracted DNA from individual mosquitoes, and after determination of the species status by species-specific PCR-based assay, sheared the DNA molecules into 250-300 bp fragments and prepared libraries for two *Anopheles punctulatus* mosquitoes, one *An. farauti* 1, one *An. farauti* 2 and one *An. koliensis.* We sequenced each library on individual lanes of an Illumina GAIIx (paired-end 51 bp) or HiSeq 2000 (paired-end 100 bp). Overall, less than 1.5% of the reads generated could be mapped to the *An. gambiae* (AG) reference genome sequence suggesting that the sequence divergence between AP and AG is too great for the latter to serve as a useful reference sequence. We therefore reconstructed large chromosomal segments ("contigs") using solely the sequence information contained in the reads. Using this procedure we successfully assembled the entire mitochondrial genome sequence for each of the five mosquitoes which confirmed the deep divergence between AP and AG but also revealed deep divergences among the AP sibling species. In addition, we assembled 50-60% of each genome into fragments larger than 1,000 bp and identified more than 40,000 DNA polymorphisms that can now be used in association studies for traits related to insecticide resistance, preference to human blood meal or capacity to transmit malaria and filariasis.

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A COMPUTER SYSTEM FOR FORECASTING WEST NILE VIRUS RISK USING EARTH OBSERVATION DATA

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Although there have been many calls to expand the use of earth observation technologies in the health sciences, there are few examples of operational systems with demonstrated impacts on public health. Our research objective was to bridge the gap between remote sensing and public health by developing decision support systems to provide health scientists and practitioners with access to environmental information for surveillance and forecasting of mosquito-borne diseases. Specific objectives were to automate the processing of remote sensing data to generate environmental metrics, analyze the predictive capabilities of these metrics using retrospective datasets of human disease cases, and develop a web-based system for visualization and analysis of the resulting products. The system was programmed using JAVA for user interface development and overall system control. Spatial analyses were carried out using Python scripts to call ArcGIS geoprocessing functions. PostgreSQL was used for the storage and manipulation of the resulting data summaries. We implemented a prototype of the system to forecast outbreaks of West Nile virus in the northern Great Plains. Environmental variables included MODIS land surface temperature (LST) and vegetation indices (e.g., NDVI, EVI) derived from the MODIS nadir BRDF-adjusted reflectance product. We also used these data to compute actual evapotranspiration (ETa) using the simplified surface energy balance method. Statistical analysis using generalized additive models (GAMs) revealed non-linear associations between interannual variability in WNV incidence and interannual deviations of cumulative LST, NDVI, and ETa throughout the spring and early summer. There was an early-season influence of the timing of spring onset (captured by NDVI) as well as a late spring/summer influence of accumulated moisture and temperature (captured by LST and ETa). Forecasts are currently being disseminated via a web atlas (http://globalmonitoring.sdstate.edu/eastweb) and will be validated using surveillance data from the 2011 WNV season.

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DYNAMICS OF *ANOPHELES GAMBIAE* POPULATIONS IN THE SAHEL: NEW PATTERNS AND NEW PUZZLES AWAIT NEW UNDERSTANDING

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Malaria remains a top public health priority across Sub-Saharan Africa, where it is transmitted primarily by *Anopheles gambiae s.s.* and *An. arabiensis*. Populations of these species exploit diverse environments including dry savannahs and semi-desert areas, where surface waters required for larval development are absent for large parts of the year. How mosquitoes survive the long dry season has been debated without resolution for over 60 years. Although recent studies provide evidence for aestivation (extended survival throughout the 4-7 month-long dry season) of M form *An. gambiae*, the role of long-distance migration from areas with year-round breeding remains unclear. Here, we analyze the dynamics of the members of the *An. gambiae* complex in the Sahelian village Thierola (Mali), focusing on the dry season and its preceding and subsequent transition periods, over a period of three years (2008-2011). The dry season mosquito populations were characterized by low overall density (<0.05 mosquito/house), and were predominantly composed of

the M form (>95%), with the remainder being *An. arabiensis*. Males were found throughout the dry season, both indoors and in swarms, albeit in very low numbers. Interestingly, the dry-season dynamics were not stable: in early April, ~2 months before the first rain, density surged up to three orders of magnitude and receded to typical dry-season density within days. This surge was observed in both 2010 and 2011 and consisted only of the M form. Five to seven days after the first rains (early June), before a new generation of adults could be produced, the M form surged again over one order of magnitude, and continued to increase gradually at an average rate of 50%/week, for several weeks. Unlike the M form, the S form and *An. arabiensis* remained virtually zero for over four weeks after the first rains; thus it is unlikely that they aestivated but would emerge only ~5 weeks after all larval sites filled. These results suggest that both the S form and *An. arabiensis* persist in the Sahel primarily by migration whilst the M form aestivate. Final analysis and implications for malaria control will be presented.

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DIRECT AND INDIRECT COSTS OF *PLASMODIUM* INFECTION ON MOSQUITO REPRODUCTIVE SUCCESS

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Infection with malaria parasites reduces the immediate reproductive success of mosquitoes, but the life-long effects, as well as their interaction with stress, are not well known. Additionally, the negative effects of infection may be exacerbated by the nutritional cost of feeding on anemic blood. We evaluated the effect of *Plasmodium gallinaceum* infection on reproductive success of stressed and unstressed *Aedes albopictus*, fed on either infected or uninfected chicken blood. Each of these treatment combinations were subdivided into three subgroups that were either fed: (i) directly on an infected (or uninfected) chicken (Live); (ii) membrane-fed on fresh blood from the same chicken (Mem_{FRESH}); (iii) membrane-fed on the same blood incubated at 4°C for 12 h (rendering infectious blood non-infectious; Mem_{UNINF}). The mosquitoes were subsequently fed two more times on uninfected blood from the same chicken. The egg batch size (EBS) of individual mosquitoes was determined 7 d after each feed. Preliminary analyses revealed that EBS was lower in infected vs. uninfected and stressed vs. unstressed mosquitoes. However, the interaction between stress and infection was not significant. Likewise, there was no significant interaction between infection and feeding type (i.e. Live, Mem_{FRESH}, and Mem_{UNINF}), indicating that the fitness costs of being fed on an infected chicken were similar in both infected-infectious (Mem_{FRESH}) and infected-non-infectious (Mem_{UNINF}) blood. We also found that the negative effects of infection and stress on EBS were not restricted to the first oviposition cycle, but rather that these factors could lead to a dramatic decline in the lifelong reproductive success of individuals. Our results highlight both the life-long and indirect (i.e. due to anemic blood) fitness costs of *Plasmodium* infection to both stressed and unstressed mosquitoes. Such costs are important from an ecological and epidemiological perspective, as they could affect evolution of resistance/tolerance mechanisms, and in turn affect mosquito population dynamics and vector potential.

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MOSQUITO COMMUNITIES AND VECTOR-ASSOCIATED MICROBIOMES SAMPLED ACROSS A HABITAT GRADIENT OF THAILAND

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Changes in biodiversity have the potential to affect the risk of infectious diseases in plants and animals, including humans, since infectious disease distribution is largely dependent inter-specific interactions. In particular, mosquito-borne diseases are well-suited to study how changes in interacting species, namely mosquitoes, their hosts, and associated microorganisms in changing habitats may affect infectious disease risk. Current knowledge of mosquitoes and their associated microbial communities in natural habitats is, however, limited. Here we explored the composition and diversity of mosquitoes and mosquito-associated microbes in relation to habitats ranging from forest to urban areas in the central plain of Nakhon Nayok province, Thailand. During the rainy season in 2008, adult mosquito collections from 24 sites using CDC light traps, BG sentinel traps, Mosquito Magnet traps, and CDC backpack aspirators yielded a total of 62,511 identifiable female mosquitoes of 54 confirmed taxa. Female mosquito abundance was highest in the rice field habitat and lowest in the forest habitat with 27,041 (43.26%) and 4,840 (7.74%) mosquitoes collected, respectively. The diversity of mosquito communities was characterized using a variety of diversity measurements including statistical sampling approaches to extrapolate species richness. In general, the rural habitat was the most diverse while the least diverse habitat varied depending on the indices used. The Vishnui subgroup of Culex species was the most common taxon found overall and also the most common in the fragmented forest, rice field, rural, and suburban habitats, while Uranotaenia sp. was the most common taxon in the forest habitat and *Cx. quinquefasciatus* was the most common species in urban settings. *Aedes aegypti* and *Ae. albopictus* were most abundant in urban and rural area respectively. To explore the diversity and composition of vector-associated microbiomes, the microbiota from three vector species *Cx. quinquefasciatus*, *Ae. aegypti*, and *Ae. albopictus* from different habitat types were studied using 454 pyrosequencing of ribosomal RNA. Patterns of microbiota community assembly in mosquitoes by habitat type and vector species using both alpha- and beta-diversity analyses will be discussed. Our results are particularly relevant for understanding the dynamics of mosquito vectors and their associated microbiomes in landscapes of Thailand.

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LINKING OVIPOSITION-SITE CHOICE TO OFFSPRING FITNESS IN *AEDES AEGYPTI*: CONSEQUENCES FOR TARGETED LARVAL CONTROL OF DENGUE VECTORS

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Maternal oviposition-site choice and its repercussions for offspring fitness are known to influence population dynamics of insects. Using four experimental container treatments (size [large vs. small] x water management [manually filled vs. unmanaged]), we tested the hypothesis that wild *Aedes aegypti* in Iquitos, Peru choose egg-laying sites to maximize offspring survival and growth. Among 80 containers located

in 20 houses, females consistently laid more eggs in large vs. small containers ($\beta = 9.17$, $p < 0.001$), and in unmanaged vs. manually filled containers ($\beta = 5.33$, $p < 0.001$). There was poor correlation, however, between oviposition preference and two components of mosquito fitness, pupation probability and adult size. Probability of pupation was higher for mosquitoes developing in small, unmanaged containers than any other container type ($\beta = 3.4$, $p < 0.001$). Adult body size decreased for individuals developing in large containers (females: $\beta = -0.19$, $p < 0.001$; males: $\beta = -0.11$, $p = 0.002$) and unmanaged containers (females: $\beta = -0.17$, $p < 0.001$; males: $\beta = -0.11$, $p < 0.001$). Our data suggest that the majority of *Ae. aegypti* eggs are laid in non-optimal sites, such that selective oviposition behavior contributes to population regulation by limiting the production and size of adults. Targeted larval control strategies removing the most productive containers may have the unintended effect of encouraging females to spread their eggs more evenly among remaining containers. By tracking egg-laying patterns of individual females inside a semi-field enclosure, we found that the probability of any container receiving eggs increased when preferred container were removed (but the total number of containers remained constant) ($\beta = 1.36$, $p < 0.001$). We suspect that in Iquitos, and possibly other locations, selective oviposition behavior by *Ae. aegypti*, along with a potential switch from clustering eggs to spreading them out, will render targeted larval control less effective than anticipated.

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TOWARDS A CONSERVED *CIS*-REGULATORY MODULE WITH CROSS-STRAIN/SPECIES APPLICATION FOR DRIVING ANTI-PATHOGEN EFFECTOR TRANSGENES: COMPARATIVE TRANSCRIPTOMICS TO DISCOVER EARLY BLOODMEAL-RESPONSIVE, CIS-REGULATORY SEQUENCES FROM MOSQUITO MIDGUT RNA-SEQ

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Empirical definition of active *cis*-regulatory elements (CRE) through classical "promoter bashing" is difficult in mosquitoes due to the time and effort required to produce transgenic mosquito strains. Bioinformatic methods combined with existing biological knowledge and quality mRNA abundance data should allow the inference of active CRE combinations, *cis*-regulatory modules (CRM), without requiring construction of transgenic mosquito strains. The ecdysone (20E) response cascade is conserved throughout insects and has been shown to drive changes in mRNA abundance following the ingestion of a bloodmeal. This supports the hypothesis that it should be possible to deduce a conserved CRM by studying bloodmeal-regulated transcript abundance across multiple mosquito species. 20E has had multiple early-response factors described previously including the ecdysone receptor (EcR), its binding partner ultraspiracle (USP), and the 20E-inducible gene E74. Other laboratories have shown that levels of 20E early-response factor isoforms vary in a time- and tissue-specific fashion in response to pulses of 20E following a bloodmeal. This allows one hormone to regulate diverse cellular responses. Tissue-specific, time-course RNA-Seq data with high temporal resolution (2 hours) will be used to compare 20E early-response factor isoform mRNA expression levels across evolutionarily distant species (*Anopheles gambiae, Aedes aegypti, and Culex quinquefasciatus*) to infer transcripts that display probable time-lagged induction by 20E and harbor known 20E early-response factor motifs. This transcript set will leverage a combined comparative-genomics and expression-profile based CRE/CRM discovery strategy to reveal putative CRMs expected to provide a better understanding of 20E-regulated transcript regulation in the midgut. The discovered CRMs will serve as the basis for validation of a set of conserved CREs that may be combined to drive robust anti-dengue effector transcription in the midguts of *Ae. Aegypti* mosquitoes directly following the ingestion of each bloodmeal.

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USING MOSQUITO SURVEILLANCE DATA TO PREDICT HUMAN WEST NILE VIRUS TRANSMISSION RISK

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West Nile virus (WNV) has become endemically established across the Americas with enzootic activity and significant human illness. Despite this, funds for surveillance and control are limited and decreasing. Predicting the risk of human infection to initiate timely preventative measures is the primary goal of public health mosquito surveillance. Many arbovirus response plans outlines recommended public health and mosquito control actions based on levels of virus activity determined by statewide mosquito surveillance. However, studies linking mosquito surveillance data to the spatio-temporal risk of human WNV infection, have rarely been attempted. We quantified the links between mosquito surveillance data and the spatio-temporal patterns of 3,827 human WNV cases reported in Colorado from 2003-2007. Mosquito data were strongly predictive for spatio-temporal variation in human WNV infections several weeks in advance in a statewide analysis, and with temporal variation within a county. Correlative and predictive relationships were strengthened by using pooled estimates of prevalence from across the state in estimating of risk early and late in the season when few mosquitoes were trapped at the local scale. However, we found that when current year prevalence data was not available, as could occur with reduced or eliminated surveillance budgets, no meaningful predictions of human risk could be made to determine appropriate public health response. Overall, our results demonstrate that mosquito surveillance provides valuable predictive data about the risk of human infection which can be used to trigger emergency response actons and allocate limited public health and mosquito control resources.

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DOCUMENTING THE POTENTIAL INTRODUCTION OF DENGUE VIRUS INTO KEY WEST, FLORIDA THROUGH AIRLINE AND CRUISE SHIP PASSENGERS FROM DENGUE-ENDEMIC LOCATIONS

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For the first time in decades, sporadic cases of locally-acquired dengue were reported in Key West in 2009 and again 2010. Current hypotheses regarding this continuance include vertical transmission, the establishment of an endemic state with undetected transmission between years, and multiple introductions via visitors from endemic countries during both years. Regarding the third hypothesis, country- and year-specific dengue incidence data (PAHO) and the numbers of airline passengers originating in dengue-endemic countries in this hemisphere with a final destination of Key West were used to estimate the relative the magnitude of potentially viremic passenger-days experienced per year. These estimates suggest multiple introductions per year are not uncommon and that potential introductions in 2009 and 2010 were higher than in 2007 and 2008 as a result of an increase in air travel and major dengue activity in the Caribbean and Central America. Both years were El Niño years that historically are associated with elevated temperatures and higher dengue activity in the region. A similar analysis of potential introductions via the cruise ship industry will also be presented.

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CRYPTIC BREEDING: A POTENTIAL CAUSE OF LOCAL DENGUE TRANSMISSION IN KEY WEST, FLORIDA

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June 2009 marked the beginning of a 2-year outbreak of locally-acquired dengue in Key West, Florida. Despite increased control efforts by mosquito control and local residents, the number of dengue cases in 2010 nearly doubled that of 2009. Surveillance on the abundance of immatures was inconsistent with magnitude of the adult population of *Aedes aegypti*. Similar disconnects(?) between immature and adult abundance in other dengue-endemic regions have been the result of cryptic breeding which occurs when mosquitoes reproduce in locations that escape control efforts. The majority of homes in Key West were built prior to municipal utilities and stored water in cisterns and disposed of waste through septic systems. Cisterns and unused septic tanks are several cubic meter in size and most are not easily accessible. Though historical maps exist, the true number of cisterns and septic tanks is unknown thus complicating control efforts. Presented here are the combined efforts of the University of Florida and Monroe County Mosquito Control to identify and eliminate cryptic breeding sources for *Ae. aegypti* in Key West.

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THE QUALITY OF DRINKING WATER IN COMMUNITIES ALONG THE MARANON RIVER IN THE PERUVIAN AMAZON

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Water is one of the world's most critical resources, however international water quality surveillance and monitoring is often not implemented, obscuring associations and etiologies of potentially related illnesses. We conducted an evidence-based approach to understand the sources and types of water contaminants as well as the overall safety of available drinking water in Peru. A comprehensive, portable, water quality assessment toolbox was used to quantify key microbial (total coliforms, *E.coli* and enterococci) and chemical (metals, anions and pesticides) contaminants. This assessment system was applied in the field to evaluate the drinking water of 20 rural villages bordering the Maranon River in the Peruvian Amazon. In total, 32 households, 32 drinking water sources, and 2 water treatment systems were assessed. All household drinking water samples and 93% of source water samples contained moderate to high levels of *E.coli* contamination. Water treatment systems varied in contaminant removal, ranging from 2.03 logs to 4.15 logs of measured bacterial removal. Multiple water samples contained chemical contaminants in excess of WHO guideline levels including phosphate (anion); aluminum, iron, and manganese (metals); and lindane (pesticide). Current international water quality screening and evaluation efforts are not adequate to address the burdens caused by the adverse health effects of waterborne contaminants, thereby demonstrating the need for portable water quality screening. In the Peruvian Amazon, results comparing source water and household contamination suggest recontamination during transport. Analysis of chemical pollutants revealed a need for water treatment to address metal contaminants. Treatment system results indicated that standardized treatment measures are required. The use of our water quality assessment toolbox provided more comprehensive detection and analysis of waterborne threats to the public. These data can help local governments and non-governmental organizations to select appropriate treatment solutions.

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INTEGRATION OF A SAFE WATER SYSTEM WITH ANTENATAL SERVICES, MACHINGA DISTRICT, MALAWI, 2010-2011

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Antenatal clinic (ANC) visits provide an opportunity to integrate additional interventions to improve maternal and neonatal health and motivate pregnant women to attend ANC services. In Malawi, although 93% of women attend at least one ANC visit, 57% deliver in health facilities, and 7% have postnatal checks. To reduce the risk of diarrhea, a leading cause of childhood mortality, we integrated free hygiene kits (safe water storage containers, water treatment solution [*WaterGuard*], soap, and oral rehydration salts) with ANC services. To receive the hygiene kit, women had to have a spouse/partner present; HIV testing was also offered to the couple. At subsequent ANC visits, up to 3 refills of *WaterGuard* and soap were provided. We surveyed 106 women receiving ANC care at baseline before program implementation and at follow-up 12 months later to assess water treatment; test drinking water for residual chlorine; observe hand-washing; and determine ANC service utilization. From baseline to follow-up, there was an increase in the percentage of women who had ever used *WaterGuard* (38% vs. 100%, $p<0.001$), knew how to use it correctly (23% vs. 81%, $p<0.001$), were observed to have a bottle in their home (3% vs. 77%, $p<0.001$), had residual chlorine in their stored water (0 vs. 71%, $p<0.001$), and were able to demonstrate proper handwashing technique (21% vs. 65% $p<0.001$). At follow-up, 89% of respondents had ≥3 ANC visits, 90% delivered at a health facility, 99% were tested for HIV, 99% of partners were tested for HIV, and 98% had disclosed their status to their partner. Women in this program showed statistically significant increases in water treatment and hygiene practices, and high utilization of ANC services and HIV testing. This evaluation suggests that integration of hygiene kits, refills, and HIV testing during ANC is feasible, can serve as an incentive to increase use of health services, and may help motivate changes in health behavior.

1266

IMPACT OF COMPLEXITY OF HANDWASHING INSTRUCTIONS ON ADHERENCE IN A LOW INCOME SETTING, DHAKA, BANGLADESH, 2010

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Handwashing reduces diarrhea risk in young children. Interventions to improve handwashing usually include instructions on how and when to wash hands. These instructions vary in complexity, with some recommending multiple steps including duration of lathering and scrubbing various aspects of the hands. To assess whether complex handwashing instructions result in reduced adherence, we conducted a randomized trial in a low-income area of Dhaka, Bangladesh. Mothers of young children were randomly assigned to one of three sets of handwashing instructions: simple, moderate, or complex. Simple instructions were to wet, lather, and rinse hands; moderate instructions included simple instructions and additional steps to scrub palms, scrub backs, and dry hands by waving them in the air; complex instructions included moderate instructions and additional steps to scrub between fingers, scrub under nails, and lather for 20 seconds. The field worker

taught the participant the randomly assigned set of instructions, without mention of the other two sets. Immediately, two days, and two weeks after the teaching, participants were asked to demonstrate handwashing to the field worker. Adherence was defined as demonstration of all of the instruction steps prescribed for the assigned treatment arm. We enrolled 244 participants (simple n=85, moderate n=75, complex n=84). Compared with the simple group, in which 100% adhered to prescribed instructions at all post-intervention assessments, the more complex groups had lower adherence at two weeks (moderate 43%, p<.0001; complex 31%, p<.0001). Adherence to air-drying hands was low at immediate, Day 2 and Week 2 assessments (moderate: 49%, 39%, and 47%; complex: 57%, 46%, and 38%). Exclusion of the air drying step from the outcome yielded adherence rates of 99%, 91% and 88% for the moderate group and 81%, 69% and 71% for the complex group. In a low-income community in Dhaka, highly complex instructions for handwashing resulted in decreased adherence. Future research should investigate whether adherence to the highly complex set results in greater hand decontamination than adherence to the simple or moderate set of instructions. When developing materials to promote handwashing behavior, handwashing promotion programs should consider the complexity of the overall set of instructions, as well as the microbiological impact and feasibility of adherence to specific instructions, such as air drying.

1267

CONSISTENT SOAP AVAILABILITY CORRELATES WITH USE OF INEXPENSIVE SOAP PRODUCTS AND IMPROVED HANDWASHING BEHAVIOR IN LOW-INCOME HOUSEHOLDS IN DHAKA, BANGLADESH

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Handwashing (HW) with soap reduces diarrhea in children < 5 years of age in low-income countries. Understanding characteristics of households with increased HW could inform interventions to increase this behavior. Amongst low-income households in Dhaka, Bangladesh, we studied consistent soap availability as a possible indicator of increased HW. Households were selected randomly from controls of a case-control study on hygiene and respiratory illness; all had ≥ 1 child ≤ 5 yrs. We visited 220 households 8 times over 4 weeks in Feb-Mar 2010. Respondents were interviewed about soap availability. Fieldworkers observed the presence of soap and water at HW stations, the cleanliness of respondents' palms and administered a validated 14-question tool on the strength of handwashing habits. We used data from structured observations, conducted several months prior, to estimate HW behavior at critical times. We used logistic regression to adjust for socioeconomic status, and compared households that had soap for HW available at 100% of visits to households that did not. Soap for HW was available in 1513 (88%) of 1716 visits to 220 households. In 110 households (50%), soap was available at every visit. Compared to those with inconsistent soap availability, households with consistent soap availability at each visit were more likely to be in the highest socio-economic status quintile (determined by principal component analysis on household assets) (OR 1.9; 95% CI 1.4- 2.4), more likely to have soap present at the HW station (OR_{adj} 1.6; 95% CI 1.3- 2.0), more likely to wash hands with soap at critical times (OR_{adj} 1.4; 95% CI 1.1 -1.7) and more likely to identify cheaper detergent soap rather than bar soap as the main HW product (OR_{adj}2.2; 95% CI 1.6- 2.9). Consistent availability of soap for handwashing was not associated with scores for the key components of habit. Households that had soap available in the home during each visit were also more likely to keep soap where it was needed for washing hands, and to wash hands more frequently at times relevant for hand contamination and pathogen transmission. Reliance on less expensive soap may facilitate consistent soap availability, underscoring the importance of promoting affordable means of increasing handwashing. Interventions that emphasize soap availability and the efficacy of inexpensive soap may be effective in increasing handwashing behavior at critical times.

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IMPACT OF INTENSIVE HANDWASHING PROMOTION ON HOUSEHOLD TRANSMISSION OF INFLUENZA IN A LOW INCOME SETTING: PRELIMINARY RESULTS OF A RANDOMIZED CONTROLLED CLINICAL TRIAL

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Although handwashing with soap decreases the risk of all-cause respiratory illness, there is little published empirical evidence for the efficacy of handwashing with soap for prevention of influenza transmission in resource-poor settings. We tested the impact of handwashing promotion on the risk of household transmission of influenza, influenza-like-illness (ILI), and fever in rural Bangladesh. In 2009 and 2010, we identified index case patients (ICPs), individuals who developed ILI within the previous two days and were the only symptomatic person in their household. ILI was defined as fever in children <5 years old and fever with cough or sore throat in persons > 5 years old. Households were randomized to intervention or control). The intervention group received handwashing stations with soap and daily handwashing motivation at critical times for pathogen transmission, such as after coughing or sneezing. We conducted daily surveillance and tested household members with fever for influenza viruses by polymerase chain reaction. Secondary attack ratios (SAR) were calculated for influenza, ILI, and fever in each arm. We used logistic regression with generalized estimating equations to estimate the significance of the SAR comparison while controlling for clustering by household. Among 274 ICPs enrolled, 33 (12%) had laboratory-confirmed influenza infections. The SARs for influenza among household contacts of ICPs with confirmed influenza virus infection were 7.5% in the control arm (10/133) and 11.0% in the intervention arm (11/100) (p = 0.362). The SAR for ILI among household contacts of all ICPs was 11.9% in the control arm (146/1,226) and 14.2% in the intervention arm (186/1,314) (p = 0.232). SARs for fever were 12.1% and 15.0%, respectively, in the control and intervention groups (p = 0.113). When an intensive handwashing intervention was initiated after illness onset in a household member, we found no protective effect against influenza virus infections. Handwashing behavior may not have changed rapidly enough to match the pace of influenza virus transmission between household members. Courtesy bias among intervention households, who received daily motivation as well as hardware to facilitate handwashing, may have led to greater reporting of respiratory symptoms. Future efforts should consider whether handwashing behavior can be changed quickly after illness onset in order to blunt household influenza transmission.

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CONSUMER INPUT TO DESIGN AND DEVELOPMENT OF A NOVEL HOUSEHOLD WATER TREATMENT DEVICE

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We collected consumer preference data in urban, periurban, and rural areas in India and Indonesia to use in design and development of a novel POU device for use in Asia. The end product incorporates a

drinking water disinfection medium (registered by USEPA-#72083-3, 2009). Consumer exposures ranged from 1-month in-home use of functional prototypes, to use-pattern questionnaires, and from household placement of life-size cut-outs of proposed designs to 3- dimensional models based on these designs. Householders showed a preference for gravity feed device configurations that: could accommodate ~ 10 L of source water; allow for collection of filtered, disinfected product water after no more than a few hours; ensured collection of clear, uncolored water with no detectable taste, taint, untoward mouth-feel or odor on immediate consumption or after storage; offered ease of use in cleaning of upper chamber filtration elements; ensured high convenience in secure replacement of the water treatment train (prefilter/filter/adsorption media/ disinfecting cartridge) after a useful life of no less than several months' daily use (i.e., > 1000L); required minimal assembly at start-up; provided for ready access to product water via a faucet/outlet with reliable, drip-free function; and (critically important) had a 'modern' and attractive appearance, enhancing the household working and living environment. From in-home observations we determined that: construction needed to be robust, include auto- shut-off at the end-of-life, plus a visual indicator of approaching termination, and include an option for 'dialing in' varying efficacy levels (up to US-EPA 6/4/3). HaloPure Waterbird emerged from this process, a gravity-feed purifier capable of 6/4/3 log reduction, auto shut-off at 1500L (± 20%), and leak-free cam-lock cartridge placement. Imperceptible halogen residual provides for continued protection of product water, in the device or upon transfer. Listening to the "voice of the consumer" can lead to enhanced product design aimed at household water treatment device development.

1270

USE AND ACCEPTABILITY OF A POINT-OF-USE WATER FILTRATION DEVICE IN HIV-1 INFECTED ART NAÏVE KENYAN ADULTS

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Among HIV-infected adults and children in Africa, diarrheal disease remains a major cause of morbidity and mortality. WHO recommendations suggest HIV-infected individuals should treat drinking water at the point-of-use. While simple and effective water filtration devices are available, limited data exist regarding the use and acceptance of these devices in this population. We enrolled ART naïve HIV-positive adults into a two-year cohort study in western Kenya. Individuals were visited in their home at least once to assess acceptability and use of a study water filtration device. Of 417 participants enrolled and subsequently visited, most were female (81%), married (64%), had at least a primary school education (72%), and had CD4 cell counts above 350 cells/µl (76%). At enrollment, participants reported the most common sources of drinking water to be shared pipe or tank source (45%) followed by well water (25%) and river or stream (25%). Among participants with a functioning device, more than half (57%) reported using the water filtration device in all of the last 5 instances of obtaining water to drink (always) and 25% reported using the device at least 3 of the last 5 times. Only 3% reported never using the device. We found household monthly income greater than 5,000 Kenyan Shillings (~$57 US) to be associated with always using the device (OR: 2.12 (95%CI 1.23, 3.65)). A trend towards an association between increasing age and always using the device was also observed (OR per 5 year increase: 1.11 (95%CI 0.98, 1.30)). While 38% of participants reported drinking water outside of the home within the last 24 hours, most (77%) reported filtering their drinking water. Almost all participants found the device very acceptable, with 97% willing to purchase the water filtration device if their current device were to break. Providing simple point-of-use water filtration devices to HIV infected adults may be an inexpensive and practical intervention to improve water quality and reduce the risk of diarrheal disease among this high-risk population.

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EFFICACY VERSUS EFFECTIVENESS OF WATER CHLORINATION IN RURAL COASTAL ECUADOR

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Chlorination can provide a low-cost method of treating drinking waters and is known to be efficacious for reducing bacterial loads, but actual effectiveness under household conditions may not reduce microbial contamination to the same extent as under lab conditions. In a previous study we found no significant differences in log reductions in drinking water of households that reported chlorination of their water in rural coastal Ecuador. We present the results of a follow-up study at the same field site in which we observed and quantified chlorination procedures at the household instead of relying on self-reported chlorination. We also tested source waters and water from control containers stored under protected conditions outside of the household. We collected three sets of samples (source water, water stored in the home, and water stored under control conditions) from 145 households: 67 that did not chlorinate, 42 that used locally available chlorine according to local practices, and 35 that used chlorine dosed to recommended standards. Covariates included physicochemical data and household level indicators. The efficacy of chlorine treatment in our field laboratory-matched control samples was higher than the effectiveness in corresponding household samples, which is most likely the result of recontamination in the household during storage. Recontamination of water in containers in the household over a 24-hour storage period was observed between pairs of household and matched control samples for both E. coli and total coliform concentrations, with mean log differences ranging from 0.4017-0.6147 ($p < 0.0001$). 63.8% of samples had greater microbial contamination in household samples than in their matched control. The reduced effectiveness can also be explained by other factors such as source water turbidity, socio-economic status, unsafe water storage behaviors. Negligible disparities were found between the two chlorine treatment groups, suggesting that dosing practices did not greatly modify the relationship between chlorination and log reduction in contamination. Household effectiveness of chlorine treatment was significantly reduced over laboratory efficacy. This research provides important new insight about the relationship between household storage practices and chlorination under village conditions.

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ROTAVIRUS OUTBREAK AMONG CHILDREN IN DAY CARE CENTER, ZAPORIZHZHYA, UKRAINE

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In the city of Zaporizhzhya the incidence of acute gastroenterocolitis (GI) has recently increased. The proportion GI illness due to rotavirus infection (RI) has increased from 21.6% in 2008 to 40.6% in 2009. Among children incidence of RI has increased 2.5 times. We investigated an outbreak of GI in a daycare center (DCC). Samples of drinking water, food and human specimens were examined bacteriologically for intestinal pathogens. Ill persons, contacts and water were tested for rotavirus antigen by ELISA and dipstick testing. During a two week period in April, 17 cases of RI were reported. Cases were identified in 8 of 11 classes. In a class which attended only three hours per day there were no cases. Through testing we identified 11 carriers (1 caregiver from class 1 and 10 children). The highest incidence of RI (4 patients and 7 carriers) was observed in class 1 which consists of children under 3 years. The primary case was identified in this class. Rotavirus was found in these 4 children. Due to a staffing shortage care-givers served food to the children against normal sanitary

regulations. Using a retrospective cohort study design we established that the route of transmission was beet salad (RR=3,5; CI 1,07-11,36). The salad was served from a single bowl and distributed to children by class. Children from the two classes in which there were no cases of RI received the salad first. The first 4 cases of RI were not identified until laboratory testing was performed. The study established that the caregiver was infected at DCC. Transmission is believed to have occurred via asymptomatic carriers. Caregivers serving food to the other classes are thought to have transmitted disease to them. No cases of RI occurred in classes that received salad before class 1 was served. This study demonstrates the necessity of strict adherence to the sanitary and hygiene regulation in DCC's and the ongoing problem of RI.

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SPATIO-TEMPORAL PATTERNS OF DIARRHEAL DISEASE CAN REVEAL TRANSMISSION PATHWAYS IN AN EMERGING URBAN REGION OF ECUADOR

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Diarrheal disease is caused by a variety of pathogens that exploit multiple transmission pathways. The patterns of diarrheal disease in space and time may reveal which transmission pathways are dominant; e.g., direct person-to-person spread produces temporary clusters of cases; whereas environmental pathways result in constant clusters around environmental sources. We explored these spatial and temporal distributions of diarrhea in Borbón, a small urban region of northwestern Ecuador. The relationship between these patterns and household and neighborhood WASH characteristics was also estimated. We conducted a series of six nested case control studies between December 2008 and May 2009. Surveys were carried out monthly to collect data on WASH factors. The river as well as all houses and outdoor latrines were mapped using GPS. We employed spatial point pattern analyses assuming an inhomogeneous Poisson process. We used the K-function to measure clustering and the ratio of intensity between cases and controls to estimate spatial variation of risk by month. Generalized linear and generalized additive models were used to estimate the association between WASH factors and household diarrhea. We found both spatial and temporal variation of diarrhea in Borbón. The spatial variation was associated with different risk factors each month; the exception to this finding was living with children under five, which was found to be a consistent risk factor. For example, early in the rainy season (December and January), use of an unimproved sanitation facility was significantly associated with diarrhea. In the middle of the season, significant WASH effects were absent. Towards the end of the rainy season (May) better household hygiene was significantly protective for diarrhea. These results provide insight on where and when improvements to WASH factors may protect from diarrheal disease, highlighting the importance of indirect transmission through contaminated latrines in the dry season.

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RAPID VIABLE DETECTION OF HUMAN-ORIGINATED FECAL CONTAMINATION USING IMS/ATP AND QPCR TARGETING *BACTEROIDES FRAGILIS*

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Human-originated fecal contamination of our drinking water source and recreational water is a continuous public health concern around the world. Timely and cost-effective ways in detecting contaminants in water is very important for protecting human exposure to possible presence of potential enteric infectious agents. This study aimed to determine the effectiveness of a rapid detection method, immunomagnetic separation coupled with ATP bioluminescence (IMS/ATP) and qPCR targeting *Bacteroides fragilis* for human-specific contamination. *B. fragilis* is a strict anaerobic bacteria and is known to be one of the predominant microbial flora in human gut. For this, an on-site wastewater treatment system was used as a testing ground. Water samples were collected from various points: septic tank effluents; after bioreactor; and after chlorine dioxide treatment. The level of *B. fragilis* were tested with IMS/ATP using *B. fragilis*-specific antibody attached magnetic beads and qPCR targeting *gyr* B gene. The *B.fragilis* (Bf) levels measured by IMS/ATP showed 1.5 log reductions after bioreactor, and 2.0 reductions after ClO_2 treatment, respectively, when compared with the original levels in the septic tank. The Bf levels determined by qPCR showed 1.6 log reduction after bioreactor and 2.3 log reduction after ClO_2 treatment. The Bf levels measured by IMS/ATP and qPCR correlated well (y=0.8834x+0.8791, R = 0.998). In summary, IMS/ATP rapidly determined the levels of Bf in an on-site wastewater treatment system with sensitivity and specificity. Thus, it can provide near real-time (1.5 hr) results of the on-site wastewater treatment efficiency prior to its release into the environment. This is the first study that the new IMS/ATP assay targeting Bf was applied for determining on-site wastewater treatment efficiency. This assay can be applied for a broad range of rapid detection of human-specific fecal contamination in water where fecal contamination is suspected.

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FURTHER INSIGHTS INTO THE PHYSIOLOGICAL MECHANISMS THAT UNDERLIE TSETSE'S BENEFICIAL SYMBIOSES

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Bacterial symbioses are ubiquitous in nature, yet to date few studies have been performed to determine the physiological mechanisms that underlie these relationships. Insects represent a group of advanced multi-cellular organisms that harbor well-documented symbiotic associations. One such insect, the tsetse fly (*Glossina* spp.), harbors 2 maternally-transmitted bacterial symbionts, mutualistic *Wigglesworthia* and commensal *Sodalis*, that are intimately involved in maintaining the overall fitness of their host. In this study we examine the functional mechanisms that underlie these symbioses by producing tsetse flies that lack all of their endogenous microbiota. The resulting aposymbiotic offspring are highly susceptible to infection with normally non-pathogenic *E. coli*, and this immuno-compromised phenotype is characterized by the absence of phagocytic hemocytes and the irregular expression of immunity-related genes. When hemocytes collected from wild-type tsetse are transplanted into aposymbiotic flies, the recipient individuals regain their refractory phenotype. We also supplement the diet of pregnant aposymbiotic females with *Wigglesworthia* and *Sodalis* in an attempt to compliment the fitness of their offspring. Our observations provide further insights into the evolutionary adaptations that anchor the steadfast relationship shared between tsetse and its symbiotic microbes.

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PUNIQUE VIRUS, A NOVEL PHLEBOVIRUS, RELATED TO SANDFLY FEVER NAPLES VIRUS, ISOLATED FROM SANDFLIES COLLECTED IN TUNISIA AND ITS POTENTIAL IMPACT ON PUBLIC HEALTH

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Sand flies are widely distributed around the Mediterranean. Therefore, human populations in this area are exposed to sandfly-transmitted diseases, including those caused by phleboviruses. While there is substantial data in countries located in the northern part of the

Mediterranean basin, few data are available for North Africa. Sand flies were collected from the site of Utique, a well-known site of visceral leishmaniasis in northern Tunisia, during the summers of 2008, 2009 and 2010. In 2008 and 2009 sand flies were captured and pooled by sex and species. A vast majority of sand flies belong to *Phlebotomus perniciosus*. Thus species identification was abandoned in 2010 and sand flies were pooled by sex. Sand flies were tested for the presence of phleboviruses by PCR. Viral RNA corresponding to a novel virus closely related to Sandfly fever Naples virus (SFNV) was detected in pools of sand flies collected in 2008 and 2009. Virus isolation in Vero cells was achieved. Genetic and phylogenetic characterisation based on sequences in the three genomic segments showed that it was a novel virus distinct from other recognized members of the species. This novel virus was provisionally named Punique virus. Viral sequences in the polymerase gene corresponding to another phlebovirus closely related to but distinct from Sandfly fever Sicilian virus (SFSV) were obtained from positive pools collected in 2008 and 2010. Isolation of this virus temporarily named Utique Virus remained to be achieved. The public health impact of those two new viruses remained to be determined.

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ANTIBODY RESPONSES OF GUINEA PIGS TO SALIVARY ANTIGENS OF *TRIATOMA INFESTANS* FOR THE DEVELOPMENT OF TRIATOMINE EXPOSURE MARKERS

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Antibody responses to salivary antigens of the most effective vector of Chagas disease, *Triatoma infestans*, offer the potential to develop exposure markers for detecting the presence of small numbers of triatomines, especially after vector control measures have been implemented. Previous studies have detected a salivary apyrase as a main candidate exposure marker using guinea pig sera, but this protein is frequently found in the saliva of different haemathophagous insects and thus not triatomine specific. Futhermore, antibody responses to saliva of different developmental stages were not considered, although the immune responses may vary if using nymphal or adult saliva. Therefore in this study, guinea pigs were exposed weekly to 5 nymphs or adults of different *T. infestans* strains from Chile, Argentina and Bolivia over a period of 11 weeks and they were bled 5 days after each exposure. IgG responses of guinea pigs to nymphal and adult saliva were detected 11 days after the first exposure and both responses differed significantly. Saliva of nymphs and adults revealed complex protein profiles that uncovered differences not only between the *T. infestans* strains but also between the developmental stages. The most prominent bands in all strains were of 85, 72, 44 and 25 kDa. Although the saliva of nymphs was richer in its protein composition than the adult saliva, more salivary proteins of adults (n=10) were recognized by guinea pig sera than nymphal proteins (n=6) during the long-term study. Four antigens (85, 79, 72 and 44 kDa) were recognized by all guinea pig sera. Candidate exposure markers, such as a truncated pallidipin-like salivary protein (gi|148469123), were characterized, identified and synthesized as recombinant protein forms. The immunogenicity of these antigens was evaluated by sera of guinea pigs from the laboratory and field studies.

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FIELD EVALUATION OF A WICKING ASSAY FOR THE RAPID DETECTION OF RIFT VALLEY FEVER VIRAL ANTIGENS IN MOSQUITOES (*DIPTERA: CULICIDAE*)

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Rift Valley fever virus (RVFV) causes outbreaks of severe disease in domestic ungulates as well as humans in Africa. There is a concern that outbreaks of RVF may continue and that this virus may spread into regions where it had not previously been detected. Surveillance and rapid detection are critical to the initiation of an effective disease control program. Here we report on the field evaluation in Kenya of the VectorTest® RVFV antigen assay, modeled on the VecTest® assay for West Nile virus. The dipsticks provided results in less than 20 min, were easy to use, and did not require a laboratory with containment facilities. Although none of the field-collected mosquitoes were infected with RVFV, the dipstick provided a clear positive result with pools of field-collected mosquitoes spiked with a single positive, irradiated (to inactivate an infectious virus) mosquito. Similarly, the dipstick was able to detect virus from pools of mosquitoes captured during the RVFV outbreak in 2007. The RVFV dipstick assay was highly specific with only a single weak false positive out of 266 pools tested (specificity >99.6%). The RVFV assay can provide a rapid, safe, easy to use preliminary test to alert public health personnel to the presence of RVFV in mosquitoes in a given area. Results from this assay will allow for more rapid medical threat assessments and the focusing of vector control measures on high-risk areas.

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IDENTIFICATION OF A NEW GROUP OF LACTATION-ASSOCIATED PROTEINS IN THE TSETSE FLY, *GLOSSINA MORSITANS MORSITANS*

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Tsetse females generate a single larva during each gonotrophic cycle. All nutrients for larval development are provided by the mother in the form of lactation products generated by the milk gland. The nutrients within the milk are primarily composed of equal amount of lipids and proteins. Four proteins have been associated with tsetse lactation products, milk gland proteins 1-3 (*gmmmgp1-3*) and transferrin. However, little is known about other protein components of tsetse milk. In this study, we performed an Illumina based transcriptome analysis of differential gene expression in pregnant flies compared to those post parturition to identify lactation-specific genes. This analysis revealed 11 transcripts that are upregulated during pregnancy including the previously identified *gmmmgp1-3* and *transferrin*. Seven new MGPs (*gmmmgp 4-10*) were identified in this analysis. These proteins appear to be related as the amino acid composition of these proteins is similar to *gmmmg2-3*. Genomic analysis of *gmmmgp2-10* revealed that they are located on the same genomic loci. Analysis of the predicted upstream regulatory regions for *gmmmgp4-10* found conserved binding sites previously identified in the regulatory regions for *gmmmgp1-3*. Search for homologus sequences to these genes has only revealed a single uncharacterized sequence from the flesh fly, *Sarcophaga crassipalpis*. The predicted amino acid sequences for *gmmmgp2-10* contain a high percentage of hydrophobic amino acids and a conserved secretory signal peptide, however they lack characterized functional domains. Expression patterns of *gmmmgp2-10* are female specific and localized to the milk gland tissue. Temporal analysis of transcript levels for these genes is similar to the other genes

associated with lactation. This expression pattern results in increased transcript levels in correlation with larvigenesis followed by immediate decline after parturition (birth). Knockdown of *gmmmgp7* utilizing siRNA injections resulted in a significant reduction of fecundity. The discovery of *gmmmgp4-10* reveals a family of genes essential for viviparity and novel in form and function.

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RETENTION OF DUPLICATED LONG WAVELENGTH OPSIN GENES IN THE GENOMES OF THE MOSQUITO VECTORS *AEDES AEGYPTI, ANOPHELES GAMBIAE* AND *CULEX QUINQUEFASCIATUS*

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Understanding the role of mosquito vision in mating, host detection and oviposition, may help to improve or develop new control strategies to reduce the incidence of vector-borne diseases. Here we report the first molecular analysis of light receptors (opsins) from three mosquito vectors - the yellow fever mosquito, *Aedes aegypti*, the malaria mosquito, *Anopheles gambiae*, and the southern house mosquito, *Culex quinquefasciatus*. Opsins are receptors that interact with photons to initiate visual processes. Typically, insects have three classes of opsins that are stimulated by ultraviolet, short, and long (LW) wavelengths. We used expression data to improve the 10 *A. aegypti* and 11 *A. gambiae* published opsins gene models, and we report the first manual annotation of 13 opsin genes from *C. quinquefasciatus*. *Opsin* transcripts were confirmed using published expression data and Reverse Transcriptase-PCR. Phylogenetic analyses predicted six putative LW opsins in *A. aegypti*, six in *A. gambiae* and eight in *C. quinquefasciatus,* suggesting an expansion of these genes in the Culicidae relative to other insect taxa. Time of divergence suggests the mosquito LW opsins originated from several duplication events between 167 to 1 million years ago (MYA), and that 15 LW genes may have originated following a duplication event that occurred approximately 126 MYA. LW opsins share approximately 100% and 60% amino acid similarity within and between mosquito taxa, which raises intriguing questions regarding the retention of these genes in the three mosquito genomes. Seven amino acids were identified under positive selection in the N and C termini, and one in a third trans-membrane domain suggestive of opsin spectral tuning. Conserved nucleotide sequence in 6 out of 38 ortholog pairs and in 8 out of 14 paralog pairs of the non coding regions, up- and or down-stream, of the LW opsins is indicative of coordinated gene regulation. We discuss potential mechanisms, including positive selection and differential gene regulation, for the conservation of LW opsins in these mosquitoes.

1281

FIELD USER ACCEPTABILITY EVALUATION OF A NOVEL, SELF-SUPPORTING, LONG-LASTING INSECTICIDAL NET (LLIN)

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Insect bed nets provide protection against arthropod-borne disease pathogens such as malaria, dengue, and leishmaniasis. United States Army service members currently have a choice between two types of bed nets to use in field environments; however, both have various limitations that preclude effective long-term use by non-mobile forces. Therefore, the US Army was faced with a challenge to develop an improved bed net that does not have any of the limitations associated with these existing bed nets. The Walter Reed Army Institute of Research has partnered with Tritons Systems, Inc. to develop a novel, self-supporting, long lasting, insecticide-impregnated net (LLIN). The purpose of this study was to evaluate the new bed net in comparison with the existing Standard and Self-Supporting Low-Profile bed nets using an acceptability threshold of 70%. Upon completion of a large scale field training exercise in which these bed nets were used over the course of several nights, soldiers completed a self-administered survey answering questions about their ease of use, setup, dismantling, and comfort. Results of this acceptability study will be presented in the context of military force health protection.

1283

IMMUNOGENIC AND BIOCHEMICAL PROPERTIES OF IXOLARIS, A TICK SALIVARY TISSUE FACTOR PATHWAY INHIBITOR

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Ixolaris is a potent Tissue Factor inhibitor from tick saliva. It binds to Factor X(a) and the binary complex Ixolaris/FX(a) interacts with FVIIa/TF thus blocking the coagulation cascade. Ixolaris has been sucessfully tested as an antithrombotic in rats, and also displays anti-cancer properties in a glioblastoma model. Because Ixolaris displays therapeutic potential, understanding its immunogenic and biochemical properties is of interest. Here we demonstrate that ixolaris elutes as approximately 18 kDa protein according to gel-filtration chromatography. Light scattering plot and ultracentrifugation experiments also indicate that Ixolaris is a monomeric protein of approximately 18 Kda. Since the predicted mol mass for Ixolari is 15.5 kDa, the discrepancy is attributed to glycosilation. This contention has been confirmed by a smear observed by SDS-PAGE and mass spectrometry analysis of Ixolaris. Elisa also demonstrate that Ixolaris is non-immunogenic in rabbits and in mice. Taken together, these results provide further support for the potential therapeutic use of Ixolaris in a number of conditions with abnormal expression of Tissue Factor, including thrombosis, cancer, sepsis and malaria.

1284

MOLECULAR MECHANISMS OF *WOLBACHIA*-MEDIATED VIRAL INTERFERENCE

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Dengue is one of the most important arboviral diseases currently threatening human populations, with over 50 million cases in tropical and subtropical regions each year. No treatment or vaccine is currently available for dengue fever. Recently, the endosymbiotic bacterium *Wolbachia* has been proposed to be used as a tool to reduce mosquito vectorial capacity for dengue viruses through population replacement. Our previous studies showed *Wolbachia* alone can induce resistance to dengue virus in *Aedes aegypti*, which was associated with a boosted basal immunity in the *Wolbachia*-infected mosquito. To understand the molecular mechanisms underlying *Wolbachia*-mediated viral interference, we examined *Wolbachia*-induced physiological changes in mosquito by comparison of genome-wide transcriptome between *Wolbachia*-infected and -uninfected *Ae. aegypti*. Experiments were also conducted to compare full scale physiological responses of the two groups of mosquitoes to dengue virus infection. We found that the Toll signal pathway was prominently activated by *Wolbachia* in response to dengue virus infection. Interestingly, the genes related to redox stress response systems and mitochondria were strickly regulated by the *Wolbachia* in *Ae. aegypti*. Further studies were also conducted to investigate how the Toll signal pathway was activated by *Wolbachia* in *Ae. Aegypti.* As the effector genes of Toll signal pathway, the defensins and cecropins genes induced by *Wolbachia* were confirmed to play roles in control of dengue infection. Our studies provide evidence to support that *Wolbachia* induces resistance to dengue virus in *Ae. aegypti* through modulation of host immunity. We discuss the results in relation to develop *Wolbachia*-based control strategies for population replacement.

1285

THE INFLUENCE OF HABITAT ON THE GENETIC STRUCTURE OF *GLOSSINA FUSCIPES FUSCIPES* IN UGANDA AND IMPLICATIONS FOR VECTOR CONTROL

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Human and animal forms of African trypanosomiasis represent a burden to the public health and economy of many African countries. For effective trypanosomiasis management, controlling its vector, the tsetse fly (Diptera: *Glossinidae*), is necessary, but long-term success in vector control efforts requires a better understanding of tsetse dispersal and breeding ecology. We have collected genetic data over several years in Uganda for a major trypanosomiasis vector, *Glossina fuscipes fuscipes* (*G.f.f.*). This genetic information coupled with publicly available environmental data (climate, hydrology, land cover) was used to assess habitat selection and dispersal and breeding capacity of tsetse in Uganda. Connection networks between *G.f.f.* sampling localities were constructed and a modified inverse distance weighting method was used on these networks to interpolate a 'landscape' of genetic variation in Uganda. Genetic variation captured in this way was used with environmental data to carry out environmental niche modeling in Maxent v. 3.3.3. The inferred distribution of *G.f.f.* represents the flow of genetic information on the environmental substrate of Uganda. We used circuit theory methods implemented in the program Circuitscape v. 3.5.4 to model genetic connectivity of the environmental landscape in Uganda and estimate environmental resistance to dispersal between *G.f.f.* populations. The environmental 'friction' estimates were used to explore local genetic structuring of tsetse flies via spatially explicit principal components analysis (sPCA) with the 'adegenet' R package. Environmentally explicit modeling of gene flow provides information about the influence of the environment on genetic variation and connectedness. Environmental-genetic inferences about habitat selection and dispersal in tsetse could substantially improve vector control by helping to identify areas to be targeted for control and minimizing the probability of re-infestation from neighboring areas.

1286

FINE-SCALE GENETIC DIFFERENTIATION OF *GLOSSINA FUSCIPES FUSCIPES* IN THE LAKE VICTORIA BASIN AND IMPLICATIONS FOR VECTOR CONTROL

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The primary vector of Human African Trypanosomiasis (HAT) in Uganda is *Glossina fuscipes fuscipes* (G.f.f.). Little information is available on genetic differentiation and population dynamics of G.f.f. in the Lake Victoria basin. We screened for genetic diversity among tsetse populations both on mainland and island sites in southern Uganda. The aim of this work is to provide empirical data to support short-term vector control efforts and inform long-term monitoring with the ultimate goal of creating tsetse free zones. We used genetic data from 19 microsatellite loci and the mitochondrial cytochrome oxidase gene (530bp) to estimate population sizes and levels and patterns of genetic differentiation and gene flow within and among 13 tsetse populations in the Lake Victoria basin. We also used mark-release-recapture data to estimate population sizes and movement patterns and related these to genetic inferences. Temporal collections from the same sites were used to evaluate seasonal fluctuations (dry vs. wet) of tsetse demography. Both nuclear and mitochondrial markers suggest the existence of past and current genetic exchange among island populations and between island and mainland sites. We observed a positive correlation between geographic and genetic distance, which suggests that open water does not necessarily act as a barrier to tsetse dispersal. Genetic data also suggest that males disperse farther than females and that populations are stable over wet and dry seasonal cycles. We will discuss the results in light of other recent genetic studies and compare them to previous ones based on ecological data.

1287

THE CELL BIOLOGY OF *CANDIDATUS* RICKETTSIA ANDEANAE

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Candidatus Rickettsiae andeanae is an incompletely characterized spotted fever group rickettsia (SFGR), first detected in *Amblyomma maculatum* and *Ixodes boliviensis* ticks collected in 2002 from northern Peru during a febrile outbreak investigation. Phylogenetic analysis of the 17-kDA, *gltA, ompB, ompA,* and *sca4* genes demonstrated alignment with SFGR, but the molecular isolates were not found to be identical to any rickettsial agent listed in GenBank, and *Candidatus* R. andeanae was deemed a novel rickettsial agent. Despite molecular characterization of the *Candidatus* R. andeanae, the successful *in vitro* cultivation of this bacterium has remained a challenge. We recently used one half of the *Candidatus* R. andeanae-positive *A. maculatum* tick collected in Portsmouth, VA to successfully infect cultures of Vero, DH82, and S2 cells. Infections were confirmed using quantitative real-time PCR (qPCR) assays, acridine orange staining, and DNA sequencing of *gltA, ompB,* and *sca4* fragments. Current investigation of the cell biology by electron microscopy of *Candidatus* R. andeanae shows that the coccobacillus is approximately 0.3 um long and 0.2 um wide, it has a double cell membrane similar to other SFGR, but it is has only been observed growing in the cytoplasm and not in the nucleus of the three cell lines assessed. Nuclear extraction studies are ongoing to more specifically determine if this agent replicates within the nucleus. The studies described herein will more fully characterize this newly discovered rickettsia, which has now been established in culture for the first time in our laboratory.

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THE ROLE OF BIOFILM FORMATION IN COLONIZATION AND TRANSMISSION OF THE COMMENSAL SYMBIONT *SODALIS GLOSSINIDIUS* WITHIN THE TSETSE FLY

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Awareness of diversity and abundance of beneficial microbes has greatly increased with the advancement of molecular technologies. Recently, the influence of beneficial microbes in onset or prevention of disease has been shown, indicating an opportunity for harnessing these microbes for control of disease. *Sodalis glossinidius* is a gram-negative commensal symbiont of the tsetse fly, the sole vector of the African trypanosome. *Sodalis* is harbored throughout the fly both intra- and extracellularly, primarily in the midgut tissue in close proximity of the trypanosome and is maternally-transmitted to offspring. The proximity and the ability to genetically manipulate *Sodalis* makes it a great candidate for paratransgenesis; i.e., expression of antitrypanosomal compounds in the *Sodalis* within tsetse's midgut. One essential aspect of paratransgenesis is understanding transmission biology of *Sodalis* and recolonization efficiency of genetically modified *Sodalis* in tsetse lines. Biofilms are dense populations of microbes that adhere to surfaces and each other secreting extracellular polymers. Only a few studies have shown the role of biofilm formation in vector born disease; i.e., *Yersinia pestis* within the flea gut. The ability of *Sodalis* to produce a biofilm was investigated using a classical microtiter plate biofilm assay and was shown to produce a biofilm under *in vitro* conditions. In this study we assessed genes important for biofilm formation in the fly gut

colonization process, transmission to progeny and trypanosome infection rates. Our studies provide enhancement of paratransgenic methodology by understanding the role of biofilm formation in both recolonization of *Sodalis* and trypanosome infections, which will guide us in applying paratransgenesis in the future.

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NEW VECTOR CONTROL MATERIALS FROM THE ARMED FORCES PEST MANAGEMENT BOARD

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The Deployed War-Fighter Protection research program (DWFP) is an initiative to develop and validate novel methods to protect United States military deployed abroad from threats posed by disease-carrying insects. Starting in 2004 and administered by the Armed Forces Pest Management Board the program is funded at $5M per year. The DWFP research portfolio is concentrated in 3 specific areas: novel insecticide chemistries/formulations, application technology, and personal protective systems. Program consists of a noncompetitive funding process for USDA ARS-based research, and a competitive grants process open to non-USDA ARS scientists (PIs from academia, industry, and military entomologists: 55 projects funded). Up to $3 million per year is given to USDA ARS National Program 104, dealing with Veterinary, Medical, and Urban Entomology. Ultimate objective is to find industry partners and get useful products into the market/military stock system. Presentation highlights DWFP products with examples of equipment, insecticides, and ~300 refereed publications.

1290

COMPREHENSIVE EPIDEMIOLOGICAL RESEARCH EFFORT ON FEBRILE ILLNESSES AND HEMOGLOBINOPATHIES ALONG THE BANGLADESH-MYANMAR BORDER

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In order to estimate the burden of febrile illnesses in the border region of Bangladesh toward Myanmar a comprehensive prevalence study on febrile illnesses and hemoglobinopathies was conducted in an area with suspected high endemicity of tropical infectious diseases. Little is known about the prevalence of febrile illnesses in the Chittagong Hill Tracts, the southernmost region of Bangladesh bordering Maynmar and India, an area with limited access to medical care due to inaccessible terrain and lack of infrastructure. Samples were collected from patients enrolled during two separate cross-sectional studies in the years 2007 to 2010 covering the same rural communities in rainy and dry season to assess seasonal trends. In a parallel ongoing hospital-based fever survey data of febrile participants from the catchment area of the Bandarban Sadar Hospital were collected. Out of a total population of 2123 enrolled in the studies 671 acute febrile patients were diagnosed for the most common infectious diseases: malaria (RDT, microscopy and PCR), typhoid fever, leptosirosis, dengue (serological assays) and influenza (RDT and PCR) as well as hemoglobinopathies. The collected data allow for an estimation of long term trends in the epidemiology of the investigated diseases as well as short-term variations such as seasonal fluctuations and emergence of rare conditions. *Falciparum* malaria remains the major health threat with a cross-sectional prevalence of 40.9% (CI: 35.4 - 46.7%) during monsoon months (May - October). However numbers vary significantly with the season and show an overall declining trend over the years. A high number of seropositive cases for leptospirosis (n= 194, 28.9%; SD: 25.6 - 32.5%) and typhoid fever (n=203, 30.3%; SD: 26.9 - 33.8%) indicate a major persistent reservoir of infection for these pathogens in the surveyed communities. Associations of disease distribution with demographic, geographic, and meteorological data were performed to define and map the prevalence and indirect estimates of incidence as the basis for assessing actual disease burden.

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INVESTIGATION OF A SUSPECTED OUTBREAK OF ACUTE FEBRILE ILLNESS IN MALINDI, KENYA IN DECEMBER 2010

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Acute febrile illness (AFI) refers to sudden illnesses with fever. It is a common clinical presentation in Kenya where its aetiology remains unknown. Information on the prevalence and causes of AFI in Kenya is limited. Walter Reed Project's (WRP) AFI surveillance site in Malindi noticed a 3 fold surge in AFI cases from Sept 2010 to Nov 2010 (Sept #10, Oct #5, Nov #15 cases). On average 2 cases are enrolled monthly. This prompted an investigation. Of note cases were from the same locality. Aliquots of malaria negative blood by RDT were sent to WRP reference lab for PCR and ELISA for Malaria, Salmonellosis, Brucellosis, Leptospirosis, Aborviruses and nasopharyngeal swabs tested for Flu. Study team: WRP and MOH. Study Area: Malindi District Hospital and Kisumu-dogo area. Investigation Period: 15 - 18 Dec. Case size: All 21 cases. A standard questionnaire was administered to all. All lab records from the period were reviewed. Data from the questionnaires was entered into an EPI-INFO database and analyzed. Majority, males (57.1%, n=12). The median age 30 yr. Most below 20 yr (42.9%, n=9). Most from Kisumundogo (23.8%, n=5). Majority presented with headache (42.9%, n=9), joint aches (28.6%, n=6) and myalgias (19%, n=4). 42.9% of cases classified as having AFI actually had undiagnosed malaria. 42.9% were malaria positive on PCR. All cases were negative for viruses by PCR, Elisas and cell culture. Importantly, the aetiology of fever remained unknown in 57.1% of cases. Malaria RDT's are not sensitive enough in low malaria transmission areas and when parasitemia is low. A negative RDT may not be enough to rule out malaria in regions of low malaria endemnisity. There is clinical and lab evidence of low parasitimia having being cleared in malaria immune patients as no PCR positive was given antimalarias but on repeat PCR all were negative for malaria. This could be explained by self treatment but all denied it. A comprehensive study to discover both common and uncommon pathogen causes of acute febrile illnesses is needed. PCR may be a complement to RDT and Microscopy in low malaria endemic areas. Continue vector control. Malaria naive persons should continue to be offered prophylaxis or preventive measures.

1292

EFFICACY, SAFETY AND PK OF ARTEMETHER-LUMEFANTRINE DISPERSIBLE TABLET IN THE TREATMENT OF ACUTE UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN INFANTS <5 KG BODY WEIGHT

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WHO recommends artemisinin-based combination therapy (ACT) as first-line therapy for infants with uncomplicated *Plasmodium falciparum* malaria who have body weight (BW) ≥5kg. However, no ACTs are indicated in infants <5kg. Poor safety profile of current standard of care,

quinine, limits its usage. Coartem (20mg artemether-120mg lumefantrine, AL), with an available pediatric formulation, has the largest clinical trial and postmarketing safety experience in infants ≥5kg to-date. This open-label, single-arm, multicenter study in Sub-Saharan Africa will enroll inpatient neonates and infants of <5kg BW with a confirmed diagnosis of uncomplicated *P. falciparum* malaria in two sequential cohorts of 15 infants each: term age >28 days (cohort 1) and term age ≤28 days (cohort 2) to minimize any theoretical risk. A joint data monitoring committee will review efficacy, safety, and pharmacokinetic (PK) data from cohort 1 and recommend whether to proceed to cohort 2, with or without dose adjustment. The primary objectives are to evaluate the efficacy and safety of AL dispersible tablet administered as 1 tablet bid over 3 days (to adjust if required), and to determine plasma levels of artemether, its active metabolite dihydroartemisinin, and lumefantrine. Exclusion criteria include severe malaria, signs and symptoms of a critical condition, hepatic or renal abnormality, and major neurological malformation. Neurodevelopment status follow-up of patients is planned until day 42 and at 3, 6 and 12 months. Primary endpoint is PCR-corrected parasitological cure at day 7. Secondary endpoints include reduction in parasite density at 24 hours; PK assessments; PCR-corrected parasitological cure at days 14, 28, and 42; time to parasite, fever and gametocyte clearance; and safety and tolerability assessments. Appropriate use of antipyretics and quinine as a rescue medication will be permitted. Protocol approval will be sought from ethics committees in Switzerland, and in each participating country. Written informed consent will be sought from all parents/guardians. Study results are expected in 2014.

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EVALUATING THE READINESS OF OUTPATIENT HEALTH FACILITIES TO MANAGE MALARIA CASES IN BENIN

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In 2008, the government of Benin and its partners began implementing a new national malaria case-management policy in 787 public health facilities (HFs) that recommended the use of artemisinin-based combination therapy (ACT). We evaluated the readiness of outpatient HFs to manage malaria cases in Benin about one year later. In late 2009, we conducted a nationally representative cross-sectional survey of a stratified random sample of 60 HFs. Surveyors observed consultations and interviewed and re-examined patients seeking care for any illness and pregnant women seeking antenatal care. In addition, health workers (HWs) were interviewed, and HFs were assessed to determine the availability of drugs and equipment. Results were weighted. Altogether, 57 HFs, 113 HWs, and 448 patients were included in the analysis. All HFs had a thermometer, 70.8% (95% confidence interval [CI]: 59.3-82.3%) had a scale for weighing children, and 66.3% (95% CI: 56.6-80.1%) of HFs had a booklet or chart with ACT algorithms. Although all hospitals could perform malaria testing, only 40.8% of non-hospitals could perform testing. In the three months before the survey, 46.7% (14/30) of hospitals and 33.3% (9/27) of health centers had stock-outs of all types of artemether-lumefantrine blister packs (i.e., none in stock) for at least three days. Adherence to the testing policy (i.e., test all patients with a febrile illness, and do not test patients without a febrile illness) was 52.9% (95% CI: 48.3-57.5%) among all 448 patients, 24.7% (95% CI: 18.2-31.2%) among 170 patients <5 years old, and 70.1% (95% CI: 64.7-75.5%) among 278 patients ≥5 years old. Nearly all of the 79 patients who tested positive were given an antimalarial (98.2%; 95% CI: 95.3-100%). However, 22.1% (17/77) of patients who tested negative were given an antimalarial. HF readiness varied. HWs prescribed antimalarials when tests were positive. However, ACT stock-outs were common, HWs did not follow testing guidelines for children <5 years old, and they sometimes prescribed antimalarials even when tests were negative.

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INVESTIGATION OF A CLUSTER OF DEATHS ATTRIBUTABLE TO MALARIA IN RURAL SENEGAL

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Since 2005, malaria control interventions, including insecticide-treated nets, rapid diagnostic tests, and artemisinin-combination therapies have been scaled up in Senegal, resulting in a large decrease in the malaria-associated morbidity and mortality. However, in Touba district, deaths attributed to malaria from September - November (transmission season) increased by 58% in 2009 compared to 2008, while admissions for malaria decreased 19%. One health center reported 80% (38/47) of the deaths. The National Malaria Control Program led an investigation of these deaths, consisting of interviews with families and care providers and retrospective chart reviews. Charts were reviewed for 38 malaria deaths, all confirmed by rapid diagnostic testing. The median age was 5 years (39% < 5 years and 37% 5-9 years) and 59% were male. Only 17% (6) sought care within 24 hours of symptom onset, with a median of 3 days. Anemia was laboratory-confirmed in 37% (14) and diagnosed clinically in 26% (10); mean hemoglobin was 3.9 g/dL in those tested, two of whom received a blood transfusion. Rapid blood glucose was performed in 18% (7) and complete blood counts in 37% (14). Of the 12 patients with elevated leukocytes, 8 received an antibiotic. Of 37 patients for whom treatment was documented, 65% received a correct medication regimen, 26% had dosing errors, and 5% died prior to starting quinine. The majority (92%) of patients died the day of admission or the following day. Recommendations included expanding the home-based management program, reinforcing preventive community based interventions, educating the community on danger signs and accessibility of treatment, and staff training to improve referral practices, performance and documentation of history and physical exams, complete blood counts and blood glucose, correct dosing of antimalarials, and differential diagnosis of fever. Additional studies would be needed to determine if the delays in seeking care and deficits in care were associated with the deaths. A standardized tool for investigation of deaths will help improve case management and the response to clusters of deaths.

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EFFECTS OF GASTROENTERITIS EPISODES ON MAINTENANCE OF POLIO VACCINE TITERS IN CHILDREN THREE YEARS AND UNDER IN RURAL COASTAL KENYA

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Evidence shows that infants with concurrent gastroenteritis (GE) are less likely to respond to oral polio vaccination than those without gastroenteritis. Our objective was to determine whether further episodes of gastroenteritis in the first three years of life had an effect on maintenance of polio titer. Children enrolled in a birth cohort in rural coastal Kenya received four trivalent polio vaccinations before 6 months of life. Sera were then drawn at 6 month intervals until age 36 months and polio titers were measured using poliovirus IgG ELISA kits. GE episodes were documented during scheduled follow-up visits and at any time of illness during the 3 year period. Student's t-test was performed to compare those with and without GE at each time point. Of 545 children in the study, 159 had 246 episodes of gastroenteritis in the first three years of life. GE episodes were more likely to occur between 6 and 18 months of life. The range of GE episodes per child was 0-4. Polio titers did not significantly differ between children with and without GE from 6 to 36

months of age. Although concurrent gastroenteritis may hamper immune response to oral polio vaccine, further episodes of gastroenteritis after the vaccination series do not appear to alter the maintenance of polio titers.

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CLINICAL IMPLICATIONS OF ADHERENCE TO WHO GUIDELINES FOR THE MANAGEMENT OF THE FEBRILE PHASE OF DENGUE

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According to WHO guidelines the use of acetaminophen is indicated during the febrile phase of dengue and aspirin or non-steroidal anti-inflammatory agents (NSAIDs) should be avoided as these drugs may aggravate gastritis or bleeding. However, there is little clinical evidence to support this recommendation. We conducted a prospective cohort study in an endemic area in Colombia to evaluate the potential association between noncompliance with this guideline and the risk of developing severe dengue. Acute febrile outpatients (less than 96 hours of onset) with dengue (confirmed by viral isolation, RT-PCR or a shift from negative to positive IgM test) were followed daily until the seventh day of disease. Subjects were excluded based on the following: diabetes, AIDS, hematologic disorders, cancer or cardiac disease and the presence of a major bleed, albumin < 3gr/dL, effusions or shock at presentation. Inappropriate Initial Treatment (IIT) was considered when the patient reported having taken NSAIDs, aspirin or dipyrone. Data collected included signs and symptoms, and daily microhematocrit determinations to recognize hemoconcentration. A complicated case was defined by the following: a platelet count ≤100.000/mm^3; any spontaneous hemorrhagic manifestation (or one positive tourniquet test); and evidence of plasma leakage (i.e. pleural effusion, ascitis, hypoalbuminemia or a variation of hematocrit greater than 10%). Of 596 patients, 97% appropriately received acetaminophen but 54% also received IIT. 63.2% (n=98) of cases receiving IIT were complicated compared with 36.8% (n=57) complicated cases in the 271 subjects treated only with acetaminophen [OR crude: 1.62 ; 95% CI: 1.96- 6.39; OR : 1.51; IC95% (1.03-2.2) adjusted by age and sex]. In conclusion, adherence to WHO guidelines during the febrile phase of dengue is important to reduce the risk of complications. This study is registered with Colciencias (Departamento Administrativo de Ciencia, Tecnología e Innovación de Colombia), number: 110245921561.

1297

MALARIA PREVALENCE AND MORTALITY IN RURAL SIERRA LEONE

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Malaria is a leading cause of morbidity and mortality in rural areas of Sierra Leone. Mortality from malaria is as high as 28% in the under age 5 group of pediatric patients. The Village Medical Project provides medical care and treatment to women and children in several villages in Gorama Chiefdom, Kono District of rural Sierra Leone The purpose of this study is to document the prevalence of malaria infection, anemia and crude mortality. The project has been working for 3 years to ascertain the success of primary treatment and prevention of malaria in a rural area of Sierra Leone. Adult and Pediatric Patients were tested for *Plasmodium falciparum* malaria and hemoglobin. Patients were selected from each village based on prior census data and followed for a 2 year period, from 2008-2010. Primary prevention with bed nets were provided for children under 5 years of age. 1043-1463 patients were seen annually over a 2 year period from 2008-2010. Overall, malaria prevalence varied from 67-97%. The overall crude mortality rate from 2008-2010 was 8%. Under age 5 mortality is 9.8%. 87% of the population is anemic based on WHO standards. Access to Medical Care and treatment remains difficult for this population We are significantly limited by lack of accurate ages, mobility of the population and changing demographics. Malaria is very prevalent in this rural area of Sierra Leone. Primary treatment and prevention has had some impact on mortality rates compared to prior studies, however there still remains a significant disease burden in this area, with significant morbidity and mortality.

1298

ARTEMETHER, DIHYDROARTEMISININ AND LUMEFANTRINE DO NOT INDUCE *IN VITRO* DRUG METABOLIZING ENZYMES AND METABOLISM OF ORAL CONTRACEPTIVES

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The goal of this study was to evaluate *in vitro* the components of Coartem/Riamet (artemether and lumefantrine) and the active metabolite dihydroartemisinin (DHA) for their potential to induce drug-metabolizing CYP enzymes and the metabolism of oral contraceptives. The experiments were conducted according to the FDA drug drug interaction guidance. The assessment was done *in vitro* in cryopreserved primary human hepatocytes of at least three individual donors. Induction of mRNA, relative to the vehicle control, was determined by real-time PCR and evaluation of changes in cytochrome P450 (CYP) enzyme activities were assessed after 48-h induction periods by LC/MS/MS analysis of CYP-selective probe substrate metabolism. Metabolism of the oral contraceptives was tested by HPLC analysis. Human hepatocytes were incubated with the three test substances up to concentrations which exceeded their therapeutic concentrations by a factor of 10. Ethinyl estradiol and levonorgestrel were selected as active ingredients of oral contraceptives and were tested at their therapeutic concentrations of 1 nM and 20 nM, respectively. Rifampicin at 0.1, 1, and 20 µM, and phenobarbital at 1000 µM were used as positive controls for induction of genes regulated by PXR and/or CAR like CYP2B6, CYP2C, and CYP3A; β-napthoflavone at 10 µM was included as positive control for AhR-mediated induction of genes like CYP1A. Artemether, DHA, and lumefantrine were determined not to be inducers of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, or CYP3A enzyme activity in hepatocytes or CYP1A1, CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP3A4, or CYP3A5 mRNA. Metabolism of ethinyl estradiol and levonorgestrel was determined not to be induced by artemether, DHA, and lumefantrine. As per FDA criteria, these conclusions were based upon the levels of mRNA or activity at least less than 2-fold, with respect to the vehicle control, and/or less than 40% of the maximal positive control induction response, indicative of a non-inducer *in vitro*.

1299

EFFECT OF DIARRHEA ON GROWTH IN INFANTS IN URBAN SLUM OF SOUTH INDIA

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Diarrheal diseases are the second leading cause of morbidity and mortality in children less than 5 years of age. The vicious cycle of diarrhea and malnutrition has long been recognized, and it has been shown that early childhood diarrhea has long term effects on growth and development. To study the association of diarrhea in early infancy with growth faltering at one year of age, longitudinal data (N = 897) from 3 birth cohort studies conducted between 2002-2011 in an urban slum of South India were analyzed. Children were followed biweekly/weekly to assess diarrhea and other morbidities. Monthly anthropometric (height/ weight) measurements were obtained. We assessed the effect of diarrhea, on acute (WHZ < - 2 SD) and chronic (HAZ < - 2 SD) malnutrition, using the WHO Child

Growth Standards 2006. The median number of diarrheal episodes among children in the cohort was 2 (1-3). At 1 yr, 33.9% of infants had chronic malnutrition and 26.9% had acute malnutrition. Three or more episodes of severe diarrhea was significantly associated with chronic (OR=2.45, *P*=0.02) and acute malnutrition (OR=2.8, *P*<0.01). Other factors associated with chronic malnutrition were living in a mud house, an indicator of lower socioeconomic status (OR=1.8, *P*<0.01), presence of an older sibling (OR=1.6, *P*<0.01). Duration of exclusive breasting, more than primary schooling as highest education in the family and being a girl offered protection of 22% (*P*<0.01), 44% (*P*<0.001), 30% (*P*<0.01) respectively. As expected, severity of diarrhea and poverty are associated with acute and chronic malnutrition, with exclusive breastfeeding and higher education being protective. Lower rates of malnutrition were noted in girls, an unexpected finding.

1300

U.S. MILITARY FORCE HEALTH PROTECTION POLICIES MAY IMPACT PEDIATRIC MALARIA PROPHYLAXIS PRESCRIBING PATTERNS

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To date, there have been no large scale systematic surveys of antimalarial prescribing practices in the United States. Although pediatric patients are at higher risk of severe disease due to malaria than adults, there is a relative scarcity of information on the prevention of malaria among pediatric travelers versus adult travelers. This study consists of a systematic search of the military health system electronic medical record system for all prescriptions of chloroquine (CQ), mefloquine (MQ), and atovaquone-proguanil (AP) to military family members 8 years of age and under in the years 2005-2010. Prescribing patterns were assessed for changes over time to identify if Department of the Army and Department of Defense policies, published in 2009, limiting the use of mefloquine in deployed forces coincided with changes in prescribing patterns for young children. A total of 3404 prescriptions were written for these medications during the study period. In total, CQ, AP, and MQ, respectively accounted for 7%, 43%, and 50% of all prescriptions. Overall prescription volume increased from a low of 507 prescriptions (60% MQ) in 2005 to a high of 726 (39% MQ) in 2010 (p <0.001). While the total volume of antimalarial prescriptions rose, this change was reflected almost entirely by an increase in the usage of AP. In 2010, in contrast to prior years, 44% of all AP prescriptions were for amounts in excess of a 30 day supply, compared to 37% for earlier prescriptions (p=0.015). This trend of progressively more prescriptions for AP in absolute and relative terms exists over the entire study period (p = 0.003). This study documents that military physicians providing pediatric travel services now prescribe less MQ relative to AP. This occurs even in settings where the duration of travel has led many experts to recommend MQ as the drug of choice. The timing of these changes suggests that military force health protection policy, as well as patient/family and provider awareness regarding adverse effects associated with MQ may be impacting prescribing practices for these medications.

1301

PRELIMINARY RESULTS OF A HOSPITAL-BASED LABORATORY SURVEILLANCE FOR INFECTIOUS ETIOLOGIES OF UNDIFFERENTIATED FEBRILE ILLNESSES IN GEORGIA

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Since 2008, laboratory-based sentinel surveillance for undifferentiated febrile illness (UFI) has been ongoing in six hospitals to establish the frequency of nine infectious causative agents of febrile illness in Georgia. Hospitalized patients ≥ 4 years of age with fever ≥38°C for ≥48 hours were asked to voluntarily participate. Blood culture and serologic testing (ELISA) were conducted for *Leptospira* spp., *Brucella* spp., West Nile virus (WNV), Crimean-Congo hemorrhagic fever (CCHF) virus, *Coxiella burnetii*, tick-borne encephalitis virus (TBEV), hantavirus, *Salmonella* Typhi and *Rickettsia typhi*. A total of 478 subjects have been enrolled in the study. Of these, 71% were outpatients and 53% were males with the mean age of 36 years. Fever of unknown origin was the preliminary diagnosis in 88% of patients. Patients also reported having fatigue (90%), rigors (87%), sweats (82%), pain in joints (48%), and sleep disturbances (40%). Acute and convalescent samples from 403 patients (n=473) were initially tested by IgM ELISA. Sixty-nine patients were seropositive for hantavirus (16%), 52 for *Leptospira* spp. (13%), 17 for *Coxiella burnetii* (4%), 16 for TBEV (4%), and 3 for WNV (0.7%). Additionally, 33 patients were seropositive for *Brucella* spp. (8%), 3 patients for *S.* Typhi (0.7%), and 8% (34) of patients showed positivity by IgG ELISA for *R. typhi*. Highest cross-reactivity was observed for hantavirus and *Coxiella burnetii,* in 13(2.8%) samples. Preliminary laboratory results indicate a high prevalence of antibodies against hantavirus, leptospirosis, brucellosis and rickettsioses among febrile patients in Georgia. This hospital surveillance for UFI has significantly enhanced laboratory capacity for the detection of specific infectious etiologies as well as established a valuable network of clinical sites that can be used for future syndromic surveillance studies. Confirmed laboratory results will allow the Georgian public health authorities to make better informed decisions regarding screening and prevention strategies.

1302

FACTORS INFLUENCING HIGH RATES OF CATCH UP GROWTH AFTER EARLY CHILDHOOD STUNTING IN CHILDREN OF URBAN SLUMS OF SOUTHERN INDIA: A COHORT STUDY

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Malnutrition and stunting in early childhood is a major public health problem in less developed countries. A lack of long term cohorts leads to a paucity of data on factors that influence catch up growth in children not enrolled in supplementary feeding programs. Our study in an urban slum in southern India investigated catch up in growth in children after early stunting. The study group was a birth cohort of 452 children,

followed intensively for three years, but at 7-8 years, 273 children were contacted in 2010. Data was collected using a structured questionnaire and anthropometry. For analysis, the cohort was divided into categories of children who were ever stunted at 12, 24 and 36 months and those who were never stunted. Of available children, 189/273 (69.2%) were ever stunted, but more than 80% of the 189 showed catch up growth by 2010. The mothers of the ever stunted group were younger by 1.4 years ($p = 0.009$), shorter ($p = 0.009$) and weighed less ($p = 0.02$) than mothers of never stunted children. Another variable that predisposed to stunting was household debt (Crude OR 1.82, 95 % CI 1.07-3.08). Ever stunted children were divided into 2 groups, persistently stunted (33, 17.5%) and children with catch up growth (156, 82.3%) at the current follow up. In univariate analyses, factors associated with catch up growth were having <3 children, use of sunflower oil, use of a ration card, schooling of child in an unaided private school and using liquefied petroleum gas as cooking fuel. After multivariate logistic regression analysis, the factor independently associated with catch up growth was use of a ration card (Adjusted OR 3.16, 95% CI 1.01-9.76). Our study shows remarkably high rate of catch up growth, which was associated with use of a ration card issued by the public distribution system, indicating that there is potential for governmental interventions to decrease malnutrition in poor urban communities.

1303

INFECTIOUS ETIOLOGIES OF ACUTE MENINGITIS AND ENCEPHALITIS IN GEORGIA

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In Georgia, there are diverse etiologies of meningitis and encephalitis, including vaccine preventable agents such as mumps virus, varicella zoster virus (VZV), *Streptococcus pneumoniae*, *Neisseria meningitidis*, *Haemophilus influenzae* type B (Hib), and others viral agents (e.g. Epstein-Barr virus (EBV), tick-borne encephalitis virus (TBEV) and West Nile virus (WNV)). Prevalence information regarding these infections in Georgia is limited. In October 2010, a hospital-based surveillance study was initiated to determine the incidence of infectious etiologies of acute meningitis and encephalitis; enhance laboratory capacity for the diagnosis of central nervous system (CNS) infections; determine antimicrobial susceptibility profiles; and describe the risk factors and clinical presentations associated with etiologic agents of CNS infections. Patients with suspected meningitis and encephalitis were enrolled from three hospitals in Tbilisi. Cerebral spinal fluid (CSF) and acute and convalescent sera were collected for bacterial culture and RT-PCR testing for HSV types 1 and 2, mumps virus, enteroviruses, VZV, *S. pneumoniae*, Hib, and *N. meningitidis.* The diagnosis of WNV, TBEV, and EBV was conducted via commercial ELISA assays. As of 21 March 2011, 66 patients have been enrolled (23 adults and 43 children) and 61 CSF samples tested. Initial laboratory results indicate the frequency of HSV-1, enteroviruses and VZV to be 43%, 38% and 2%, respectively. For both TBEV and WNV, the frequency was determined to be 7%. Nine samples were positive for TBEV and seven samples were positive for EBV in 131 pairs of acute and convalescent sera. One *S. pneumoniae* case was cultured from CSF. These preliminary study results suggest the presence of a wide-spectrum of pathogens among patients with suspected meningitis and encephalitis. This surveillance study serves as a model for enhancing patient care through understanding disease prevalence, building laboratory diagnostic capacity and designing future syndromic surveillance projects in Georgia.

1304

CLINICAL MANAGEMENT OF DENGUE: A PHYSICIAN EDUCATION PROGRAM TO IMPROVE CLINICAL OUTCOMES, PUERTO RICO

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In 2007-08, the Centers for Disease Control and Prevention (CDC) Dengue Branch and the Puerto Rico Department of Health (PRDH) conducted a survey to assess physician's knowledge of dengue and clinical management practices. The survey identified limited knowledge of warning signs for severe dengue and early signs of shock and non-standard treatment practices including use of corticosteroids and non-isotonic crystalloid solutions. A review of fatal dengue cases in 2007 corroborated these findings. In 2008-09, CDC Dengue Branch developed and pilot tested a physician training course to address the deficiencies identified by the survey and fatal case review. Focus groups and interviews were conducted with attendees of the pilot course to evaluate instructional process and content, and the course was revised accordingly. The course was approved by CDC and accredited by the Accreditation Council for Continuing Medical Education for 4 CME credits in February 2009. The Secretary of Health of Puerto Rico mandated that physicians take the training as a prerequisite for re-certification by 2013. To fully implement the case management course, 52 physicians were selected, trained and certified as Master Trainers. From February 21, 2009 to December 31, 2010, 55 courses attended by 8,301 of the 12,929 licensed physicians in Puerto Rico were conducted. Most physicians (6,294, 76%) were trained between September 1 and October 31, 2010 in response to another mandate from the Secretary of Health that all primary care physicians be trained immediately due to the increased number of dengue fatalities. An evaluation of the impact of training on clinical practices will be conducted in the Fall of 2011. Findings from this evaluation will be used to redirect continuing training efforts and to develop an online dengue clinical management course. Lessons learned from the implementation of this training initiative will be shared with dengue endemic countries planning to train physicians on the clinical management of dengue.

1305

PERFORMANCE OF STABILIZATION TUBES FOR EXTENDING TIME TO ANALYSIS OF COMPLETE BLOOD COUNTS FROM TRIAL PARTICIPANTS AT A RURAL FIELD SITE IN MALI

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Stabilization tubes (ST, Beckton Dickinson, Franklin Lakes, NJ) extend the pre-analytical stability of whole-blood (WB) specimens taken for CD4 measurements by utilizing a proprietary supplement targeted at

cell surface membranes and clusters of differentiation (CD) on white blood cells (WBC). We hypothesized that this membrane-protective effect might confer specimen stability by acting on other membrane-bound cellular components. ST were assessed using twenty (n=20) WB specimens collected during a malaria vaccine clinical trial in Mali, West Africa. WB specimens were collected into ST and EDTA Vacutainer tubes for comparison, and complete blood counts (CBC) were conducted at day 0 and then every 24-hrs for 7 days thereafter. All measurements of WBC parameters deteriorated (> 10% erroneous or missing values) after 24 hours post-collection, while all red blood cell (RBC) parameters remained largely unchanged through 6 days post-collection. Data analysis revealed that ST do not provide stability of WB after collection in our setting. Further investigations validating and implementing novel technologies in the field are greatly needed to ensure quality specimens are analyzed in clinical research.

1306

EVALUATING BLOOD CULTURES IN GUATEMALA AFTER IMPLEMENTATION OF A DEDICATED PHLEBOTOMY TEAM

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Blood cultures (BCs) are important diagnostic and surveillance tools to identify invasive bacterial diseases. In January 2008, we established automated BCs at a rural hospital in Guatemala and provided frequent trainings, job aids and all supplies and materials. After the first year of implementation, we found poor adherence to protocols and high contamination rates. In August 2009, we implemented a dedicated, round-the-clock phlebotomy team. To determine whether this intervention decreased contamination rates and improved pathogen isolation, we conducted segmented regression analysis of a monthly time series of BC outcomes from the laboratory database. A blood culture was defined as one or two blood culture bottles filled with blood taken from the same site. We collected data on 2,140 BC prior to intervention (January 2008-July 2009) and 1,525 blood cultures after the intervention was fully implemented (October 2009 to September 2010). There was an increase in the median number of BC per month among children <10 years old (41 per month pre-intervention vs. 58 per month post-intervention, p=0.05) but not among persons aged 10 years and older (63 per month pre-intervention vs. 68 post-intervention, p=0.69). Among 858 BC in children <10 years old during the pre-intervention period, 14% of cultures were contaminated and 7% produced a pathogen, compared to 10% contaminated and 4% with a pathogen among 695 cultures post-intervention. Among persons aged 10 years and older, 3% of 1282 cultures taken pre-intervention were contaminated and 8% yielded a pathogen, compared with 1% contaminated and 4% yielding a pathogen of 830 cultures taken post-intervention. Segmented regression analysis showed no impact of the intervention on the contamination rates among children <10 years old (β=-15.3, p=0.13) or persons aged 10 years and older (β=-2.5, p=0.45). Similarly, there was no effect of the intervention on pathogen isolation rates among children <10 years old (β=-2.2, p=0.62) or persons aged 10 years and older (β=-3.6, p=0.55). The results from this evaluation suggest that contamination rates among young children are unacceptably high and may be preventing isolation of pathogens. In addition to strengthening efforts to reduce contamination during venipuncture, particularly of young children, a review of laboratory protocols and procedures may identify further opportunities to reduce contamination and identify meaningful pathogens.

1307

"LOOKING FOR GOLD, FINDING MALARIA" THE 2010 MALARIA SURVEILLANCE OF THE SURINAME GOLD MINING MALARIA CONTROL PROGRAM

Hedley Cairo

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Malaria is endemic in the forested interior of Suriname. Since 2006 malaria cases have declined tremendously with dispersed foci remaining in the gold mining areas. Currently the majority of malaria infections occur among persons (ca. 15,000) engaged in small-scale gold mining and related activities. Because there were no formal health services in these remote areas, a Global Fund supported malaria program was initiated in 2009 to fill the gap. This control program builds further on a surveillance system established in 2006 as a pilot under the Medical Mission Malaria Program. The surveillance system gathers weekly information from the Tourtonne diagnostic and treatment facility in the Capital city Paramaribo and from a network of 18 home-based diagnostic and treatment facilities (Malaria Service Deliverers) in the gold mining areas. Malaria cases are diagnosed by blood film or rapid diagnostic tests. A descriptive analysis of preliminary surveillance data of 2010 will be presented and compared with data from the previous year. The system recorded 1548 cases of malaria in 2010 among gold miners; 1388 (90%) confirmed by microscopy and 160 RDT cases. This number represents a decrease of 31.5% from the 2259 cases reported for 2009. *Plasmodium falciparum* 39%, *P. vivax* 52%, mixed *P. falciparum* plus *P.vivax* (7%) and *P. malariae* (2%) were the species identified. Among the 1548 cases 961 (62%) were classified as imported from neighboring countries and 26 (2%) were of unknown origin. 40 cases were reported in pregnant women of which 8 were *P. vivax* relapse. The Annual Blood Examination Rate (ABER) was 52.83, Slide Positivity Rate (SPR) was 17.51 and the Annual Parasitic Index (API) calculated from autochthonous cases was 30 in 2010 compared to ABER 48.34, SPR 22.45 and API 39 in 2009. In comparison to 2009 a notable decrease in the number of malaria cases from gold mining areas was reported in 2010. Conveying the importance of adhering to appropriate preventive measures for malaria to the population at risk is mandatory for the decrease in malaria cases to be sustainable.

1308

ADULT REFERENCE VALUES FOR COMMONLY USED BIOCHEMICAL AND HEMATOLOGICAL TESTS IN CENTRAL GHANA

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Laboratory results and clinical examinations, provide useful information in screening, diagnosing and monitoring of diseases. Interpretation of laboratory results depend on reference values obtained from apparently healthy individuals from the population they are intended to serve. The reference values obtained from healthy residents of the communities used for clinical studies will help in determining eligibility and assessing the safety of those participating in these studies. This study was aimed at establishing gender-specific haematological and biochemical reference values for healthy adults in central Ghana. A total of 625 adults between the ages of 18 and 60 were enrolled within Kintampo and its environs. The medians, 2.5th and 97.5th percentiles were determined for five haematology and five biochemistry parameters commonly considered during screening/enrolment and follow up monitoring of individuals who usually participate in clinical trials and also for health management.

The Clinical Laboratory and Standards Institute (CLSI) guidelines for defining reference values were used. Values established in this study were compared with those derived in the developed countries. The percentage of our healthy population which had out of range values based on the data from the United States and the United Kingdom were determined. The red blood cell (RBC)) parameters (haemoglobin, haematocrit and RBC count), total leucocyte and platelet counts and urea values were significantly lower compared to values derived in the developed countries. Higher values were, however, obtained in our study for parameters such as Alanine aminotransferase, aspartate aminotransferase and total bilirubin. Up to 53% and 75% of the haematology and biochemistry values, respectively from our healthy population would have been declared as abnormal results if data for the developed countries were to be used. The results from this survey support the need to establish reference values using individuals from the population it intends to serve. This will help reduce the inappropriate exclusion of potential clinical trial participants based on reference values derived in the developed countries.

1309

CLINICAL OBSERVATIONS OF HUMAN MONKEYPOX INFECTIONS IN THE DEMOCRATIC REPUBLIC OF THE CONGO

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We describe the results of an observational study of the clinical natural history of human monkeypox (MPX) infections at the remote L'Hôpital Général de Référence de Kole in the rainforest of the Congo River basin of the Democratic Republic of the Congo (DRC). The cardinal observations from 244 subjects enrolled in the study are summarized. All subjects who were positive by pan-orthopox MBG PCR -- utilizing an onsite quantitative real-time assay (LightCycler) -- were also positive by a MPX-specific MGB PCR assay, suggesting that MPX may be the only poxvirus circulating in the area. Sequencing of MPX DNA from one subject's scab showed only 17 nucleoside changes from the MPX Zaire 79 strain (collected in 1979) which was circulating during the WHO clinical characterization studies of 25 years ago when the case-fatality rate (CFR) was about 10%. This is the same isolate USAMRIID has used to develop non-human primate MPX models for drug and vaccine evaluation. The spectrum of disease severity in our study was broad as evidenced by lesion counts ranging from 2 to 8,617, viremia (by PCR) ranging from undetectable to 6.3×10^7 genomes ml/blood, and clinical status ranging from very mild to critically ill. The CFR to date is only 0.9% in subjects aggressively treated with antibiotics, antimalarials, antiparasitics, anti-inflammatory drugs, and IV fluid. A strong correlation appears to exist between maximum lesion count and maximum viral load. Low albumin and total protein levels, as well as elevated liver enzyme and alkaline phosphatase levels, were seen in nearly all cases. In one case, in which viremia was detected before the onset of clinical illness, the maximum viral load occurred before the appearance of lesions and coincident with the onset of symptoms. Fetal demise due to maternal transmission of MPX infection occurred in three of four cases of pregnancy. A high percentage of cases involved transmission within households. The severity of disease within families varied widely without discernable pattern. This observational study is expected to lead to future hypothesis driven studies.

1310

ETIOLOGY OF UNCOMPLICATED FEBRILE ILLNESS AMONG CHILDREN 2 - 59 MONTHS OLD ATTENDING A PRIMARY HEALTH CARE CENTRE IN ZANZIBAR

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A better knowledge of non-malarial fevers is a critical component for improved case management of childhood fevers in the new context of low malaria transmission in Zanzibar. We therefore conducted a health facility based prospective cohort study of the relative frequencies of infectious disease etiologies of uncomplicated febrile illness in North A District, Zanzibar. We enrolled 650 children aged 2 to 59 months old presenting to a primary health care center with confirmed fever (axillary temperature ≥37,5°C) or history of fever within the preceding 24 hours. Patients were clinically managed according to the newly adapted Zanzibar Integrated Management of childhood illness (IMCI) guidelines, which include a Rapid Diagnostic Test (RDT) for malaria. Naso-pharyngeal and rectal swabs were collected for PCR-detection of respiratory tract infection and diarrhoeal etiologies. Further, full blood count, malaria microscopy, C-reactive protein, chest X-rays, urine cultures with antibiotic susceptibility testing, rapid detection of pneumococcal antigen in urine and of Group A Beta-Hemolytic Streptococci from throat swabs were analyzed. Preliminary results from a sub-sample of 292 patients of whom 41% had measured fever show the following distribution of clinical diagnoses (a patient can have >1 diagnosis): 52% pneumonia, 50% upper respiratory tract infection, 22% diarrhea, 8% tonsillitis, 4% skin infection, 3% ear infection and 2% dysentery. After laboratory testing no RDT or microscopy confirmed malaria infection was found. Streptococcus A rapid test was positive in 8% of all patients. Chest x-ray was performed in patients classified as pneumonia according to IMCI (cough and/or difficult breathing and increased respiratory rate). Radiological pneumonia consolidation was verified in 5% of those with pneumonia as initial diagnosis. Urine culture from non-diarrheal patients showed significant growth in 8%. These preliminary results indicate that a majority of the fever episodes were related to respiratory tract infections and diarrhea. However, most IMCI classified pneumonias could not be confirmed with chest x-ray. Further PCR analyses will provide insight into the viral and bacterial etiologies of these febrile episodes.

1311

PRE-TRAVEL VACCINATIONS, PRESCRIPTIONS AND COUNSELING FOR MEDICAL MISSIONARIES AND RESEARCHERS

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Overseas volunteers and researchers face unique risks related to their travel purpose and duration. We sought to characterize these travelers and identify whether they received appropriate vaccinations, prescriptions, and counseling on travel-related issues. Boston Area Travel Medicine Network is a research collaboration of five travel clinics in the greater Boston area that sees ~7,500 travelers per year. We evaluated characteristics of travelers who reported their reason for travel as "missionary/volunteer" or "researcher/student." Between March 2008-July 2010, 15,440 travelers were seen in BATMN clinics. Of these, 1451 (9.4%) were missionaries/volunteers, 1216 (7.9%) researchers/students, and 65 (0.4%) reported both reasons. The median age of all 2,732 was 24 years (range 8-85), and 907 (33.2%) were male. The median travel duration was 21 days (range 1-1,096). Among 4,035 destinations, the most common were Haiti (308; 7.6%), India (228; 5.7%), Kenya (195; 4.8%), China (190; 4.7%), and Tanzania (176; 4.4%). Documentation was available for up to date vaccination status, evidence of immunity, or vaccine receipt at the clinic visit for 1,466 (53.7%) for Td/Tdap, 1,835 (67.2%) for hepatitis B, 2,323 (85.0%) for hepatitis A, 1,088 (39.8%) for influenza, and 199/286 (69.6%) for meningococcus (among persons going to at-risk countries). Commonly prescribed medications included ciprofloxacin (1599; 58.5%), azithromycin (590; 21.6%) and levofloxacin (50; 1.8%) for traveler's diarrhea and atovaquone/proguanil (850; 49.0%), mefloquine (174; 10.0%) and doxycycline (120; 6.9%) for those 1732 persons traveling to chloroquine-resistant malaria risk countries. HIV post-exposure prophylaxis was prescribed for 1,235/1,598 (77.3%) travelers, and 385/1,943 (19.8%) had documentation of tuberculin skin testing. Blood-borne pathogen counseling was documented for 1,235/1,599 (77.2%), evacuation insurance counseling for 1,243/1,878 (66.2%), and rabies or animal bite counseling for 215/1,940 (11.1%). Missionaries, volunteers, researchers and students make up less than 20% of BATMN travelers. Although it is likely that not all of these travelers had direct patient contact overseas, there are still critical gaps in the vaccinations and counseling they receive.

1312

SUCCESSFUL USE OF MODIFIED HEIMLICH VALVE USING PLASTIC GLOVE FOR MANAGING TUBERCULOUS BRONCHOPLEURAL FISTULA

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Sequellae of pulmonary tuberculosis (TB) include pleural effusion, empyema, and bronchopleural fistula. After thoracostomy and appropriate medical therapy, failure of lung reexpansion may signify bronchopleural fistula due to underlying pulmonary destruction, which often results in recurrence of empyema, sepsis and death. We present a 23-year old HIV-negative male in Cameroon with smear-positive TB complicated by empyema and bronchopleural fistula who was successfully managed using a modified Heimlich valve made from the finger of a plastic glove. The patient presented with left-sided empyema, which was drained with a chest tube under water seal. Sputum was positive for acid-fast bacilli, and anti-TB therapy and antibiotics were initiated. After two weeks, a persistent air leak remained and chest radiography showed failure of lung reexpansion. The chest tube was trimmed to 4 centimeters and the 5th finger of a plastic glove with both ends cut was attached to the end and lubricated with Vaseline, which allowed for one-way exit of air and fluid. The tube was left in place for three months to create a chest window, after which the tube was removed, leaving an epithelialized passage between the pleural space and external environment. Scant fluid continued draining from the valve and the chest window during the treatment course but no other complications were noted. Bacteriological cure was confirmed by negative control sputum at two and five months. After 12 months the window had closed spontaneously, and the lung had completely reexpanded. Radiographs illustrate the entire clinical course. The method described here using a widely available resource–a non-sterile plastic glove–to make a modified one-way valve was successfully used for the treatment of tuberculous bronchopleural fistula and persistent pneumothorax. Ongoing drainage of chronic empyema and formation of a chest window is thus possible without advanced thoracic surgical intervention. The glove drain is worth considering in resource-limited settings for this challenging complication of pulmonary TB.

1313

THE BURDEN OF CHRONIC HEPATITIS B IN IMMIGRANTS IN QUEBEC, CANADA: A POPULATION BASED STUDY

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Immigrants have higher mortality from chronic hepatitis B (HBV) and hepatocellular carcinoma as compared to those born in Canada. Despite this disparity there are no screening programs to detect chronic HBV, and HBV vaccine is not routinely given to immigrants after arrival in Canada. This is because there is no population based data describing the burden of chronic hepatitis B in immigrants. To fill this gap we created a cohort of all cases of hepatitis B reported from 1991-2008 in Quebec through linking administrative databases. We linked the MADO (Quebec Reportable Disease database), the MICC (Quebec Landed Immigrant database) and the RAMQ (Quebec provincial health insurance and physician billing database). For incidence rate estimates, denominators for immigrants were obtained from the MICC database (N=757,650 newly arrived immigrants from 1991-2008); for non-immigrants, denominators used 1991, 1996, 2001, and 2006 Quebec census data (immigrants removed). Rates and rate ratios and 95% CI were calculated using the Poisson distribution. 13,889 cases of chronic hepatitis B were reported during the study period. Non-immigrant cases were older (mean age 43.4 vs 33.4 $p < 0.01$) and were more likely male (69% vs 51%, $p < 0.01$). The rate of chronic hepatitis B overall was 10 fold higher in immigrants as compared to non-immigrants [rate ratio; 95% CI = 10.0 (9.7-10.30) and rates/100,000 person years (PY) were 73.9 vs 7.4]. Rates were highest for immigrants from East Asia/Pacific [rate/100,000 PY 95% CI = 280 (268-293)], Sub-Saharan Africa [280 (262-298], Eastern Europe [86 (77-94)]; they ranged from 38-42/100,000 PY for immigrants from South Asia, the Middle East/North Africa and Latin America. Immigrants are at increased risk for chronic hepatitis B and its associated sequelae, including potential transmission to close contacts. Immigrants would therefore likely benefit from screening for chronic hepatitis B and verification of hepatitis B immune status, so that appropriate treatment of chronic infection and vaccination of susceptible contacts can be provided.

1314

CORRELATION OF MALARIA RAPID DIAGNOSTIC TESTING WITH CLINICAL-BASED ALGORITHM IN A RURAL VILLAGE IN UGANDA

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Uganda has the world's highest malaria incidence and mortality. The Engeye Clinic was created in 2006 as a U.S./Ugandan non-governmental organization based in the Ddegeya Village. In this resource poor setting lacking microscopes and trained technicians, rapid diagnostic testing (RDT) was initiated to confirm clinically suspected malaria. Issues facing this community include little to no use of mosquito nets, failure to complete treatment and/or use of paracetamol substituted for malarial treatment by village merchants. The purpose of this study was to evaluate the implementation of a clinical algorithm in a resource-limited setting for the diagnosis of malaria compared to RDT. Over a two week period in February 2010, 344 patients were assessed by the on-site clinician using a clinical algorithm for the diagnosis of malaria. This included fever, chills, sweats, headaches, muscle or abdominal pains, nausea and vomiting for greater than 48 hours with no other obvious cause. RDT was performed on patients meeting clinical criteria for malaria by obtaining whole blood samples using immunographic testing. Treatment for suspected malarial cases was initiated with artemether/lumefantrine based on weight and pregnancy/lactation status. 117 patients met clinical criteria for malaria diagnosis. All clinically diagnosed cases were positive when confirmed with RDT for the detection of parasite specific antigens for *P. falciparum*. The prevalence of malaria as a cause of presenting symptoms was 34% in this cohort. This clinical algorithm was found to be highly specific. The specificity and the positive predictive value of the clinical algorithm was 100% when compared to the RDT in this cohort. In patients without clinical criteria for a diagnosis of malaria there were no positive RDT results. It was determined that the clinical algorithm could be used by rural health care workers to accurately diagnose malaria as misdiagnosis leads to a delay in treatment causing an increased mortality and unnecessary prescription of malarial medications and increased drug resistance.

1315

LYME DISEASE AND FILARIASIS - A *WOLBACHIA* CONNECTION: A CASE REPORT

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Health care providers must consider neglected tropical and regional endemic diseases. Lyme disease, commonly found in North America requires a diagnosis of erythema migrans with confirmatory serology. Lymphatic filariasis, endemic to Africa, is a neglected chronic disease that can be easily overlooked in immigrants coming to North America. We present a case of a 21 year old Liberian male complaining of one week of right knee pain and swelling during the summer. He had a history of malaria and filariasis. He immigrated to Albany, New York eighteen months ago as a refugee from civil war and was taking isoniazid and pyridoxine for a positive tuberculin test. He had no known tick exposure or trauma. He was afebrile and hemodynamically stable complaining of right knee discomfort, warmth, swelling and decreased range of motion. His white blood cell count was 4.7 with 52% neutrophils, 26% lymphocytes, 9% eosinophils and 12% monocytes. C-reactive protein (101 mg/L) erythrocyte sedimentation rate (73 mm/hr) and IgE level (4123 U/ml) were elevated. Knee radiograph showed joint effusion. Synovial fluid aspiration contained 116.5 tho/cmm white cells (95% neutrophils) but gram stain and culture were negative. Lyme PCR from synovial fluid was not submitted for analysis. Lyme C6 peptide was positive as was confirmatory Western blot which demonstrated IgG bands 18, 23, 30, 31, 39, 41, 58 and 93. Antifilarial IgG4 was positive indicative of past or chronic infection with filariasis. A course of Doxycycline was initiated for the management of both acute Lyme arthritis and chronic filariasis. Antifilarial therapy with Doxcycline was directed toward the symbiotic bacteria, *Wolbachia* associated with microfilaria. While it is possible our patient's positive Lyme serology reflected cross reacting antigens to filaria; his clinical presentation was consistent with Lyme associated arthritis. This case is unique in that antimicrobial management of one endemic infection was useful in the management of a geographically separate pathogen.

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STRATEGIES TO PREVENT MEASLES, MUMPS AND RUBELLA AMONG NEWLY ARRIVED ADULT IMMIGRANTS AND REFUGEES IN CANADA: A COST-EFFECTIVENESS ANALYSIS

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Adult immigrants are an unrecognized group at risk for measles, mumps and rubella (MMR). They have been over-represented in rubella outbreaks and most cases of congenital rubella (CRS) in Canada have occurred in infants born to foreign-born mothers. Despite this gap, MMR vaccine is not routinely given to adult immigrants after arrival in Canada. We performed a cost-effectiveness analysis to define the optimal strategy to prevent MMR in this population. We constructed a decision analysis (Markov modeling) to compare the cost-effectiveness of four preventive strategies using MMR vaccine, compared to no intervention (status quo). Interventions tested were: 1 and 2) Vaccinating all adult immigrants with either 1 or 2 doses of MMR vaccine without prior serotesting, or 3 and 4) serotesting for MMR then vaccinating those found to be susceptible to one or more of the diseases with either 1 or 2 doses of MMR vaccine. A hypothetical cohort of 250,000 newly arrived immigrants 18 years of age or older was modeled over a 20-year period. The expected number of cases of MMR, associated complications and costs (health care system perspective) for each of the strategies were calculated with 3% discounting for costs and clinical outcomes. Vaccinating all adult immigrants with 2 doses of MMR vaccine without prior serotesting saved 41 million Canadian dollars and avoided 6,362 measles cases, 9 measles deaths, 5,051 mumps cases, 8,181 rubella cases, 1 rubella death and 38 cases of CRS over a 20-year period, as compared to no intervention. Comparing both interventions involving vaccination without serotesting, the 2-dose strategy cost an additional $1.69 per person (total of $422,500) compared to the 1-dose strategy but prevented an additional 273 measles cases, 405 mumps cases, 273 rubella cases and 1 CRS. Both strategies that involved serotesting before vaccination were cost-saving compared to no intervention. However, both serotesting strategies were more costly and less effective than the vaccination-only strategies. All new adult immigrants should be given two doses of MMR vaccine without prior serotesting: this strategy is cost-saving, prevents individual morbidity and mortality, and decreases the potential for outbreaks.

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DETERMINANTS OF ANEMIA AMONG YOUNG CHILDREN IN WESTERN KENYA

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Anemia among children in developing countries is often attributed to iron deficiency caused by low dietary iron intake. However, the causes

of anemia are multi-factorial and interlinked. In sub-Saharan Africa, sickle cell disease (SCD), α-thalassemia, and infections are widespread and are known risk factors for anemia. Data on multiple risk factors for anemia are needed to design more effective prevention and treatment programs. We conducted a cross-sectional cluster survey of 841 children aged 6-35 months in 60 randomly selected villages in Nyando District, western Kenya. Anemia prevalence (hemoglobin 8.3 mg/L) 75%, vitamin A deficiency (retinol binding protein (RBP) 10 mg/L) 30%, reported fever in the last 24 hours 27%, stunting (height-for-age z-score<-2) 30%, wasting (weight-for-height z-score<-2) 3%, sickle cell trait 17%, SCD 2%, heterozygous α-thalassemia genotype 38% and homozygous α-thalassemia genotype 9%. In bivariate analysis, anemia was associated with iron deficiency, vitamin A deficiency, malaria, inflammation, fever, stunting, wasting, homozygous and heterozygous α-thalassemia genotypes, age<30 months, male sex, and low socioeconomic status (SES) ($p<0.05$). In linear regression, accounting for cluster design, the best fit model included TfR, RBP, malaria, CRP, SCD, homozygous α-thalassemia genotype, male sex, age<30 months ($R2= 0.59$, $p<0.0001$). Age <30 months, homozygous α-thalassemia genotype, and CRP modified the relationship between iron deficiency and hemoglobin. Fever, height-for-age z-score, height-for-weight z-score, and low SES were not significantly associated with hemoglobin when included in the best fit model and did not confound the relationship between TfR and hemoglobin. Interventions designed to prevent anemia should utilize an integrated approach, ensuring optimal iron intake while also addressing malaria and other infections.

1318

COMPLICATIONS OF MONKEYPOX INFECTIONS IN HUMANS

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We report on the complications of monkeypox infections in humans as observed during a 4 year (2007-2011) study at the remote L'Hôpital Général de Référence de Kole in the rainforest of the Congo River basin of the Democratic Republic of the Congo (DRC). The study was conducted jointly by the Institute National de Recherche Biomedicale (INRB) and the US Army Medical Research Institute of Infectious Diseases (USAMRIID). Human monkeypox infections were first identified during the final stages of smallpox eradication when laboratory testing determined that some cases clinically presenting as smallpox were actually caused by monkeypox virus. The present study was conducted at one of the two previous WHO MPX study sites (1981-1986) staffed by the same Spanish medical order of Catholic sisters who have continued the treatment of MPX patients after others in the country stopped hospitalizing such cases. A total of 244 patients were consented and enrolled into our study. Generally, patients presented with fever, chills, sore throat, pox lesions, and general malaise, fatigue. The number of pox lesions ranged from two lesions to more than eight thousands lesions. Complications included death, coma and other neurologic manifestations, co-infections, infected wounds, secondary dermatitis, miscarriages, keratitis, staphyloma, and caseation of eye lesions. The case fatality rate was 0.9% in our study. Case histories with appropriate graphics, including photographs will be presented, when appropriate, to demonstrate findings.

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MID-UPPER-ARM CIRCUMFERENCE IS A USEFUL TOOL FOR ASSESSING NUTRITIONAL STATUS IN YOUNG CHILDREN WITH DIARRHEAL DEHYDRATION

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Undernutrition is implicated in over half of all child deaths related to diarrhea. Cohort studies which assess the relationship between diarrheal disease and nutritional status typically use weight-based anthropomorphic measures. However, such measures are not useful to study the acute nutritional changes induced by diarrhea because children with diarrhea are often dehydrated. Mid-upper-arm circumference (MUAC) is a cheap, simple tool often considered to be a better indicator of acute malnutrition than weight. MUAC measures musculature, a proxy for protein stores. If truly unaffected by interstitial water content, or dehydration, MUAC could be used to assess the acute nutritional changes induced by diarrhea. In February 2011, we initiated a case control study in Mirzapur (Bangladesh), Kolkata (India), and Basse (The Gambia) to determine whether diarrheal dehydration affects MUAC. A case, enrolled at a clinic, was defined as a child 0-59 months old with acute (< 7 days) diarrhea (≥3 abnormally loose stools in the previous 24 hours) who had ≥1 of the following: sunken eyes, skin tenting, dysentery, IV rehydration, or hospitalization, and underwent rehydration therapy. A healthy control matched for age and gender was enrolled at home within 3 days of case enrollment. Weight and MUAC were measured for each case and his/her matched control at enrolment and again 4 hours later. To date, 66 cases and 66 controls have been enrolled. Cases had substantial weight gain within 4 hours, which was significantly more than the controls within 4 hours [mean kg 0.29 (0.16, 0.42) vs. 0.10 (0.02, 0.19) p=0.02]. In contrast, the change in MUAC before and after rehydration in cases was comparable to that in controls measured at two time points 4 hours apart [mean cm 0.03 (-0.01, 0.07) vs 0.01 (-0.01, 0.04) p>0.09]. These preliminary results suggest that rehydration therapy results in weight gain but does not affect MUAC, thus providing a useful tool for epidemiologic studies.

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EMERGING BIOMARKERS FOR THE DIAGNOSIS OF SEVERE NEONATAL INFECTIONS APPLICABLE TO LOW RESOURCE SETTINGS

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Severe neonatal infections are one of the most significant causes of pediatric mortality, resulting in more than 500,000 deaths each year, of which 99% occur in low-resource settings. Compared to clinical algorithms, new point-of-care diagnostics that could distinguish neonates with or without severe infections may have potential to substantially improve the global management of severe neonatal infections. This review sought to characterize promising biomarkers for the diagnosis of severe neonatal infections. Biomarkers extensively reviewed elsewhere (procalcitonin, C-reactive protein, tumor necrosis factor-α, interferon-γ, and interleukin-6 and 8) were not re-reviewed. Hundreds of other biomarkers have been associated with "sepsis"; this review focused exclusively on biomarkers with published performance data for the diagnosis of severe neonatal infections. We identified infant diagnostic

performance data on 21 biomarkers: seven acute phase reactants (Serum Amyloid A(SAA), LPS Binding Protein(LBP), Inter-α Inhibitor Proteins(Iα-Ip), Antithrombin, Soluble E-Selectin, Fibronectin); five pro-inflammatory cytokines (Interleukin-1α, Interleukin-1β, Interleukin-12p70, Interleukin-18, Granulocyte Colony Stimulating Factor(G-CSF)); two anti-inflammatory cytokines (Interleukin-10, Interleukin-1 Receptor Antagonist(IL-1RA)); five chemokines (Growth Related Oncogene α, Interferon-γ-Inducible Protein 10(IP-10), Monokine Induced by Interferon-γ, Regulated upon Activation Normal T cells Expressed and Secreted, Monocyte Chemoattractant 1); one soluble cell surface marker (soluble intercellular adhesion molecule-1); and one molecule involved in triglyceride metabolism (apolipoprotein CII(apoC2)). Seven soluble biomarkers (G-CSF, IL-RA, IP-10, SAA, LBP, IαIp, apoC2), compatible with point-of-care immunodiagnostics (defined as a concentration > 1ng/ml), emerged as promising candidates, with sensitivity and specificity generally > 90% (range 33 to 100%). These biomarkers seem particularly attractive for future prospective studies of diagnostics for severe neonatal infections.

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OUTCOME OF FOUR PREGNANCIES IN CONGOLESE WOMEN WITH MONKEYPOX INFECTION

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The outcomes of four pregnancies in women with clinically apparent, PCR-confirmed, community-acquired monkeypox (MPX) virus infections are described. During 2007 to 2011 we studied the clinical features of human MPX infections in Kole, the Democratic Republic of the Congo. 244 subjects were enrolled of which four were pregnant. The outcomes of these four pregnancies along with the maternal pox lesion counts and the PCR-confirmed viremia were carefully documented. This is the first report of intrauterine demise due to complications of human monkeypox. In Case 1, MPX viremia rose rapidly and abruptly upon cessation of fetal movement at the 18th week of gestation, some 24 days after onset of rash. Marked fetal hepatomegaly and peritoneal effusion (*hydrops fetalis*) were seen at necropsy. In Case 2 a spontaneous miscarriage occurred in a subject without significant viremia or remaining lesions at the 6th week of gestation, 22 days after rash onset. The third spontaneous miscarriage occurred at 7 weeks of gestation and the 10th study day in a mother with over 1,000 lesions and viremia exceeding 10^5 genomes/mL of blood. (No pathology examination was performed and no determination of viral load was made on the aborted material for Cases 2 and 3.) The fourth pregnant subject was enrolled for observation as a healthy family member of an index MPX case. She was then noted to be about 14 weeks pregnant. On her second study day she was noted to have MPX lesions on her genitals. Her lesion count never exceeded 20 and she had a low level viremia by PCR. At her study day 75 follow-up visit (24 weeks gestation), the fetus was alive. Although the sample size is small, we observed a very high abortion rate in cases of maternal MPX infections, but death is not inevitable.

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QUASISPECIES VARIANT ANALYSIS OF A 2010 DENGUE 3 VIRUS FROM KAMPHAENG PHET, THAILAND

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All four serotypes of dengue viruses exist as quasispecies. Quasispecies are described as a spectrum of variants ('candidate genomes'), genetically linked through mutation, creating an interactive population where selection acts on the population rather than the individual variant. We explored an assertion of quasispecies theory that the fitness (ability to infect and cause disease) of a particular viral sequence is determined more by its freedom to mutate than by its ability to replicate. A quasispecies from dengue virus serotype 3 (DENV3) was cloned from a single mosquito collected within a cluster of human dengue infections (100 meter radius) in Kamphaeng Phet, Thailand, in 2010, to understand diversity and mutational effects apparent in the population. Sequences were combined with other published DENV3 sequences and maximum likelihood phylogenetic analysis revealed quasispecies populations removed from the baseline 'consensus sequence' diversity of human DENV3 circulating in Thailand. Mutational analysis showed a high proportion of nonsynonymous mutations and 2.8% of the population was evolving faster per site than average despite overall low diversity. Forty-four percent of the sequences were under positive selection while 19% were under purifying selection. Quasispecies analysis identified amino acid substitutions that have been reported to lead to phenotypic changes in viral like particle assembly, prM/E protein production, glycosylation and/or antibody binding ability. Other uncharacterized amino acid substitutions identified are predicted to be deleterious. The diversity of the quasispecies suggests there are variants with altered abilities to infect and disperse with overall diversity being constrained in the mosquito. An altered ability to infect or disperse will potentially affect how the population responds to selective pressures such as innate immunity and vaccine implementation.

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VALIDATION OF DENGUE SEVERITY PREDICTIVE ALGORITHMS DERIVED FROM PRIMARY CARE AND HOSPITALIZED CASES IN AN ADULT SECONDARY CARE COHORT

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Dengue is the most prevalent arthropod-borne infection worldwide. In well-resourced centers where diagnosis can be rapidly established, the next crucial step is to triage for appropriate care. Singapore has primarily adult dengue disease and recent epidemics have led to development of predictors to guide admission to secondary care. We validate three algorithms in a prospective cohort of 137 laboratory confirmed adult cases referred to a hospital-based dengue clinic. Cases that have already fulfilled severity criteria at presentation are excluded from analysis. First, the decision tree classifier developed from a febrile (<=72hrs) primary care cohort with laboratory-confirmed dengue fever, as reported previously: a cycle threshold of real time reverse-transcriptase polymerase chain reaction <=20.9 with positive dengue IgG at presentation, or platelet

count of <108 000/mm3. Reported sensitivity (Sn) was 78.2% and specificity (Sp) 80.2% in predicting a platelet nadir of 50 000/mm3. In our cohort, Sn/Sp=88.9%/66.7% in an identically defined subgroup (n=30), with no significant difference between previously published and our Sn/Sp. Second, comparison was made with a decision tree developed from a retrospective hospitalized cohort to predict dengue hemorrhagic fever (DHF) (Lee et al Trop Med Int Health. 2009 Sep;14(9):1154-9). Reported Sn/Sp=100%/46% using any of a history of bleeding, serum urea >4 mmol/L, or serum protein <=67g/L. In our cohort (n=115), Sn was significantly lower at 85.7% but difference in Sp at 49.4% was not significant. Last, the predictive equation for DHF using history of bleeding, serum urea, serum protein and lymphocyte proportion from the same cohort (Lee et al, J Clin Virol. 2008 May;42(1):34-9) had Sn/Sp=97.6%/60.3%. Our Sp was significantly lower at 32.2% but Sn was not significantly different at 100%. While our cohort was more severe than the hospitalized training cohort and the primary care cohort (24% vs 4% vs 2.6% DHF), it is reassuring that sensitivities remain high. Given the wide spectrum of dengue disease and varying presentations in different populations, a thorough exploration of the utility of prognostic algorithms taking into account population and clinical factors such as time to presentation will be required to safely triage dengue patients. We showed that the utility of predictors may vary even within the same country depending on source of patients.

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RAPID DIAGNOSIS OF DENGUE IN A HOSPITAL-BASED COHORT

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Accurate and rapid dengue diagnosis is vital to triage and management. The World Health Organisation (WHO) proposed in 2009 an updated clinical definition of probable dengue replacing 1997 criteria for suspected dengue fever. Definitive laboratory diagnosis of dengue is not always possible, and newer methods such as testing NS1 antigen are undergoing evaluation. We prospectively enrolled 205 adult suspected dengue cases referred to the Communicable Disease Centre, Singapore to comprehensively evaluate methods for rapid diagnosis of dengue. Clinical and laboratory criteria were evaluated, including daily PCR, NS1 antigen, and IgM/IgG serology for those positive by PCR or NS1 on presentation. Confirmed dengue cases (n=142) were positive by PCR/NS1 or by IgM seroconversion by ELISA at 3-4 weeks. Non-dengue cases (n=20) were negative by PCR, NS1 and IgM ELISA in paired sera. Forty-three cases could not be assigned an acute dengue diagnosis because of a lack of paired sera or elevated IgM/IgG without seroconversion. The sensitivity (Sn) of PCR at presentation (median fever duration 5 days, range 2-9 days) was 70.4% and specificity (Sp) 100%. Median duration of viremia was 6 days (range 3-11 days). For NS1, Sn/Sp=89.4/100%, with median duration of antigenemia of 7 days (range 2-10 days), significantly longer ($p<0.001$) than median viremic duration. Using <=5 days of fever as a cutoff for early illness, the Sn/SP of PCR (n=84) was 88.9/100% vs 51.4/100% late in illness (n=78), compared to NS1 of 90.3/100% early and 88.6/100% late in illness. Only 2 cases (1.4%) were detected only by IgM seroconversion with negative results by PCR and NS1. WHO 1997 criteria for dengue fever had Sn/Sp=98.6/20.0% while the recent 2009 criteria for probable dengue Sn/Sp=97.9/20.0%. Laboratory diagnosis using NS1 antigen had consistently high Sn/Sp, with markedly improved Sn compared with PCR after day 5 of fever ($p<0.001$), and was positive for a mean of 1.1 days longer than PCR. Assessing seroconversion did not substantially increase the sensitivity of diagnosis in hyperendemic Singapore. Both clinical guidelines had similar test characteristics: very sensitive but with poor specificity in a cohort of referrals for suspected dengue.

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GENETIC DIVERSITY OF DENGUE VIRUS IMPACTS TO THE DETECTION SENSITIVITY OF RT-PCR BASED METHOD, ONE CAUTION FOR METHOD DEVELOPMENT AND QUALITY CONTROL ASSESSMENT

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Dengue virus (DENV), transmitted by Aedes mosquitoes, causes the disease in 50-100 million people per year in tropical and subtropical regions worldwide. Four DENV serotypes (DENV-1 to -4) can cause infections ranging from asymptomatic or mild febrile illness to severe hemorrhagic disease. Various RT-PCR techniques have been developed for rapidly detecting and typing DENV. The rapid diagnosis allows early initiation of patient care and specific preventive health measures. RT-PCR is also a useful diagnosis tool for surveillance studies. Our laboratory has used the modified Lanciotti's conventional RT-PCR method as one of the diagnostic tests for DENV since 1994. The method was evaluated and classified as an optimal method for DENV diagnosis and surveillance by an international External Quality Control Assessment (EQA). However, the analysis of PCR results of 13,532 dengue confirmed cases by ELISA tested over 11 years (2000-2010) showed 17-42% negative PCR results. Among these negative PCR results, 36% were from acute sera from patients within 0-4 days of illness onset, typically a viremic period with a high percentage of virus isolation. We tested 300 samples from patients with dengue confirmed by ELISA and with negative conventional PCR results with in-house TaqMan RT-PCR which was classified as a 'need of improvement' method by EQA as part of a quality improvement effort. One hundred and eighty samples (60%) showed TaqMan positive results including 129 DENV-1 (43%), 39 DENV-2 (13%), 4 DENV-3 (1.3%) and 8 dual infections of DENV-1 and -2 (2.7%). Sequences of conventional PCR primers binding sites of 9 TaqMan positive samples including 4 DENV-1, 3 DENV-2, and 2 DENV-3 revealed points of mismatch between primers and templates that likely effected the sensitivity of the detection. These data indicated that the genetic diversity of DENV impacts the sensitivity of RT-PCR based methods, a critical concern during method development and quality control assessment.

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CONSIDERATIONS FOR CHANGING PRNT DENGUE 4 REFERENCE VIRUSES: SUB-OPTIMAL IMMUNITY TO DOCUMENTED INFECTIONS

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The plaque reduction neutralization test (PRNT) is considered to be the "gold standard" to characterize and quantify circulating levels of anti-dengue virus (DENV) neutralizing antibody. The PRNT is used to define the immunogenicity of dengue vaccine candidates, support dengue seroepidemiologic and pathogenesis studies. Despite numerous efforts to standardize the assay and normalize data to better compare data between studies and natural and vaccine infections, there are several sets of reference viruses around the world. The Armed Forces Institute of Medical Sciences in Bangkok utilizes DENV-1 (16007), DENV-2 (16681), DENV-3 (16562) and DENV-4 (1036) reference strains. In 2006 the dominate

serotype in circulation in Thailand was DENV-4. In cohort studies we observed poor or absent PRNT titers using the 1036 DENV 4, genotype 3 strain (originally isolated in 1976 in Indonesia) to documented DENV-4 infections. New candidate DENV-4 reference viruses were selected from isolates collected in the last 10 years. These viruses were tested using a bank of sera from documented DENV-4 infections including homologous sera from the individuals from which the strains were isolated. A candidate reference strain was selected based on PRNT titers achieved, low cross reactivity, and the ability of the virus to produce large well-formed plaques. More than 300 samples were tested with the old and new reference virus. Geometric mean titers were increased 4.2 fold. Using the new reference virus enabled identification of additional inapparent infections in cohort studies and has enhanced our ability to characterize the DENV-4 immune response. This study illustrates the need to continuously monitor the performance of viral strains in reference assays. Furthermore, this data suggests that dengue viral evolution may have a profound effect on tests that utilize reference strains.

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SAFETY OF A RECOMBINANT LIVE ATTENUATED TETRAVALENT DENGUE VACCINE IN HEALTHY ADULT VOLUNTEERS

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Dengue (DEN) virus threatens over half the world's population, causing debilitating dengue fever, dengue hemorrhagic fever and dengue shock syndrome leading to over 20,000 deaths every year. DENVax is a tetravalent live attenuated dengue vaccine that is based on the DEN-2 PDK-53 genetic backbone. DEN-2 PDK-53 has been tested previously in humans and was found to be safe and immunogenic. Recombinant DENVax-1, DENVax-3 and DENVax-4 strains were generated in which the prM and E genes of PDK-53 were substituted with those of DEN- 1, -3 or -4 viruses. These recombinant viruses retain the genetic attenuation markers present in PDK-53 and direct the immune response to the other three serotypes. A single center, placebo-controlled, randomized study assessing the safety, tolerability of tetravalent DENVax formulations was performed in Rionegro, Colombia, a high altitude area with no *Aedes aegypti* and no dengue exposure. One of two dose levels (low or high) of DENVax was administered subcutaneously or intradermally to healthy male and female subjects with no pre-immunity to flaviviruses. Two doses of DENVax or placebo were administered, separated by an interval of 90 days. Safety was assessed as the frequency and severity of adverse events through physical examination, injection site examination, lab examinations, and subject diary cards. Clinical laboratory assessments included serum chemistry, hematology and urinalysis. The safety data demonstrate that both tetravalent formulations were well-tolerated by either route of administration. To date, the most frequent adverse events were local reactogenicity at the injection site for both dose levels and both routes of administration. Systemic adverse events were mild to moderate headache, muscle pain, nausea and fatigue. There were no meaningful laboratory changes. This study highlights the safety of the tetravalent DENVax formulations in healthy adults. Further clinical trials to assess safety, tolerability, and immunogenicity in other age groups and in dengue exposed individuals are being planned.

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DEVELOPMENT OF A RECOMBINANT TETRAVALENT DENGUE VACCINES (TDV) THAT LINKS INNATE AND ADAPTIVE IMMUNITY

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We have previously demonstrated that the domain III of West Nile virus envelope antigen (EIII) fused to flagellin of *Salmonella typhimurium* (STF2, a TLR5 ligand) is immunogenic and efficacious against lethal WNV infections in mice (McDonald *et al*., 2007, J. Infect Dis. 195, 1607-1617). To develop a tetravalent dengue vaccine, we have designed, purified, and evaluated similar and alternative flagellin-EIII fusion vaccine formats, which differ in the site of antigen attachment to the flagellin. These fusion proteins can be efficiently and economically manufactured in *E. coli* fermentation systems. Here we report immunogenicity results of recombinant dengue vaccine candidates in monovalent, bivalent, and tetravalent formulations. BALB/c mice were immunized s.c. three times at 2 or 3 week intervals, and bled at various times post boost. In an efficacy study, AG129 mice lacking receptors of types I and II interferons were immunized with two or three doses of a monovalent DENV-2 vaccine candidate, and challenged with 2,100 LD_{50} of DENV-2 (strain NGC). Serum neutralizing antibody titers were determined by 50% plaque reduction neutralization test ($PRNT_{50}$). Survival rates, weight changes, and viremia, as measured by qRT-PCR, of infected mice were determined. Our results indicated that immunizations of BALB/c mice with these vaccine candidates at doses of 2-15 µg elicit robust homotypic neutralizing antibody responses. Furthermore, a monovalent DENV-2 candidate conferred partial protection against a lethal DENV-2 challenge and significantly reduced viremia and weight loss in infected AG129 mice. The DENV-2 candidate was also found to elicit high $PRNT_{50}$ titers in rabbits. Finally, BALB/c mice immunized with tetravalent dengue flagllin-EIII formulations developed strong neutralizing antibodies to all 4 serotypes of DENV (GMTs of $PRNT_{50}$ = 200 - 3000). In conclusion, VaxInnate flagellin-EIII vaccine candidates are highly immunogenic in mice and rabbits and are effective in protecting AG129 mice against a lethal DENV-2 challenge, thereby justifying further development of a TDV.

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IDENTIFICATION OF HOST FACTORS THAT INFLUENCE DENGUE VIRUS INFECTION IN HUMAN PRIMARY MONOCYTES AND MONOCYTE-DERIVED DENDRITIC CELLS

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Dengue virus (DENV) is a flavivirus in the family flaviviridae that infects up to 50-100 million people per year, with 2.5 billion people at risk. The burden of disease is significant, with a clinical primary infection manifesting as fever, rash, severe headaches, and intense myalgia and arthralgia that persist for approximately one week. Elucidating the interactions between host cell proteins and the dengue virus is critical to the development of targeted and effective antiviral drugs. Learning the details of these interactions will be essential to be able to rationally design drugs targeting viral proteins, or to identify compounds that will interfere with host processes critical to DENV infection. The first step towards this end is to identify which host proteins interact with the dengue virus in a clinically relevant system. Proteomic evaluations of host cells following

dengue virus infection have been performed in liver cells and endothelial cells but not the described primary target of dengue virus infection, primary human monocytes and dendritic cells. We used a proteomics-based approach to identify host factors relevant to dengue virus infection in primary human monocytes and monocyte-derived dendritic cells. After infecting these cells with DENV serotype 2 strain 16681 we compared their proteome to that of uninfected cells using the Beckman Coulter PF2D system, a fluid-based system analogous to a 2D-gel that separates proteins by isoelectric point (pI) followed by hydrophobicity. After comparing infected cells to uninfected cells we found approximately 75 proteins that either increased or decreased in abundance by greater than 2.5 fold in the presence of DENV. These unidentified proteins were then subjected to Mass Spectrometry analysis. Proteins down-regulated in the presence of DENV in both monocytes and monocyte-derived dendritic cells were chosen for further analysis to elucidate their role in the pathogenesis of dengue virus.

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SEROTYPE-SPECIFIC DENGUE VIRUS CIRCULATION AND DENGUE DISEASE IN BANGKOK, THAILAND FROM 1973 TO 2010

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Since 1962, the Queen Sirikit National Institute of Child Health (QSNICH) and AFRIMS have cooperated in a mutual public health effort to accurately diagnose dengue infections including serologic determinations of antibody patterns and identification of dengue serotypes. The epidemiologic data included all patients admitted to the dengue ward of QSNICH with suspected dengue fever and dengue hemorrhagic fever who were subsequently proven to have dengue infection by serology or virus detection. Available data from 1973 t0 1999 have been analyzed and published previously (Nisalak et al, 2003). We report on data for the expanded years from 1973 to 2010 including many more cases of DENV-4 infection than were observed previously. Findings that were reconfirmed from the previous report: 1) primary cases are increasing relative to secondary cases; 2) symptomatic primary cases were most likely due to DENV-1; 3) Primary non-infant hospitalized cases were less severe than secondary non-infant hospitalized cases. The mean age of DHF cases are noted to be increasing. In 1973-1982, the mean age of primary and secondary infection was 4.5 years and 8.0 years, respectively. In 2001-2010, it was 6.1 years and 8.0 years. These findings highlight the longitudinal epidemiology of dengue over a uniquely extended period of observation. Further spatial analysis is planned to elucidate transmission dynamics.

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THE ROLE OF ROS SIGNALING IN MOSQUITO CELLS THAT SURVIVE DENGUE 2 VIRUS INFECTION

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Dengue virus (DENV) is naturally transmitted by *Aedes* mosquitoes between humans and replicates efficiently in mosquito as well as in mammalian cells. However, the fate is distinct between the two types of cells in response to the infection. Cytopathic effects (CPE) in mosquito cells are generally trivial compared to that occur in mammalian cells that usually end up with apoptosis. In spite, production of ROS resulted from mitochondria dysfunction occurs in both cell types. It was demonstrated that the survival of mosquito cells is beneficial form up-regulation of genes related to antioxidant defense, such as glutathione S transferase (GST). The anti-apoptotic effect plays a role as the second defense system on protection of mosquito cells from DENV infection. It was eventually regulated by inhibitors of apoptosis (IAPs) that are the upstream regulators of caspase 9 and caspase 3. C6/36 cells with double knockdown of GST and IAP showed a synergistic effect on activation of caspases, causing a higher rate of apoptosis rate (>20%) than those with knockdown of each single gene (~10%), after infection by DENV. Compared with mammalian cells, residual H_2O_2 after anti-oxidation in DENV-infected C6/36 cells may serve as the signal up-regulating the expression of IAP. Taken together, two defense systems including antioxidant defense and anti-apoptotic effects exist in mosquito cells; which were linked by ROS, *i.e.*, H_2O_2 signaling.

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EPIDEMIOLOGY OF DENGUE IN MALAYSIA

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The first major epidemic of dengue fever in Malaysia occurred in 1973, and since that time dengue epidemics have become more frequent, and more virulent. We analyzed data collected by the Ministry of Health Malaysia between 2001-2010 and describe increasing incidence from 68.2/100,000 in 2001 to 159.7/100,000 in 2010, with a spike of 176.5/100,000 in 2008. Analysis of surveillance and notification data collected between 2005-2010 showed that the DHF/DSS:DF ratio was 1:19 in 2005 and 1:10 in 2010, with higher rates of severe disease in secondary versus primary infections. The mean age of DHF/DSS cases was 28 years. Age-specific incidence was highest in adults aged 20-29 years and incidence rates were higher in males, with a male to female rate ratio of 1.397 (95% CI: 1.390 - 1.404; p<0.005). Between 2005-2010 there was a shift to increased transmission in urban settings, with an increase in urban:rural rate ratios from 1.5 in 2005 to 2.0 in 2009, based on urban incidence rates of 170.4/100,000 compared to 98.7/100,000 in rural areas with an urban and rural rate ratio of 1.727 (1.719 - 1.735; p<0.005). Analysis of approximately 700 virus isolates collected between 2005-2010 showed that all 4 DENV serotypes circulated in Malaysia during this period, with all four serotypes detected in each year. DENV-1 genotype I, a virus which has circulated in Southeast Asia since 2003, reemerged as the predominant serotype in 2010. We describe a marked increase in dengue epidemic activity in Malaysia within the last decade, characterized by hyperendemic transmission of all four dengue serotypes, increasing rates of severe disease and increasingly urban transmission.

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AN ISLAND-WIDE DENGUE EPIDEMIC - PUERTO RICO, 2010

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Dengue, a potentially fatal febrile illness caused by four mosquito-transmitted dengue viruses (DENV-1-4), is endemic in Puerto Rico. In January, 2010, the number of suspected dengue cases reported to the Puerto Rico Department of Health/CDC passive dengue surveillance system exceeded the epidemic threshold. To characterize this epidemic, surveillance data were used to describe all reported cases. Suspected cases were patients with a serum specimen submitted for dengue testing. Laboratory-positive cases had (i) DENV identified via reverse transcriptase polymerase chain reaction (RT-PCR) in an acute specimen, and/or (ii) anti-DENV IgM detected in a convalescent specimen. Laboratory-negative cases had no anti-DENV IgM in a convalescent specimen and an acute specimen that was either RT-PCR-negative or not submitted. Indeterminate cases were RT-PCR-negative in an acute specimen and had

no convalescent specimen submitted. In 2010, 23,622 suspected dengue cases were reported, of which 10,956 (46.4%) were laboratory-positive, 2,588 (11.0%) were laboratory-negative, and 9,999 (42.3%) were indeterminate. Of 7,424 RT-PCR-positive specimens, DENV-1 (69.0%) and DENV-4 (23.6%) were detected more frequently than DENV-2 (7.3%) and DENV-3 (<0.1%). Of all laboratory-positive cases, nearly half (46%) were adults ≥ 20 years of age, 4,173 were hospitalized, and 254 had met the 1998 WHO definition of dengue hemorrhagic fever. Enhanced surveillance detected 38 laboratory-positive dengue deaths, yielding an incidence of 3.5 laboratory positive deaths per 1,000 laboratory positive cases; 89% of these deaths were in adults. The 2010 epidemic was long in duration, high in magnitude, and resulted in the most dengue-related deaths since surveillance for dengue began in Puerto Rico in the late 1960's. For this reason and using lessons learned from the 2007 epidemic in Puerto Rico, CDC implemented an initiative to train Puerto Rico clinicians in the management of dengue patients to minimize morbidity and mortality in future epidemics.

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SEROLOGICAL SURVEY OF DENGUE INFECTIONS AMONG INDIVIDUALS IN RAYONG, THAILAND

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Dengue and DHF have been a major public health problem in Thailand over the past 50 years. Clinical dengue (DF and DHF) has traditionally affected children with rare cases among adults. Even though the incident number of DHF cases does not seem to have decreased, a shift towards older age groups has been observed over the past years. The reasons for this shift have not been elucidated. We report the results of an age-stratified serological study conducted among school aged children living in the district of Mueang Rayong in Rayong, Thailand. Schools and classrooms were sampled probabilistically from all schools serving the district. A total of 1812 children (approximately 140 per age group) from 25 schools were enrolled and provided a blood sample. Samples were analyzed using single dilution neutralization testing (SDNT), an assay that differentiates between primary and secondary infection and is serotype specific for those subjects that have only been exposed to one dengue serotype. Preliminary results (n=720) show that 72% (95%CI 61-82%, n=71) of children have been exposed to dengue by age 10 years and that 16% (95%CI 2-30%, n=25) of children have only undergone primary exposure by 18 years of age. These results are significantly different from a similar study conducted by Sangkawibha et al. in Rayong in 1980, where 97% (95%CI 93-100%, n=65) of sampled children were seropositive by age 10 years. This change is consistent with an overall decrease in transmission intensity (force of infection) of dengue in Rayong over the last years. Analysis of the full dataset will explore geographic heterogeneity and factors associated with seropositivity.

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ENHANCED SURVEILLANCE FOR FATAL DENGUE IN PUERTO RICO

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Dengue has been endemic in Puerto Rico (PR) for four decades, and data suggests disease severity increased. Trends are monitored by the passive dengue surveillance system (PDSS) operated by the Centers for Disease Control and Prevention's (CDC) Dengue Branch and the PR Department of Health (PRDH). Suspected cases, including fatals, are reported to the PRDH or the PDSS, and reporting to PDSS requires submission of a serum sample. Prior to 2010, fatal cases were also detected by review of death certificates (DC) that had dengue as cause or contributing cause of death. However, a 2007 evaluation of PDSS found limited ability to detect deaths because it collects data early in the course of disease and few clinicians revise the PDSS report when a patient dies (<10%). In addition, <50% of laboratory-confirmed deaths identified by PDSS had "dengue" on their DC. Deaths are difficult to diagnose as many die on day 4 or 5 of illness when standard diagnostic tests are often unable to detect dengue virus (DENV) or IgM anti-DENV. Although tissue diagnosis is more sensitive in fatal cases, <40% of cases in 2007 had tissue submitted and collection was not systematic. To improve detection and diagnosis of fatal dengue cases, CDC developed an enhanced surveillance system in collaboration with Institute of Forensic Sciences of PR and CDC Infectious Diseases Pathology Branch. All patients who die with a dengue-like, acute febrile illness are identified through weekly calls to hospitals and at death investigation, autopsy, or DC review, and tissue specimens and autopsy findings are collected. During 2010 epidemic, 122 suspected fatal cases were identified of which 38 were laboratory-confirmed, more than twice the number previously identified. The majority (65%) of suspected cases submitted tissue, a higher percent than in any other year which notably reduced the proportion of laboratory-indeterminate cases. Only 16% of the 38 laboratory-confirmed cases had dengue on their DC. In spite of conventional wisdom, dengue deaths appear to be under-reported even in endemic areas.

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DENGUE FEVER DURING THE 2005 AND 2007 EPIDEMICS IN PUERTO RICO: EXPERIENCE OF A TERTIARY LEVEL HOSPITAL

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Dengue Fever is now the most important arthropod borne disease worldwide. It is caused by four serotypes of the Dengue virus. It can be a non-specific febrile illness without complications or it can progress to severe disease with plasma leaking, shock, bleeding and severe organ damage. In Puerto Rico there have been island wide epidemics since 1915 and these have increased in frequency and severity over the past 20 years. Until 2010, the 2007 epidemic had been one of the largest and most severe, presenting many diagnostic and management challenges. The goal of our study was to characterize the epidemiologic and clinical features of pediatric Dengue Fever admissions to the Hospital Episcopal San Lucas in Ponce during the 2007 epidemic and compare with the 2005 outbreak. The study was based at Hospital Episcopal San Lucas, a tertiary level hospital in southern Puerto Rico from January to December of 2007. Pediatric residents reviewed the records of 163 cases with a diagnosis

of Viral Illness with Thrombocytopenia that met the World Health Organization definition of Dengue Fever. The study included a comparison with Dengue Fever admissions (n= 71) during the 2005 outbreak, when data was available. Female/male distribution was 49%/51% in 2007 and 44%/56% in 2005. About 90% of patients were between the ages of 3 to 18 years in 2007 and in 2005. Platelet counts under 50,000 were more frequent in 2005 (74.3% versus 38.7%). During the 2007 epidemic 100% patients presented with a history of fever, 59% had vomiting and 39% had abdominal pain. Maintenance hydration therapy in 2007 included fluids that are considered hypotonic in 65% and isotonic in 16% of cases. Our study reveals that the pediatric age group most frequently affected in both epidemics was the school age and adolescent. Difficulties in the diagnosis during the 2007 epidemic may be related to less thrombocytopenia and the presence of gastrointestinal symptoms. Plasma leaking complications in 2007 may be due to disease severity complicated by hypotonic hydration fluids.

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EVALUATION OF COMMERCIALLY AVAILABLE DENGUE DIAGNOSTIC TESTS: NS1 AND IGM RAPID TESTS AND NS1 ELISAS

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The World Health Organization (WHO) and the Pediatric Dengue Vaccine Initiative (PDVI) established a network of 7 worldwide laboratories with dengue diagnostic expertise to provide an independent evaluation of currently available commercial kits for dengue diagnostics. Each laboratory contributed serum samples to develop a well characterized panel for testing dengue non-structural protein 1 (NS-1) antigen and dengue virus IgM antibodies (IgM anti-DENV). Asia and America region panels of similar composition were developed to evaluate NS1 and IgM anti-DENV test kits. The NS1 combined panel consisted of 192 sera from 147 patients defined as dengue positive and 142 negatives by culture or PCR. The IgM anti-DENV panel had 228 positive and 155 negative sera as defined by reference MAC-ELISAs at Mahidol University and CDC. Seven companies submitted 3 NS1 microplate ELISAs, 1 IgM microplate ELISA, 4 NS1 rapid diagnostic tests (RDT) and 4 IgM anti-DENV RDTs for evaluation. All kits were evaluated at the network sites using region-specific panels. The panel was coded so that technicians performing the evaluation were blinded to the reference assay results. Evaluation results were analyzed to determine sensitivity, specificity, inter-laboratory agreement, inter-reader agreement, lot-to-lot variation and ease-of-use. Preliminary results showed that the 3 NS1 ELISAs had sensitivities ranging 52-46% and specificities of 71-80 %. In comparison, the NS1 RDT had sensitivities ranging 28-59% and specificities of 71-76%. Of the 4 IgM RDTs, sensitivities ranged 52-95% with specificity from 83-90%. Sensitivity of IgM ELISA was 96% and specificity 84%. The range of acceptable sensitivity or specificity from the combined Asia/America panels is being considered by a panel of experts convened by WHO/PDVI. The threshold for acceptable performance may vary by the purpose of testing and by local epidemiology and final recommendations will be distributed as a report upon completion of the analysis.

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A RETROSPECTIVE GEOCODING STUDY ON THE IMPACT OF URBANIZATION ON INCIDENCE RATES OF DENGUE FEVER WITHIN BORNEO, KUCHING DIVISION, FROM 2009 TO 2010, USING ARCGIS

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Review of available data indicates that the impact of urbanization has not been extensively analyzed as it effects disease transmission of Dengue. Understanding urbanization, its meaning on the vector habitat, and how it contributes to disease transmission is vital. The districts of Bau, Lundu and Kuching, all have documented cases of Dengue and the repository of epidemiological data at the Divisional Health Office in Kuching, Sarawak is extensive. By incorporating Geographic Information Systems (GIS) to retrospectively analyze urbanization within these districts of Sarawak, and their cases of DF and DHF from January 2009 to June 2010, projections of the impact of urbanization on the vector distribution and density can be extrapolated and aid in the development of future prevention strategies. Spatial and descriptive analyses methods were used as was two sample t-test. Specific to Kuching District there is a direct correlation between urbanization and an increase in the vector responsible for dengue. Additionally, urban areas experienced higher rates of disease transmission. Three areas of the Kuching, Sarawak Division will be selected; Kuching, Bau and Lundu Districts. The selection criterion is based on the presence of Aedes mosquito breeding, and the frequent reporting of cases within these areas. All clinically diagnosed dengue cases, reported to the Health Office from Jan 2009 - Jan 2010, from Kuching Division will be included for GIS geocoding. Only geocodable addresses using ArcGIS will be included. An interactive method using data abstraction from case records, use of additional maps and street reference will be used to determine geocode within ArcGIS. For Kuching Division, an overall description of area (urban, semi-urban or rural), basic facilities, and geographical profiles, population, population density, and average annual population growth rate as provided by the Malaysian Census will be included. Incorporation into ArcGIS will be based on census tract files availability for 2009 - 2010. Clearly, supplemental GIS studies are warranted and should include habitat, climate and soil moisture modeling and variability in entomological parameters. In order to mitigate further disease transmission, an integrated approach is required for the endemic areas and developing areas of SE Asia.

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CHARACTERIZATION OF DENGUE FEVER IN SCHOOL CHILDREN IN MEDELLIN, COLOMBIA

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Dengue fever is the arboviral disease with the most significant impact in public health. In Colombia, the largest outbreak ever recorded occurred in 2010 with at least 151,774 cases. Understanding the factors and rates of transmission in schoolchildren are needed towards characterizing the burden of disease in the community and defining strategies for epidemic control. In this study, the incidence, seroprevalence and circulating serotypes of DENV were determined in schools from three different neighborhoods of Medellin (San Javier, Poblado and Laureles). A cohort containing 2,340 volunteer students from two public and one private school including primary and high schools (ages 5-19) was established. In the cross sectional study, blood samples were obtained from all admitted students and specific dengue IgM ELISA were performed. The longitudinal study involved surveillance of absenteeism of enrolled students due to febrile illness shorter than 7 days. Standardized physical exam were performed and venous blood samples were obtained from ill

students during both acute and convalescent stages. Dengue diagnosis was confirmed using RT-PCR and IgM ELISA. Among the 2340 students enrolled, 53% were women and students of all grades were represented. In the cross sectional study, 69 (2.9%) students were positive for IgM antibodies. Their mean age was 11.4 years (range = 5 to 19 years) and the distribution of cases by sex was the same. In the longitudinal study, among the 146 students declared ill because of absenteeism due to febrile illness, 12 (8.2%) had IgM antibodies against dengue and DENV-1 serotype was detected by RT-PCR in three of them. The highest frequency of DENV seropositive cases was detected in San Javier's school (3.9%), followed by the Laureles' school (3.4%). In this first phase of the study, a high incidence of dengue fever was found in school children mirroring the large outbreak experienced in 2010.

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ASSOCIATION OF POLYMORPHIC VARIANTS IN TNF-A, IL-6 AND IFN-Γ GENES IN PATIENTS AFRO-DESCENDANT AND MESTIZOS WITH DENGUE INFECTION, COLOMBIA

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Dengue is an important problem of health public in tropical and sub-tropical countries. On the other hand, the response to dengue infection is influenced by the genetic background of the host. In this study was evaluated the association of polymorphic in TNF-α, IL-6 and IFN-γ genes between two ethnic groups with dengue. The study was carried in Antioquia and Chocó, two departments of Colombia. The study population consisted of 122 Afro-descendents patients and 104 mestizos patients with dengue infection. The ethnic group was based using 19 ancestry informative markers (AIMs). The clinical form more frequently was dengue fever (90.3%). Comparisons between ethnic groups showed significant differences. In Mestizos was significantly more frequently the cases of dengue hemorrhagic fever (16.3% vs. 4.1%, p=0.003), more patients had thrombocytopenia (66.7% vs. 47.3%, p=0.012) and more patients were hospitalized (63.5% vs. 23.8%, p< 0.000) compared with afro-descendents patients. The difference between the average of ancestral component was obtained with an ANOVA, showing that the European component had effect above IL6 genotypes distribution, G/A (0.213 ± 0.135) G/G (0.3 ± 0.216) p=0.038. The African component was higher in dengue fever (0.526 ± 0.26) than in dengue hemorrhagic fever (0.376 ± 0.234), it confers protection until 50% (OR=0.49; IC=0.27-0.9; p=0.023). Of the 5 candidate loci (IL64589, TNF -376, CD209-336, INF-Y4100, INF-Y 78), in INF_Y G4100T and TNFα G376A, minor allele frequency were lower to 5%; at this respect, in an association analysis, was compared allelic and genotypic frequencies, finding significant differences for IL6 4589 locus between predefined disease groups, been homozygous G/G frequency higher in DF than in DHF (83% vs. 68%, respectively). This was the first evidence that IL6 polymorphism can be implied in protection/susceptibility to the infection for dengue virus. These results provide evidence about the susceptibility genetic to the infection for dengue virus. However further studies are still necessary.

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ASSOCIATION BETWEEN PRE-EXISTING DENV ANTIBODY AND THE OCCURRENCE OF SYMPTOMATIC ILLNESS DUE TO DENV-4 INFECTION, IQUITOS, PERU

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Dengue fever is caused by infection with any of four distinct viral serotypes (DENV-1 through DENV-4). Antibody induced by infection with one serotype can influence the clinical outcome of subsequent infections. In general, however, details of modifying effects are poorly understood, even though they are potentially critical determinants of transmission dynamics and disease severity. We analyzed data from an on-going longitudinal study of DENV transmission in Iquitos, Peru, to evaluate the relationship between infection history and disease outcome. Iquitos has been the site of intense DENV transmission since the early 1990s, with large outbreaks due to DENV-1, DENV-2 and DENV-3 since then. In 2008, DENV-4 was introduced into the city and became the dominant serotype over the 2008/9 and 2009/10 transmission seasons (>99% of all cases). During that time, 1,397 participants in the longitudinal cohort seroconverted to DENV-4 (35% of the study population). Of these, 5.7% experienced a clinical case of dengue fever as detected by active door-to-door febrile surveillance. No effect of pre-existing antibodies against heterologous DENV serotypes was observed on DENV-4 seroconversion rates. However, we observed that primary and secondary infections resulted in a higher rate of symptomatic illness (8.7% and 9.0%, respectively) than third infections and fourth infections (3.1% and 3.3%, respectively). These data suggest that although pre-existing antibodies did not confer sterile immunity to heterologous serotypes, there may have been a cumulative protective effect against symptomatic illness. Further analysis is being conducted on the effects of specific serotypes, levels of neutralizing antibodies and time interval between infections.

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CIRCULATION OF DIFFERENT LINEAGES OF DENV-2 IN GUATEMALA DURING RECENT DENGUE EPIDEMICS, THEIR EVOLUTIONARY TIME-SCALE AND SELECTION PRESSURE ANALYSIS

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Dengue is the most common arboviral disease worldwide. Dengue is caused by dengue virus (DENV), which exist in nature as a complex of four different viruses or serotypes (DENV-1 to 4), belonging to the genus Flavivirus. DENV serotypes have been classified into different genotypes based on phylogenetic analysis from sequences of different viral regions. Dengue is endemic in Central America and is present in Guatemala since at least 1978. Here we report the phylogenetic relationships of the first fully sequenced DENV-2 from Guatemala (GU/FDA-GUA09/2009) with strains representing all known DENV-2 genotypes. Phylogenetic analysis of the envelope (E) protein and whole coding region sequences by maximum-likelihood and Bayesian inference methods revealed that at least two lineages of the American/Asian genotype of DENV-2 have circulated in that country during the 2007 and 2009 epidemics, and have possibly co-circulated during these and other epidemic periods. We found that the time to most recent common ancestor for Central

American DENV-2 of American/Asian genotype existed about 18 years ago, and Bayesian Skyline analysis revealed that the genetic diversity of this DENV-2 genotype in the region has increased since 2005. Site-specific selection pressure analysis revealed positive selection in the NS2A, 4B and 5 proteins but none in E protein. The study of dengue evolution in endemic regions is of importance, since nucleic acid technology (NAT) assays has been developed and implemented in the detection of DENV in these countries, as well as in places in which the disease is imported and can cause autochthonous transmission, as it happened recently in the US. Even though primers and probes for these assays are designed to target the most conserved regions of the viral genome, there is always a risk that the assay could fail to detect variants with mutations located at the target area. Therefore, the use of whole genomic sequences in molecular epidemiology studies appears to be more suitable for identifying mutations that may occur throughout the viral genome, some of which could potentially impact the performance of detection assays.

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EVALUATION OF LOW DOSE MONOVALENT DENGUE VACCINES IN HUMAN VOLUNTEERS

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Infections caused by the four serotypes of dengue virus represent a substantial burden of vector-borne disease. Globally, 3.6 billion persons are at risk for dengue infection; outcomes range from a self-limited febrile illness to a fatal shock syndrome. As part of the NIH dengue vaccine development program, we performed phase I clinical trials on two live attenuated monovalent dengue vaccines, DEN1Δ30 and DEN2/4Δ30, at a dose of 10^1 PFU to further evaluate the safety profile and to determine the human infectious dose 50% (HID_{50}) of these candidates. Results were compared to prior studies at higher doses of each vaccine (10^3 PFU). Flavivirus-naïve healthy adult volunteers were dosed with 10^1 PFU of DEN1Δ30 or DEN2/4Δ30 (15 each) or placebo (3 each) and followed for 6 weeks. Subjects were screened for viremia for 16 days and seroconversion was determined on days 28 and 42. For the two vaccines at lower dosages relative to the higher dosages, no significant differences were observed for headaches, myalgias, arthralgias, rash, or neutropenia. Nearly identical seroconversion levels were achieved for DEN1Δ30 at both doses: 93% for 10^1 PFU and 95% for 10^3 PFU. However, peak geometric mean titers were lower (91.5 vs. 160.6, p=0.029) at 10^1 PFU. Transient viremia was similar for DEN1Δ30: 8 (53%) volunteers for 10^1 PFU and 9 (45%) for 10^3 PFU. The duration of viremia was the same (2.8 days), but the mean day of onset was delayed for the low dose cohort (D12 vs. D 10). In contrast, for DEN2/4Δ30, only 53 vs. 100% seroconverted at the 10^1 vs. 10^3 dose (18.6 vs. 120, p=0.001 Peak GMT), though fewer vaccinees were viremic (33 vs. 55%). Viremia onset was delayed for the low dose (D13 vs. D9)s, but again the duration was similar (3.5 vs. 3.2 days). These data suggest that the human infectious dose (HID_{50}) of the DEN1Δ30 candidate vaccine is $\leq 10^1$ PFU, however that of rDEN2/4Δ30 is approximately 10 PFU, indicating lower infectivity of this candidate. These data suggest a higher dose of DEN2/4Δ30, relative to other components, may be required in a tetravalent formulation.

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PHYLODYNAMICS AND CHARACTERIZATION OF NATURAL ATTENUATION IN A SOUTH PACIFIC DENV-2 OUTBREAK

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Dengue is an arboviral disease that has seen a recent increase in activity throughout the tropics in recent decades, marked by more frequent and severe epidemics. While the causes of this reemergence are probably multifold, including geographic expansion of both vectors and viruses, the importance of virus strains with greater fitness, epidemic potential and possibly virulence, has been implicated. This prompted us to investigate the role of virus molecular evolution in driving epidemics. Our study was a series of outbreaks of American genotype DENV-2 in the South Pacific beginning in 1971 in Tahiti and Fiji, which became increasingly severe in New Caledonia and Niue Island in 1972. In Tonga in 1974, however, it became dramatically attenuated, with near-silent transmission. To elucidate the relative contribution of viral genetic change in outbreak dynamics, we conducted whole-genome phylogenetic analysis of DENV-2 strains collected during the South Pacific sweep paired with *in vitro* assays of comparative viral infection phenotype. Because all islands were equally immunologically naive for dengue, this study offers an opportunity to isolate the effects of viral genetic variation from differential herd immunity on epidemic behavior. We studied 17 low-passage DENV-2 strains isolated during outbreaks on the islands of Fiji, Tahiti, New Caledonia, American Samoa and Tonga. Each isolate was subjected to whole genome sequencing and phylogenetic analysis then compared for infection efficiency, replication rate and productivity in cell culture. We found variations in the coding portion of the dengue genome, particularly the pre-membrane gene (prM) and the nonstructural genes, NS2A and NS4A that correlate with the attenuation of the Tongan strains of virus. Phenotypic characterization of viruses bearing these genetic substitutions, in terms of their potential to account for different epidemic dynamics, will be discussed. Our analysis indicates a significant role for viral genotypic change in dengue epidemic severity.

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DENGUE VIRUS INFECTION MODULATES EXPRESSION OF REGULATORY COMPLEMENT FACTORS IN HEPG2 CELLS

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Several organisms, including flaviviruses, exploit the regulatory mechanisms of the complement system to evade innate immune responses. Elevated activation of complement system is present in severe dengue cases. Hepatocytes express several soluble complement factors and receptors; and liver damage is often found in severe dengue patients. CD46 and CD55 are cellular regulatory proteins that inhibit complement activation on the host cell surface and protect healthy cells from inflammation, whereas gC1qR is a C1q receptor that has been shown to be involved in modulating cellular anti-viral responses by disrupting MDA5 and RIG-I signaling. We hypothesized that dengue virus (DENV) exploits the complement regulatory mechanism to evade direct attack of the complement system compromising the homeostatic control of complement activation. Thus, we investigated the regulation of CD46, CD55 and gC1qR and complement deposition in a hepatoma cell line HepG2 infected with DENV serotypes 2 (Thailand 16681) or 3 (H87). The cells were analyzed both for virus infection and for the expression of above-mentioned receptors by flow cytometry. The frequency of infected cells (DENVpos) was approximately 50% for both viruses. The expression of complement receptors in DENVpos and bystander cells (DENVneg)

revealed that, regardless of the serotype, the expression of CD46, CD55 and gC1qR on DENVpos cells was increased, whereas on DENVneg cells the expression was decreased. Consistent with this finding, we observed an increased deposition of complement factors, iC3b and C6, on cells with lower expression of CD46, CD55 and gC1qR. These findings suggest that DENVpos cells are protected from direct complement attack, whereas DENVneg cells are more susceptible. Overall, these results reveal a mechanism to allow virus replication in DENVpos cells by protecting them against complement attack and inhibiting the intracellular factors for virus detection and suggest an indirect effect of virus infection on bystander DENVneg cells making them more vulnerable to inflammation and liver damage.

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DIFFERENTIATING THE EFFECTS OF DENGUE VIRUS INFECTION AND *AEDES AEGYPTI* SALIVARY PROTEINS IN DENDRITIC CELL IMMUNITY

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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-hemostatic, 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. Dengue virus (DENV), a mosquito-borne flavivirus, is also known to modulate various components of the immune response to infection. As such, it is necessary to differentiate the immuno-modulatory effects of the virus from those of the mosquito and to examine their possible synergism. The effects of *Aedes aegypti* saliva on the immune response profile of monocyte-derived dendritic cells were examined using multiplex cytokine immunoassays and ELISA. To reduce the variance between samples often observed in immunological studies of multiple donors, the human monocytic leukemia cell line THP-1 was used in place of primary human peripheral blood mononuclear cells. Treatment with DENV type 2, strain 16803, results in down-regulation of numerous cytokines involved in the innate immune response as well as those that would shift the response toward Th2. Treatments with saliva and virus each raised the secretion levels of TNF-alpha and IL-8, and the combined effects of treatment with saliva and virus further increased expression. Additionally, IL-1beta was significantly up-regulated in treatments including saliva while virus alone had no such effect. The continued characterization of both the isolated and synergistic effects of saliva and virus is vital to the understanding of the immunological environment during infection establishment as well as developing possible therapeutic applications.

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HYPERENDEMIC TRANSMISSION OF DENGUE IN NORTE DE SANTANDER, COLOMBIA

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Dengue virus is a significant international public health threat with the potential to become a health security issue as it continues to emerge throughout the tropics and reaches across national borders. The incidence of dengue infections in people is increasing within endemic regions across the globe. Coincident with this emergence, dengue is also expanding into less recently afflicted areas with increasing effect (including autochthonous transmission establishment). Currently the department of Norte de Santander, Colombia is experiencing the highest rate of DENV cases in the country. Specifically, in Cucuta and Los Patios, transmission has intensified. In cooperation with local hospitals and the health department of Norte de Santander, we tested serum collected from suspected dengue cases. It was believed that dengue 3 was absent from the area, largely based on the incidence of this serotype in the neighboring Venezuela. We show that not only is D3 circulating and infecting people in Norte de Santander, but it is at a comparable rate as dengue 1 and 2, speaking to the continuing expansion of the virus. Dengue 4 is largely overshadowed by the other serotypes, though this report confirms the co-circulation of all four serotypes in this area. Of particular interest was the presence of four double positive individuals. Three individuals in Los Patios (D1/D2, D1/D3, D1/D2) and one individual in Cucuta (D2/D3) tested positive for two serotypes. Preliminary sequencing shows that D1 and D3 are genotypes that have been circulating in Colombia since 2008. D2, however, has 100% homology with a Venezuelan strain isolated in 2007. Further sequencing will inform phylogenetic relationships and possibilities of temporal and spatial patterns of viral movement. The presence of all four serotypes indicates this is a hyperendemic area with high levels of transmission. These interactions require further investigation to further inform public health officials on dengue transmission in this region.

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SEASONAL PATTERNS OF DENGUE VIRUS TRANSMISSION IN IQUITOS, PERU

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Understanding periodicity in disease dynamics is fundamental to predicting outbreaks and reveals factors involved in disease etiology. Dengue virus (DENV) has been shown to exhibit annual and multi-annual patterns of transmission, presumably driven by climate. We examine ten years of laboratory-confirmed, acute DENV infections captured by passive, clinic-based surveillance and routine entomological monitoring of adult *Aedes aegypti* population densities in Iquitos, Peru where there is minimal climatic variation (daily temperature = 25.9ºC ± 2.2). Wavelet analysis of weekly DENV cases indicates a strong, annual signal of increased transmission and weak evidence of periodicity on a three-year scale. The number of cases peaks annually in December, is lowest in July, and is strongly correlated with mean daily temperatures, which consistently dip in early July. As has been observed in Thailand, however, mean temperatures do not significantly differ between high transmission and low transmission seasons (26.9ºC vs 25.6ºC), but neither does the daily temperature range (10.3ºC vs 10.3ºC). Thus it appears that temperature variation is insufficient to explain annual periodicity in Iquitos, because although the short period of low temperatures in July could effectively slow viral replication in mosquitoes, transmission rates decline well before that time (~March) and do not elevate until well after (~October). Furthermore, precipitation decreases during June and July (0.25 inches/day, annual mean 0.38 inches/day), but otherwise is constant throughout the year, and variation in *Ae. aegypti* adult population densities does not show any consistent pattern that fluctuates with trends in transmission. Viral fade out in the human population appears to be strongly influenced by near-annual fumigation campaigns organized to control DENV outbreaks, while annual amplification in the population remains unexplained. We present and discuss a number of alternative models explaining annual amplification as well as inter-annual variation in the shape and magnitude of epidemic curves.

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ENVIRONMENTAL AND AGING INFLUENCES ON ANTIBODY-ENHANCED DENGUE DISEASE OUTCOMES IN AN IMMUNOCOMPETENT MURINE MODEL

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T-lymphocytes are proposed to promote clearance during primary dengue virus (DENV) infection but contribute to immunopathology during heterologous infections. Since an enriched environment enhances T-cell activity during viral infections and active older adults show less functional decline in T cell adaptive immunity, we hypothesized that enriched environment and aging would increase disease severity. To induce multiple infections of a single serotype or antibody-enhanced disease as it may occur in human infection, serial i.p. injections with DENV3 (genotype III) infected brain homogenate or anti-DENV2 hyperimmune serum followed 24h later by DENV3 (genotype III) infected brain homogenate were done. Control mice received anti-DENV2 hyperimmune serum followed 24h later by uninfected brain homogenate. Compared to antibody-enhanced dengue disease, clinical signs after one serotype infection were less apparent and survival periods longer. After antibody-enhanced dengue disease significant differences in the survival probability curves ($p = 0.031$) were found and both young and aged subjects from enriched environment showed higher mortality, intense clinical signs and hyperplasia of T cells in liver and lungs than subjects with impoverished environment. We propose that an enhanced immune response is occurring in the subjects of the enriched environment and in line with this concept glucocorticoids reduced these adverse outcomes

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LONGITUDINAL CHARACTERIZATION OF ANTIBODY RESPONSE TO DENGUE VIRUS IN BANGKOK THAILAND

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The plaque reduction neutralization test (PRNT) is the gold standard used to characterize the serologic immune response during and after infection with dengue virus (DENV). Few studies have described the trajectory of PRNT titers to all four serotypes after infection. We illustrate the antibody response to infection in a cohort of Thai children through parallel analysis of longitudinal PRNT results for all four serotypes of DENV. One hundred and eighty children from 1 to 15 years old seen at 2 hospitals in Thailand with RT-PCR confirmed DENV infection were followed up for dengue virus antibody response. Blood samples were collected daily from enrollment to the day after defervescence. Subsequent samples were collected at one week, six months, and yearly, with a maximum of three years of follow-up. Children were categorized based on their first PRNT measurement as undetectable ($PRNT_{50} < 10$ to all DENV serotypes), monotypic ($PRNT_{50} > 10$ to only 1 DENV serotype) or, multitypic ($PRNT_{50} > 10$ to more than 1 DENV serotype). We modeled the mean rate of ascent, time to peak, and rate of decline for children in all three initial PRNT categories. We observed a consistent response across all serotypes characterized by an approximately linear rise in log titer followed by a peak 5-10 days after return to normal body temperatures. Through analysis of the complete PRNT trajectory of those with a secondary infection, we find evidence that the serotype with the highest titer at first sample dominates the secondary neutralizing response thus supporting the theory of original antigenic sin. Furthermore, we find a rise in titer trajectories beginning two years after secondary infection that may be explained by subclinical tertiary or quaternary infections. These results help characterize the longitudinal serologic immune response to infection with dengue virus and can assist in the interpretation of PRNT results taken at different times from infection.

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WHAT DO PEOPLE KNOW AND DO ABOUT DENGUE AND PROTECTING THEMSELVES FROM IT IN IQUITOS, PERU?

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As part of a community-randomized trial to evaluate the effectiveness of insecticide-treated curtains (ITC) for dengue prevention in Iquitos, Peru, we applied a survey between October-December 2009 to 1334 study participants to examine their knowledge, attitudes and practices (KAP) associated with dengue and its prevention. Most of our respondents were female (73.9%), had finished secondary school (78.4%), and had a median age of 39 (range: 16-88). Although most participants knew that dengue was transmitted by a mosquito bite (85%), only 16.5% recognized that this mosquito bites during the daytime, 19.3% knew that its legs have white stripes, and 14.7% knew dengue is transmitted by *Aedes aegypti*. The most commonly recognized symptoms of dengue were fever (86.5%), headache (76.4%), muscle or joint pain (57.2%), and nausea or vomiting (25.0%). The most commonly identified preventive practices included getting rid of unusable items that might collect water (37.3%), and use of products to kill or repel mosquitoes (13.5%). More than half the respondents knew someone who had had dengue at some point (65%), and amongst these individuals, the median number of people they knew was 2. When people were asked what one should do if one has dengue, only about half (54.1%) knew to take paracetamol. Most common practices for mosquito control people mentioned were cleaning their homes (46.9%), picking up unusable items that might collect water (37.3%), covering water containers (26.2%), fumigating their homes (17.8%) and using insecticides around their home (13.5%). Use of insect repellent was minimal (2%). Despite (1) dengue endemicity in Iquitos for the past decades, (2) that the Regional Health Authority routinely fumigates and places larvicide in water containers, and (3) that there have been different types of health education messages at the community level disseminated through various media (radio, signs on buses and other public places), knowledge about dengue and its transmission, as well as household level practices to reduce dengue, could be improved in Iquitos.

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LEPTOSPIROSIS AND DENGUE FEVER CO-INFECTION: A REPORT OF THREE REPRESENTATIVE CASES

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Leptospirosis and dengue fever cause overlapping symptom profiles leading to mis-diagnosis, higher morbidity and mortality. Concurrent leptospirosis and dengue infections have not been widely studied. We report 3 representative cases of co-infection during the 2008 dengue epidemic in American Samoa. Hospital infection control records from

January to September were analyzed for dengue and leptospirosis co-infection cases. Three cases representing a spectrum of disease and treatment in outpatient, inpatient and critical care settings were identified. Patient medical records were reviewed retrospectively. Data included demographic information, history and physical exam findings, laboratory and imaging results, treatment, length of hospital stay, severity of illness, complications and final outcomes. Of the 132 dengue IgM+ patients, and 17 leptospirosis + patients identified during the study period, six were identified to have a co-infection. Representative cases demonstrate disease, treatment, and hospital setting variability. Case one is of a 41 year-old male presenting to the emergency department (ED) with fever, chills, and headache. Laboratory analysis during two outpatient visits demonstrated leucopenia, hemo-concentration, thrombocytopenia, elevated lactate dehydrogenase (LDH) and creatinine phosphokinase (CPK). Concomitant lower extremity cellulitis complicated decision making. Symptoms resolved with supportive outpatient care. Case two is of a 33 year-old female presenting to the ED with chills, dizziness and nausea. Initial laboratory assessment demonstrated leucopenia, hemo-concentration, thrombocytopenia, and mild AST and ALT elevations. On day two she demonstrated a fine erythematous rash, critical thrombocytopenia, alkaline phosphatase (ALP), and LDH elevations. Critical thrombocytopenia resolved with two days of supportive inpatient care. Case three is of 32 year-old female evaluated in the ED and admitted to the Critical Care Unit for septicemia and multi-system organ failure. Critical thrombocytopenia developed with resultant hemorrhagic disease and eventual death. Comparative case descriptions, laboratory analysis and treatment reviews are provided. In conclusion, challenges identifying dengue fever-leptospirosis co-infections result in treatment delays and adverse outcomes. Dengue epidemics in American Samoa may require routine testing for both diseases.

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SEA SURFACE TEMPERATURE MONITORING FOR DENGUE EARLY WARNING IN ECUADOR

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Dengue fever, a mosquito-borne viral disease, is one of the most important emerging tropical diseases in Ecuador. We report a statistical model for assessing the importance of climate as a driver for inter-annual variability in dengue fever in southern coastal Ecuador. Climate variables from a local meteorology station (precipitation, relative humidity, min/max/mean air temperature) and Pacific sea surface temperature (SST) anomalies were used to predict annual dengue fever incidence (1993-2010). Non-climate confounding factors such as serotype introduction were also considered. During El Niño events (positive Pacific SST anomalies), southern coastal Ecuador experiences warmer and wetter conditions, while during La Niña events (negative Pacific SST anomalies), the climate is cooler and drier. Preliminary results indicate that years with an above normal incidence of dengue fever were associated with El Niño events and years with below normal incidence of dengue were associated with La Niña events. Increased rainfall and warmer temperatures increase the availability of breeding sites and the development rate of the dengue mosquito (*Aedes aegypti*). Due to time lags involved in the climate-disease transmission system, monitoring El Niño / La Niña evolution in the Pacific Ocean could provide some predictive lead for forecasting dengue epidemics. This is the first study of dengue fever and climate in this region. This research provides the foundation to develop a climate-driven early warning system for dengue fever in Ecuador.

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ECO-EPIDEMIOLOGICAL EVALUATION OF CHAGAS DISEASE PREVALENCE IN THE VILLAGE OF LAGARTERA GRANDE, REPUBLIC OF PANAMA

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Chagas disease has historically been endemic to the village of Lagartera Grande, especially in children. Previous data collected in 2004 revealed that 2.9% of the village children tested seropositive for *Trypanosoma cruzi*, the parasite responsible for Chagas. Once infection was detected in this community, health officials began to implement preventive measures. The Gorgas Memorial Institute conducted health education projects in the community in cooperation with the Japan International Cooperation Agency (JICA) with particular focus on educating school children on various aspects of the disease. The Department of Vector Control of the Ministry of Health began sporadic spraying of infested houses and developed an efficient system of entomological surveillance with community participation. This study serves to evaluate the progress accomplished since 2004 by assessing the current prevalence of Chagas within Lagartera Grande. Seventy-seven members of the community completed the Knowledge, Attitudes and Practices (KAP) survey for Chagas disease, the vector *Rhodnius pallescens*, and the vector's arboreal habitat the royal palm *Attalea butyracea*. Serum samples were also collected from all the children in Lagartera Grande above the age of six months to assess the current prevalence of infection in the community. Samples were screened with 3 different serological tests: a commercial recombinant enzyme-linked ELISA (ELISA Chagatest, Wiener Laboratory, Argentina), a recombinant ImmunoComb commercial test (Orgenics, Israel), and an immunoblotting technique using a crude epimastigote antigen preparation derived from a Panamanian *T. cruzi* strain. All samples collected tested negative, suggesting the successful implementation of preventive measures in the community. Continued surveillance and monitoring was recommended since favorable conditions for transmission are still present in Lagartera Grande.

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IDENTIFICATION OF ANTHROPOLOGICAL AND SOCIOCULTURAL RISK FACTORS FOR CUTANEOUS LEISHMANIASIS IN CAPIRA DISTRICT, PANAMA

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Leishmaniasis is a disease with multiple clinical presentations that affects millions of individuals around the world. This parasitic disease is complex and difficult to control due to the intricacies of its transmission cycle, the variety of animal host reservoirs, the many species of sand flies that act as transmission vectors, and diversity of ecosystems involved. In Panama, there is an increasing incidence of the ulcerated cutaneous form of the disease and it is suspected that there is an underestimate of the actual number of people affected due to lack of access to healthcare by poor and disadvantaged populations. This project aimed at identifying risk factors for cutaneous leishmaniasis that can be useful in establishing a prevention plan tailored to the community where cutaneous lesihmaniasis is endemic. The population studied was from the village of Trinidad Las Minas in Capira District, Republic of Panama. One hundred and twenty-five individuals older than 12 years of age were surveyed between November and December 2009 in the village. Twenty-four households were randomly chosen and the characteristics of each household were recorded to identify risk factors. The variables studied included the floor material,

the roof material, the presence of cracks on the walls, the presence of surrounding royal palms, the presence of other animals around the home, and the presence of screens on the windows. The data was analyzed using descriptive statistics and univariate logistic regression. On univariate analysis the only variable associated with cutaneous leishmaniasis in the home was the presence of dirt floors (χ^2 = 6.8, P = 0.01). Multiple logistic regression will be carried out to determine whether this is an independent factor. In conclusion, the floor material should be further studied as a potential risk factor for cutaneous leishmaniasis. The results may be useful in developing a community prevention plan with the goal to decrease the incidence of the disease.

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SUDANESE *LEISHMANIA DONOVANI* POPULATION STRUCTURE: UNCOVERED BY MULTILOCUS MICROSATELLITE AND SEQUENCE TYPING

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The population structure of *Leishmania donovani*, the etiological agent of visceral leishmaniasis (VL), was investigated. VL is highly endemic in countries such as India, Brazil and Sudan. Sudan has suffered serious epidemics in the past, particularly in the East and South. Here we had typed a large panel of Sudanese strains using multilocus microsatellite typing (MLMT) (n=14 markers, 103 isolates) and multilocus sequence typing (MLST) (n=11 targets, 50 isolates). Both genetic typing methods agreed on the presence of two main groups of L. donovani in Sudan: group R which contained 3 subpopulations as estimated by MLMT (SDA, SDB and SDD) or 2 subpopulations as estimated by MLST (SDA and SDB+SDD) and group G (~1:1 canine: human isolates) which contained 2 subpopulation (SDC and SDC-outliers) as suggested by both typing methods. All subpopulations showed a significant deficiency of heterozygosity that cannot be explained by a Wahlund effect except SDA which showed higher than expected heterozygosity for MLMT markers only. In addition, subpopulation SDB of MLMT Group R was not significantly departed from Hardy-Weinberg equilibrium, was in linkage equilibrium and had an inbreeding index (FIS= 0.20), selfing rate (s=0.33) and panmictic index (f= 0.02) values compatible with recombination. In this study, it is tempting to suggest that this subpopulation is an intermediate between SDA and SDD, but this hypothesis needs to be further investigated. Furthermore, all MLST subpopulations showed none to minimal pairwise linkage disequilibrium after sequential Bonferroni correction (α= 0.05). The equal number of isolates from dogs and humans in group G suggests a role in transmission for domestic dogs, at least in eastern Sudan. However, subpopulations SDC and SDC-outliers were moderately subdivided to effectively isolated from Group R subpopulations (FST values ≥ 0.40). An association was suggested between the subpopulation and pathology, which if holds true after further investigations, may be very useful for disease diagnosis and pathology-specific designed drugs. This study provided a plethora of new information regarding the population genetics of Sudanese L. donovani, and raised several hypothesis regarding important aspects of the epidemiology of leishmaniasis in Sudan, and possibly other endemic areas.

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MULTIPLE ETIOLOGIC AGENTS; THE POSSIBLE CAUSE OF CUTANEOUS LEISHMANIASIS IN GHANA

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The emerging and/or re-emerging focus of cutaneous leishmaniasis (CL) in Ghana since 1999 has in recent times, seen more than one species of the parasite identified and implicated as the etiologic agent. Leishmania *major* was first to be identified as the agent of the infection in 2006 in the endemic focus in Ghana. An unknown and uncharacterised *Leishmania* species was identified in 2007 but did not use species linked to African leishmaniasis as positive control. The recent work done in 2008 using *Leishmania* species associated with infections in Africa as positive controls identified *Leishmania* contrary to the species previously recognized, as one of the possible species influencing disease in Ghana. This study aimed at the identification of species of *Leishmania* parasites responsible for CL focus reported in Ghana. The endemic focus is located in the south-eastern part of Ghana, which borders three countries in the West African sub-region. Twenty lesion aspirate and scraping samples were taken from active patents lesions for the study. Primers A1 and A2, were used to amplify a fragment of ~1500 bp of the intergenic region between the ribosomal protein genes RPS7A and RPS7B on chromosome 1 and second primers B1 and B2, were used to amplify an internal fragment of ~1350bp in a nested PCR. These nested PCR products obtained were digested using restriction enzyme *MspI* and the products run on 2% agarose gel. The bands produced from some samples showed a match to one of the control sample *L. aethiopica*, hitherto is found to be associated with leishmaniasis in the eastern part of Africa. Comparing this preliminary results to previous works by other investigators, one can somewhat say that there could be more than one agent responsible for CL focus in Ghana.

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TRYPANOSOMA SPP. OF RODENTS AND OPOSSUMS IN A CHAGAS DISEASE ENDEMIC REGION OF NORTHERN PERU

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An estimated 192,000 Peruvians are infected with *Trypanosoma cruzi*, the etiologic agent of Chagas disease. In an endemic region of northern Peru in the province of Cutervo, intervention methods, including spraying, began in 2001 that seemingly decreased the incidence of Chagas disease by the next year. Despite these efforts, new acute cases (2 months- 2 yrs) were reported from 2004-2008 and prevalence from a 2004 study was still 27.7% in individuals <15 years of age. Many communities in Cutervo are in close contact with the sylvatic environment having mud brick homes in the middle of the Andean high jungle ecosystem. Considering the presence of new cases and the sylvatic environment, the goal of the current study was to determine the role of wildlife reservoirs in *T. cruzi* transmission within the region. Rodents (*Rattus norvegicus* and *R. rattus*) and opossums (*Didelphis albiventris*) were live captured from within homes and surrounding areas in five communities (Casa Blanca, Pindoc, Esperanza, Rumiaco, and Nuevo Guayaquil). At the time of collection, trypomastigotes were observed by bright-field microscopy in 22 of 50 rodent whole blood samples, while no parasites were observed in opossums (n=7). A panel of PCR, including gene targets for the minicircle

and 28S, 18S, and region from 18S to 5.8S rDNA, was run to identify false negatives by microscopy and determine the *Trypanosoma* spp. present. Of the rodents, 16/22 positive by microscopy were infected with *T. lewisi*, while the *Trypanosoma* spp. of the remaining six rodents could not be identified due to sample loss. *T. cruzi* was identified in 4/7 opossums; these animals were hand-captured south of the Esperanza community. While the sample size of the current surveillance study is small, two main observations were made: 1) Rats do not appear to play a role in the *T. cruzi* transmission cycle within Cutervo and 2) the opossum may have a potential role as a wildlife reservoir for *T. cruzi* in the area.

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EPIDEMIOLOGICAL CHARACTERISTICS OF LEISHMANIASIS IN PERU 2004-11

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Leishmaniasis is a parasitic disease of significant public health importance. Considered by WHO as one major Neglected disease, this disease is Transmitted by sand fly vector. In Peru, leishmaniasis is an endemic disease affecting several departments and is the second endemic tropical. Is reported an annual average of 2500 cases. There is a need to provide information for the management of the disease. The study Focus on the determinate characteristics epidemiological and distribution of cases. Notified leishmaniasis is near mandatory public health services. All suspected cases were recorded in the Epidemiological sheet and reported to the epidemiological surveillance system in the country. From 2004 until 2010, were reported 19113 cases of leishmania. Of all cases 92.6% were cutaneous leishmaniasis and the remaining 7.4% mucocutaneous leishmaniasis. Most have come from Cusco with 3318 cases (16.1%), Ancash with 1836 cases (8.9%), Piura with 1816 cases (8.8%), Junín with 1797 cases (8.7%) and San Martin with 1711 cases (8.3 %). Until week epidemiology 13-2011, 1471 cases have been reported, which makes a national incidence rate is 101.6 per 100 000 inhabitants. The endemic area extends through the Andes and the valleys between 600 and 3 000 meters above sea level, for cutaneous leishmaniasis, and areas of high and lowland forest below 2000 meters for mucocutaneous leishmaniasis. The age group was most common entre 20 and 49 years old (67.56%). The male / female ratio was 1.6. The majority of patients were farmers. The unique lesion was The Most Frequent (69.8%) and the Majority of injuries was in extremities (58.2%). In conclusion, leishmaniasis is endemic in tropical kind in Peru and is distributed in poor areas of various departments. This produces a negative social and economic impact in the economically depressed. In addition, the destructive consequences it causes, particularly the mucocutaneous form the effect of isolating the individual, its irreversibility.

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CAN WE REDUCE PERSONAL RISK OF RHODESIAN SLEEPING SICKNESS? ANALYSIS OF FACTORS CONTRIBUTING TO THE PROBABILITY OF BEING BITTEN BY TSETSE

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Rhodesian sleeping sickness, the zoonotic form of Human African Trypanosomiasis found in east and southern Africa, is often associated with game parks and wilderness areas where tsetse flies (*Glossina* spp.) and wild reservoir hosts are abundant. People living and working within or near such areas have limited options for controlling HAT due to concerns about the cost, feasibility and environmental impacts of tsetse control. In the Mana Pools National Park of Zimbabwe, we carried out studies to identify the circumstances in which people are most likely to be bitten by infective tsetse. Our results show that, contrary to expectation, people were more likely to be bitten by tsetse (*G. morsitans morsitans*, *G. pallidipes*) in the vicinity of their houses rather than in the woodlands (total catches = 375 *vs*. 264 tsetse caught over 221 days) where tsetse are apparently more abundant. For tsetse from houses, 44% were female and of these, 30% were old enough to be able to carry mature infections. Moreover, natural repellents (human body odour, woodsmoke) produced by humans which are highly effective outdoors, are ineffective against indoor-biting tsetse. For humans in woodlands, the numbers of tsetse were greatest if the human was mobile and not in the vicinity of a natural non-human host: humans walking without an ox caught 20 tsetse/day compared to 0.2 tsetse/day for a stationary human accompanied by an ox, albeit the flies were younger and hence less likely to be infected (18% of females were old enough to be able to carry mature infections). We suggest that for people in areas where Rhodesian sleeping sickness poses a risk, interventions designed to prevent or kill tsetse entering houses or vehicles might reduce personal risk of HAT significantly.

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POPULATION STRUCTURE OF *LEISHMANIA TROPICA* IN NORTHERN PAKISTAN AND NEIGHBORING COUNTRIES

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In Pakistan, Anthroponotic Cutaneous Leishmaniasis (ACL) caused by *Leishmania tropica*, has a broad distribution occurring focally in the Northern areas and Azad Kashmir. Reportedly, some isolates of *L.tropica* are heterozygous. This project aims to investigate the intra-specific diversity of *L.tropica* in Northern Pakistan using Multilocus microsatellite typing (MLMT). Further, the population structure and phylogenetics of the parasite will be mapped by taking into account isolates from Pakistan and from other countries lying between the Mediterranean Sea and the Bay of Bengal, namely Syria, Afghanistan, Iran and India. As a tool to enhance our ability to identify *L. tropica* from *L. major*, which is sympatric in this region, we have developed a novel PCR that distinguishes between these two species. Samples have been collected from 3 major hospitals of Peshawar, Khyber Pukhtoon-Khwa (KPK),Northern Pakistan. These include isolates in culture, biopsies and filter paper impressions. These samples have been typed for species and followed by MLMT for *L. tropica* isolates. MLMT analysis of clinical isolates from Pakistan and other countries in the region (confirmed as *L. tropica*), plus the WHO strains will be presented. Results providing an estimate of the ACL presentation rate at Peshawar hospitals will also be discussed briefly.

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TRANSMISSION DYNAMICS OF *TRYPANOSOMA CRUZI* LINEAGE I IN TWO ENDEMIC PROVINCES OF ECUADOR

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Trypanosoma cruzi infection affects an estimated 230,000 people in Ecuador. Recent reports indicate limited effectiveness of insecticide-based vector control interventions, due to re-infestation by sylvatic triatomines. Previous studies demonstrated that the lineage I (TcI) of *T. cruzi* is the predominant lineage circulating in Loja (Southern Andes) and

Manabi (Central Coastal) provinces. Furthermore, in southern Ecuador (Loja province) microsatellite analyses of TcI isolates showed two main parasite populations exist: one related with domestic and peridomestic environments and a second one related with sylvatic environments. The aim of this study was to evaluate TcI isolate divergence within and among Loja and Manabí using Multi Locus Sequence Typing (MLST). We sampled vectors and mammals and a wide geographic area within each province. Our results corroborate that the presence of two different parasite populations in Ecuador, according to habitat: In Loja province, the previous separation in two populations (domestic/peridomestic and sylvatic) was confirmed. In Manabí province, this tendency was also seen, where the sylvatic population was separated from the peridomesic population. However, in both cases, limited genetic flow was evidenced. Interestingly sylvatic samples of both provinces cluster together, suggesting genetic flow among sylvatic populations, while genetic separation was evident between domestic/peridomestic populations of both provinces. These results suggest that similar transmission dynamics are taking place in both provinces where albeit at different rates, there is limited genetic flow between sylvatic and domestic/peridomestic *T. cruzi* populations within a small geographical area. Therefore, control strategies need to be adapted to the intrinsic characteristics of a small geographic scale.

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DISTRIBUTION AND NATURAL INFECTION OF CHAGAS DISEASE VECTORS IN DOMESTIC, PERIDOMESTIC AND SYLVATIC HABITATS IN SOUTHERN ECUADOR

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Chagas disease is endemic in 70% of the Ecuadorian territory. The main vectors responsible for *Trypanosoma cruzi* transmission in the Southern Andean region of the country are *Rhodnius ecuadoriensis, Triatoma carrioni, Panstrongylus chinai* and *P. rufotuberculatus.* This study aims to describe the triatomine distribution and natural trypanosome infection in domestic, peridomestic and sylvatic habitats in Loja Province. Active triatomine searches were conducted in domestic and peridomestic habitats in rural villages and in nearby sylvatic areas throughout the province. 11,115 live triatomines were found infesting domestic units in 68% of the 92 rural communities while 1,923 live triatomines were found in 52% of the 23 sylvatic localities examined. Nine percent of the domestic units (n = 3,191) were infested with one or more triatomine species and 12% of the sylvatic nests (n=1,219) were infested with *R. ecuadoriensis*. Nymphs were observed in 80% of both infested domiciles and nests. Triatomines were found in all ecological regions below 2,200 meters above sea level. In the domicile *R. ecuadoriensis* and *T. carrioni* were found mostly in bedrooms while in the peridomicile these species were abundant in chicken coops located near the house. Established colonies of *P. chinai* and *P. rufotuberculatus* were found restricted to the domicile. Sylvatic triatomines were found mainly in squirrel and mouse/rat nests, and to a lesser extent in bird nests. *T. cruzi* infection was found in 10% of the domestic/peridomestic triatomines (n=775) and 64.7% (n=300) of sylvatic triatomines analyzed. Mixed infections with *T. cruzi* and *T. rangeli* were found in 8% of sylvatic triatomines. To date, limited vector control efforts have been implemented in this area. Although, the application of insecticide-based vector control could be effective in reducing domestic and peridomestic populations it must be complemented with constant surveillance to detect and control post-intervention reinfestation by sylvatic triatomines. Our findings highlight the need for a systematic, sustained, and monitored vector control intervention in the region.

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ANTI-TRIATOMINE SALIVA IMMUNOASSAYS FOR THE EVALUATION OF IMPREGNATED NETTING TRIALS AGAINST CHAGAS DISEASE TRANSMISSION

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Insecticide-impregnated nets can kill triatomine bugs, but it remains unclear whether they can protect against Chagas disease transmission. In a field trial in Quequeña, Peru, sentinel guinea pigs placed into intervention enclosures covered by deltamethrin-treated nets showed significantly lower antibody responses to saliva of *Triatoma infestans* compared to animals placed into pre-existing control enclosures. Our results strongly suggest that insecticide treated nets prevent triatomine bites and can thereby protect against infection with *Trypanosoma cruzi*. Anti-salivary immunoassays are powerful new tools to evaluate interventions against Chagas disease.

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PROXIMITY BETWEEN DOGS AND *TRYPANOSOMA CRUZI* INFECTED TRIATOMINES AS A RISK FACTOR FOR THE PERSISTENCE OF CHAGAS DISEASE

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Chagas disease is a vector-borne disease transmitted by triatomine bugs and caused by the *Trypanosoma cruzi* parasite. It is one of the most neglected tropical diseases. Insecticide application campaigns to eliminate vectors are the most effective intervention to stop transmission of the parasite, and are routinely conducted in Arequipa, Peru. After these campaigns, re-infestation can occur, and areas where vectors and *T. cruzi* infected mammals overlap can be the starting point for re-initiation of disease transmission. Dogs have been described as reservoirs of *T. cruzi* in Argentina, Brazil, Mexico, and Venezuela. Our objective was to determine whether the presence of seropositive dogs can explain the clustered re-emergence of *T. cruzi* in vectors. The study was designed as a cross-sectional serological screening to detect antibodies against *T. cruzi* in dogs, entomological collection of vectors from households to determine their infection status, and georeferencing of households to determine proximity between dogs and triatomines. The main outcome was canine seropositivity. Its association with other factors was analyzed with multivariate logistic regression for demographic and household risk factors and with spatial techniques for clustering and proximity correlation. Canine seroprevalence in the area was 12.3% (SE=2.6, N=154). The statistical results show that seropositivity in dogs was positively associated with proximity to *T. cruzi* infected triatomines, with proximity to high numbers of triatomines, regardless of their infection status, and with dog's age. The presence of *T. cruzi* seropositive dogs

could explain the persistence of Chagas disease in endemic areas of Peru. Massive insecticide campaigns allow for the collection of triatomine data that are used to determine high-risk areas for humans. Interventions based on these entomological data should include the presence of dogs around houses where infected triatomines were collected.

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GENETIC DIVERSITY OF *RHODNIUS ECUADORIENSIS* (*HEMIPTERA: REDUVIIDAE*) POPULATIONS IN THE CENTRAL AND SOUTHERN ANDEAN REGIONS OF ECUADOR

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Rhodnius ecuadoriensis is the most widespread vector of Chagas Disease in Ecuador. Effective control of this disease requires a good understanding of the epidemiological cycles, including a reliable analysis of the genetic structure of populations of this important vector. *R. ecuadoriensis* occupies domestic, peridomestic and sylvatic habitats and is a widely distributed species in the central Coastal (Manabí province) and southern Highlands (Loja province) regions of Ecuador. These two regions are phytogeographically and climatically different and correspondingly, bugs collected from these areas demonstrate differences in several phenotypic characters (i.e., body size, antennal sensilla and wing geometry morphometrics), as well as in behavioral traits (feeding and defecation patterns, and life cycle). To evaluate the genetic relationships among *R. ecuadoriensis* populations between these regions, we sequenced the mitochondrial cytochrome b (Cytb) gene in 168 insects collected from both regions (n=95 in Loja) and (n=73 in Manabí). We found 34 Cytb haplotypes determined by 53 variable sites. Only three haplotypes were shared between the two provinces (15 were exclusive for Loja and 16 for Manabí). A moderate genetic differentiation was observed between the two geographical regions (G_{ST}=0.05622) and remarkably, a third genetically different group within the Loja province was found. Our results support the hypothesis of disruptive selection acting upon *R. ecuadoriensis* populations, probably due to geographical isolation. The genetic patterns observed in this work contribute to the knowledge of genetic variability of *R. ecuadoriensis* across different geographical regions and provides background for interpretation of routes of dispersion and isolation.

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DEMOGRAPHIC AND SOCIOECONOMIC DETERMINANTS OF MOSQUITO NET USAGE IN MALARIA ENDEMIC REGION OF BANGLADESH

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Use of mosquito net is a proven strategy for malaria control. One of the vector control components of the National Malaria Control Program in Bangladesh was to promote insecticide treated bed net to ensure prevention or reduction of malaria mortality and morbidity. However, use of mosquito net as well as insecticide treated bed net varies among households. We investigate associated factors in bed net usage at Kuhalong and Rajbila unions under Bandarban Upazila, south-east region of Bangladesh. We utilized data of an ongoing demographic surveillance from 4567 households with population size 20755. Both the unions were divided into 12 clusters (C). Overall 99.2 % (4529) households possessed mosquito nets. Most cited reason for not using bed net while sleeping in 38 households was unavailability of a net. In Kuhalong and Rajbila respectively 89% and 87% of population slept under bed net at previous night of the interview whereas use of insecticide treated bed net was about 80% in both. Lowest percentage of last night bed net users were in C5 (63.5%) of Kuhalong and in C9 (80.2) of Rajbila. Insecticide treated net use was lowest in C11 (59.3%) and C4 (69%) of Kuhalong and Rajbila respectively. Person per bed net was as high as 3-4 in few areas of the unions. Nontribals, household heads, females, married persons, children <5 years of age, individuals from family size <5 were more likely to use bed net in previous night of the interview (P <0.001). Use of insecticide treated bednet was significantly higher among tribal counterpart and individuals from family size <5 (P<0.001). Last night bed net users were higher among persons from households that own radio/tape recorder (<0.001) or dwelling unit ≤2 (P <0.001). However, insecticide treated bed net users were also significantly higher among members of households having bamboo floor/wall (P <0.001) or using oil/kerosene lamp ((P <0.001). In both unions, there was no significant variation between malaria positives and last night bed net user. Gap between insecticide treated bed net user and nonuser should be reduced and more emphasis should be given to seek malaria associated risk factors in these areas.

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COMMUNITIES IN NETWORKS MAPPING MALARIA MOVEMENT IN KENYA

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With malaria eradication back on the global agenda and subsequent elimination targets for various low endemic countries, control strategies require a strong quantitative evidence base. The failure of previous elimination efforts has shown that human population movements are important for infection exchange between different transmission areas. For countries, that have overall low transmission but a few high transmission hotspots, population movements from high to low transmission zones may threaten imported infections, therefore local control agendas and challenge larger scale elimination efforts. Here, a unique and extensive mobile phone records dataset was analyzed with network analysis tools, a countrywide *Plasmodium falciparum* transmission map and previously developed transmission models to assess communities within Kenya linked by infection flows. The likely principle sources of imported infections within national boundaries, which may threaten onward transmission or have clinical significance, were mapped at a settlement level. Clusters of settlements were identified and compared to approximate "natural" malaria-relevant migration boundaries, splitting the country into regions that share malaria-relevant movement characteristics. With elimination as the ultimate goal for Kenya, we provide a quantitative platform for strategic control planning, by targeting control resources at defined spatial and temporal scales.

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SOCIO-DEMOGRAPHIC RISK FACTORS FOR MALARIA IN A NEWLY ESTABLISHED SURVEILLANCE REGION IN THE CHITTAGONG HILLS TRACTS OF BANGLADESH

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Until recently, the Chittagong Hills have been hyperendemic for malaria but in recent years has been hypoendemic. A new study initiated in two

unions (population=20,563) near Bandarban, Bangladesh in 2009 was designed to improve knowledge of malaria transmission, to monitor malaria interventions, and serve as an area for developing new control strategies. The project included: (a) demographic surveillance system, (b) periodic surveys of knowledge, attitude, and practice, (c) geographic information system, (d) weekly active and continuous passive surveillance for malaria infections, (e) monthly mosquito surveillance, (f) daily weather measures. The program included both standard and molecular methods for case detection. Between October 2009 and January 2011, 151 cases of malaria were detected. 83% were symptomatic infections (97% chloroquine resistant *Plasmodium falciparum*, 2.5% *P. vivax,* and 0.8% mixed infection). Malaria infections were highly clustered geographically and seasonally. Risk factors associated with higher malaria rates included age, pregnancy, education and occupation. In univariate analysis, there was increased odds of high-season malaria disease in children aged 1-4 (95%CI 1.1, 3.8) and 5-14 (95%CI 1.7, 4.0) compared with those aged >14. Tribal people had a 4-fold higher odds compared to Bengalis (p=0.019) living in the same unions. These effects were independent and of similar magnitude and significance in a multivariate model. After controlling for the effects of union of residence, age and sex, there was an increased odds of malaria amongst people doing day labor (2.5-fold, p=0.046) and a hillside agriculture practice by tribal groups called "jhum" (4.3-fold, p=0.002), but not other agricultural occupations (p=0.304). These results reveal socio-demographic risk factors for symptomatic malaria during the high-season in this Chittagong Hills surveillance region that will: (1) serve as a basis for future hypothesis-driven epidemiological studies, or (2) target future intervention strategies to high risk groups.

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INCIDENCE OF MALARIA IN THE FIRST YEAR OF LIFE IN A HIGH MALARIA TRANSMISSION AREA IN GHANA

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Malaria in pregnancy is a risk for abortions and low birth weight. Placental malaria may also have an impact on the infants susceptibility to parasitemia, clinical malaria and anemia. A prospective birth cohort study was carried out among pregnant women and their infants to determine the incidence of malaria and its effects on the infant's health in a high malaria transmission area in Ghana. At birth, placental biopsy was taken to determine placental malaria. The infants were followed up for a period of one year. Each infant was followed up monthly for malaria parasitemia, anemia and passively for clinical malaria. The mean total IgG levels to the *Plasmodium falciparum* CSP antigen (NANP6) were also determined by ELISA in a subset of the birth cohort at birth, 3 and 6 months of age. A total of 2810 pregnant women were identified and followed up till birth. The average age of mothers was 27 years with 25% and 75% being primigravidae and multigravidae, respectively. Twenty-two (22) percent of the pregnant women were in the very poor quintile of socioeconomic status. The coverage of intermittent preventive treatment with at least one dose of SP was 95% and insecticide treated net (ITN) use was 38%. The prevalence of placental malaria was 37.3%, 95% CI 35.22-39.44. A total of 1605 infants contributed to 1079.0 PYs of followed up. The mean coverage of ITN use among the infant cohort at any point of contact was 36%. The incidence of malaria parasitemia was 0.50PYRS (95% CI 0.46 -0.55 unadjusted), 0.68PYRS.(95% CI 0.47-0.97 adjusted); incidence of anemia was 3.34 95% CI 3.17-3.52 unadjusted, 4.13 95% CI 2.22 -7.68 adjusted and incidence of clinical malaria was 0.22 95% CI 0.20 - 0.25 unajusted, 0.45 95% CI 0.16 -1.23 adjusted. The mean total IgG levels to CSP at birth, 3 mo and 6 mo were 1091.20 95% CI 952.21 -1249.51; 203.9 95% CI 174.11 -238.89, 179.4 95% CI 147.53 -218.18 respectively and the mean total IgG levels to GLURP RO at birth, 3 mo and 6 mo were 257 95% CI 187.38, 352.46, 38.5 95% CI 28.38, 52.21, 36.9 95% CI 26.56, 51.20 respectively. In conclusion, the incidence of malaria among infants in the first year of life is low while the incidence of anemia is high. In addition IgG levels at birth was higher to CSP than GLURP and decayed to lower levels at month 3 and month 6th in the infant cohort.

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ASSESSING THE CLINICAL EFFICACY OF MALARIA VACCINES IN TRIALS AND THEIR IMPACT IN THE FIELD: A MULTIFACETED APPROACH TO USING SIMULATION MODELS FOR PREDICTION

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Assessing vaccines against *Plasmodium falciparum* in clinical trials and predicting their impact after licensure comes with many complications and open questions. RTS,S, the vaccine furthest in clinical development with initial Phase III trial results expected before the end of the year, may be licensed in the next few years. During trials of this type of vaccine and others (such as vaccines directed towards the blood-stage cycle of malaria) important questions are raised concerning how to adequately define endpoints to assess efficacy, and if the impact of the vaccine will vary with transmission setting, age-group immunized, or delivery strategy. In this work, we examine these questions using an ensemble of simulation models for malaria epidemiology and control. We discuss the advantage of model ensembles to quantify uncertainty about predictions and show how uncertainty in predictions varies with transmission setting, with simulations suggesting greater confidence in predictions of health effect for lower transmission settings than for higher ones. We discuss simulation results that show the choice of clinical endpoints used to assess the efficacy of vaccines in trials, especially for blood-stage vaccines, impacts the perception of the success of a vaccine. In addition, we show how ensemble models might be used to study new approaches for delivery of pre-erythrocytic vaccines like RTS,S, with results indicating mass vaccination strategies, even at modest coverage, substantially reduce transmission compared to immunization of infants alone and contribute to much greater health effects per dose. Our multifaceted approach to modelling and simulation of malaria vaccines, with multiple models for predictions at the population level and within-host level, offers not only a pragmatic way to predict their impact and cost-effectiveness, but also allows decision makers to appraise alternatives for delivery and for efficacy assessment to those considered in trials.

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MICRO-GEOGRAPHIC ENVIRONMENTAL RISK FACTORS FOR CHILDHOOD MALARIA DURING THE DRY SEASON IN LIWONDE, MALAWI, 2010

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Despite the importance of environmental factors to *Plasmodium* transmission and the re-emergence of spatial epidemiologic methods for studying malaria, the role of fine-scale environmental heterogeneity in rural locations has been relatively unexplored, particularly during periods of seasonally low transmission. During the dry season of 2010 in Liwonde, Malawi, children attending Machinga District Hospital's (MDH) under-5s clinic were studied for household environmental characteristics that predicted malaria. *P. falciparum* infection in children were determined using Parachek® Pf Rapid Diagnostic Test (RDT), with demographic and environmental data collection occurring at the residence. House location

and elevation were recorded with a global positioning system (Garmin eTrex Venture® HC), as were locations of water sources. Four distinct land cover categories, along with materials used in house construction, were assessed by direct observation. In multivariate logistic regression, children who lived within a 25 m radius of actively cultivated agricultural land were more likely to have malaria when compared to children who did not, after controlling for age and elevation (odds ratio = 2.39, 95% confidence interval: 1.12, 5.10). No spatial clustering or autocorrelation of malaria cases was found, perhaps indicating that increased risk from proximity to agriculture is independent of geographic location across the study region (Moran's I = -0.20, P value = 0.66). This study provides preliminary evidence of continued *Plasmodium* transmission during the dry season, and of various environmental factors that influence malaria risk in rural Malawi. The implications for attempting to eliminate malaria are explored.

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STANDARDIZING A NATIONAL MALARIA BULLETIN FOR TAPPING THE POTENTIAL OF ROUTINE HEALTH MANAGEMENT INFORMATION SYSTEM DATA IN AFRICA: PROCESS AND RESULTS FROM ZAMBIA

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For high-burden African countries, routine information systems offer many often untapped opportunities to present local-level information on malaria impact, logistics and service delivery. The information needs and opportunities of the Zambia National Malaria Control Programme are reviewed with respect to the routinely-reported health information system. A standard indicator set, methods of data collection, data systems for storage and most importantly, analysis and presentation of results were developed from national HMIS system data to support the information needs of an ever-complex malaria epidemiological situation over the period 2001-2010. The resulting national malaria bulletin serves, based on a consolidated District Health Information System 2.0 information system platform, as a model for malaria endemic-African countries to improve analytic capacity of the Ministry of Health and malaria control partners to promote best-practice malaria monitoring, evaluation, and surveillance for improved decision making for malaria control at national and local levels.

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USING CENSUS METHODS TO PREPARE FOR EVALUATING THE IMPACT OF UNIVERSAL SCALE-UP OF MALARIA INTERVENTIONS: CASE OF LIKOMA DISTRICT, MALAWI

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Malaria Indicator Surveys (MIS) and Demographic Health Surveys (DHS) are traditionally used to document coverage and impact of interventions on disease burden. Malawi carried out its first MIS in 2010, but sampling excluded Likoma Island, which is situated in Lake Malawi. The Malawi National Malaria Control Programme planned to pilot a campaign to achieve universal coverage of long-lasting insecticide treated nets (LLINs) in 2010 in Likoma district; comprising Likoma and Chizumula Islands. We conducted a population census during the low transmission season prior to distribution and tested all children under five for malaria parasites and severe anemia. A repeat of the enumeration and testing is planned after the campaign. The census was carried out using a shortened version of the MIS questionnaire programmed on personal digital assistants. We geo-referenced all households to analyze spatial patterns. We enumerated 2,189 households, which included a total population of 11,079 including 1330 under five years children. Insecticide-treated-net (ITN) coverage was higher on Likoma Island (64.9%) than the national estimate from the 2010 MIS (58.2%). ITN use among under five years old children, was lower (45.9%) than the 2010 MIS estimate (55.4%). ITN use among pregnant women was 35% compared to 49.4% in the 2010 MIS. Bio-marker samples were collected from 904 out of 1330 children (68.0%) aged 6 to 59 months, of which 835 (62.8%) were included in parasitological reading. Samples were not collected from over 30% of children due to frequent travel to the mainland. Malaria prevalence was 9.2% and severe anaemia (Hb < 8g/dl) prevalence was 7.5%. Spatial analyses are currently being conducted to assess disease clustering. As National Malaria Control Programmes is planning universal coverage of LLINs and other interventions, it is important to explore new and creative ways to best measure the impact of interventions. In locations with small confined populations, a population census that includes malaria parasite testing of all children under five provides important data on the spatial distribution of disease. We conducted this census in the dry season, when malaria parasite infections are more likely to be spatially clustered. When combined with health facility incidence data, census data such as ours can help elucidate disease dynamics and support operational research into how best to improve control.

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MALARIA CONTROL INTERVENTIONS: A COST-EFFECTIVENESS ANALYSIS

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With a human burden of more than 500 million people every year, the need for cost-effective malaria control is outstanding. The objective of malaria control is to significantly reduce the rate and number of cases of parasitic infections and clinical malaria. This study analyzes a number of control interventions and presents a template for decision makers looking to reduce the burden of malaria in a cost-effective manner.Twenty two malaria control interventions are analyzed. Cost data from WHO-CHOICE were used to calculate the current DALY value was calculated using twelve scenarios, differing in β (age weighting parameter), K (age weighting modulation factor) and r (rate of discounting). Standard life expectancy was calculated using WHO tables. Total current DALYs were calculated using the current DALY value and age group population data for each country from the United Census Bureau International Data Base. DALYs averted were then calculated by multiplying the coverage rate of an intervention and its efficacy with the total current DALY. Efficacy rates were determined using WHO cited literature but were varied to model three scenarios for each intervention. The cost-effectiveness of interventions was analyzed by considering the average cost per DALY averted. The most cost-effective intervention under every scenario is case management with artemisinin-based combination therapy; the least cost-effective intervention is insecticide-treated bed nets. Results from the cost-effectiveness analysis suggest that marked increases in funding for and supplies of insecticide treated bed nets may be misguided from a cost-effectiveness standpoint. Recent pushes to scale up artemisinin-based combination therapy policies fall in line with the findings of this study, especially in sub-Saharan Africa.

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UNEXPECTED PATTERNS OF PRIMARY SYMPTOMATIC MALARIA AND NON-MALARIA ILLNESS EMERGE IN A BIRTH COHORT OF GHANAIAN CHILDREN DESPITE WIDESPREAD USE OF INSECTICIDE-TREATED BEDNETS (ITNS) AND STANDARD ARTESUNATE COMBINATION THERAPY (ACT)

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To gauge the extent and impact of a national malaria prevention/control strategy based on insecticide-treated bednets (ITNs) and Artesunate Combination Therapy (ACT), all-cause illness, symptomatic malaria, and death was monitored in a cohort of 2,279 live births from March, 2006 to October, 2008, in the Kassena-Nankana District of northern Ghana. Ownership of ITNs in this cohort rose from 76% at 4 mos. to 95% at 16 mos. and malaria prevalence in young infants was 15% in households with an ITN compared to 31% in households without (OR: 2.7; 95% CI: 2.0, 3.6). Despite high ITN coverage and free, standard Artesunate + Amodiaquinetreatment for uncomplicated malaria, *Plasmodium falciparum* was detected in 44% (608/1,375) of all inpatient (IP) and 35% (6,327/18,223) of all outpatient (OP) visits. Several unexpected findings emerged: 1) Firstborns had a significantly lower incidence of primary symptomatic malaria and a longer time to this event than infants of multigravid mothers, but suffered a higher incidence of non-malaria illness; 2) Between the two dominant ethno-linguistic groups (Kassem and Nankam), onset of primary malaria illness in infants of Nankam ethnicity came two months earlier and infections amounted to an estimated 500 more OP malaria cases/thousand, but paradoxically, these children accrued grossly lower annualized rates of severe malaria and non-malaria illness requiring hospitalization; 3) More high parasitemias were seen in females, but males accounted for a significantly greater proportion of severe malaria anemia cases (2.6% vs. 1.9%; P = 0.04). Malaria was associated with 36% of cohort deaths that occurred in the hospital but case fatality rate for children with malaria was 2.5% compared to 5.7% (P = 0.02) for admissions with no detectable parasitemia. All symptomatic malaria was seen in the post-neonatal period, associated with 20% of IP deaths in infants, 53% of IP deaths in children >12 mos., and collectively in 36% of all IP deaths. Our cohort all-cause infant mortality rate of 37/thousand, which may owe ~20% of its deaths to malaria, is well below the infant mortality rate of 68/thousand for the Upper East Region, and the national rate of 71/thousand calculated by the Ghana Multiple Indicator Survey in 2006. These results, derived from real-life practices and outcomes in a rural community may be indicative of new trends in malaria prevention and child survival that are occurring widely in sub-Saharan Africa.

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ASSOCIATION BETWEEN VECTOR CONTROL COVERAGE, CLIMATE VARIABILITY AND THE SPATIAL DISTRIBUTION OF MALARIA AT THREE TIME POINTS IN ZAMBIA

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Three malaria indicator surveys (MIS) have been conducted in Zambia since 2006 to evaluate intervention scale-up. Coverage of insecticide-treated mosquito nets (ITNs) and indoor residual spraying (IRS) has increased since 2006. However, while malaria infection and anemia prevalence in children younger than 5 years dropped in 2008, results from 2010 indicate that levels have rebounded in several parts of the country. We sought to ascertain the relative effects of ITN coverage, IRS, and climate variability on the spatial distribution of malaria infection and anemia in 2006, 2008, and 2010. We fit Bayesian geostatistical models to assess the effect of intervention coverage on malaria infection and severe anemia prevalence, while adjusting for climatic and socioeconomic factors. We assessed the spatial dependence of disease distribution through time with spatial random effects for each survey. Model fit was conducted with Markov chain Monte Carlo simulation. Malaria infection and severe anemia prevalence rose from 2008 in six of nine provinces, and from 12% to 20% across rural areas nationally. Parasite prevalence increased by the largest percentage in Luapula (132%), Northern, (97%), and Eastern (137%) provinces. Household ITN possession fell 31% in Luapula province and 29% in Northern province, but remained constant in Eastern province. Parasite prevalence also increased in Central (by 19%), Western (96%), and Copperbelt (22%) provinces, even though ITN coverage also increased, by 45%, 121%, and 9%, respectively. 20-day cumulative rainfall estimates two months before each survey were positively associated with odds of malaria parasite infection; rainfall was highest preceding the 2010 survey, and lowest in 2008. In 2010, greater ITN age was associated with greater odds of malaria parasite infection. Spatial dependence increased with each survey year. These results suggest that a combination of climatic factors, lower ITN coverage, and ITN age contributed to the rebound in parasite infection prevalence in some areas. Unusual rainfall patterns in the early part of 2010, perhaps related to moderate El Niño conditions, may have contributed to this increase. We emphasize the importance of accounting for climate variability and spatial heterogeneity when using cross-sectional data for malaria evaluation efforts.

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FACTORS AFFECTING ACCESS TO ACT TREATMENT FOR UNCOMPLICATED MALARIA IN WESTERN KENYA

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Effective case management is central to reducing malaria mortality and morbidity worldwide, but only a minority of those affected by malaria, have access to prompt effective treatment. In Kenya, the treatment policy for malaria has changed from chloroquine (CQ) to sulphadoxine-pyrimethamine (SP) as the first-line antimalarial drug for uncomplicated malaria in 1998, and then from SP to artemisinin-based combination therapies (ACTs) in 2004. Despite this, these three classes of antimalarial drugs are being used by residents in endemic areas in Kenya; however the extent of ACT usage is unknown. We have surveyed 1,100 households of 5,775 individuals and 1,930 antimalarial prescriptions/treatments in three district in western Kenya in 2003 and 2010. We found that the SP and amodiaquine (AQ) based antimalarials accounted for 88% of prescriptions/treatments in 2003, 4% of the cases were treated with quinine (QN) and the rest were with CQ. Malaria treatment-seeking occurs mostly in the formal sector, i.e., government-run health centers and hospitals. In 2010, 58% of the cases were treated in government-run health centers/hospitals. Overall, only 60% of the antimalarials used in 2010 were first-line government recommended drugs. Shortage of ACTs in stock at government hospitals and clinics and less cost for CQ and SP drugs in private sectors are the major reasons for the patients to obtain them from the private sectors.

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HOUSEHOLD SURVEYS OF MALARIA INFECTION, FEVER AND ANEMIA IN A BIRTH COHORT OF GHANAIAN INFANTS REVEAL TRENDS IN ITN OWNERSHIP, BENEFIT AND PARENTAL ACTION IN SEEKING HEALTH CARE FOR THE BABY

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Prospective study of illness and death in a birth cohort of 2279 Ghanaian infants was complemented by cross-sectional surveys timed to correspond with the end of low (April-May) and high (Nov.-Dec.) malaria transmission seasons. Home visits during Oct.-Nov. 2006, when infant's age averaged 4 mos., found ITN ownership in 75% of households (range: 62-83%). Malaria prevalence associated with ITN ownership was 15%, ranging from 3% (town) to 21% (non-irrigated, rural). Malaria prevalence among infants lacking ITNs averaged 31% and ranged from 12.5% (town) to 42% (irrigated rural). Although ITN ownership was similar in the four ethnic groups (74-89%), malaria prevalence was significantly higher among Nankam infants (22%; P = 0.011). Fever was reported by mothers in 29% of *Plasmodium falciparum* infections but actually measured in 13.5% of these cases. Prevalence of shaking, diarrhea, vomiting, cough, or breathing difficulty was similar in infants with and without parasitemia but fever and anemia was more prevalent in children with *P. falciparum*. Records showed that only 27% of infants with parasitemia were brought to clinic for treatment within 7 days of the home visit. Severe anemia, fever and older age distinguished those brought to clinic. Analysis also revealed that malaria in these infants was associated with multiple maternal factors: older age, less education, no education, fewer ANC visits, and no use of ITN during pregnancy. By Oct.-Nov. 2007, when children averaged 15 months old, ITN ownership had increased to 95% but prevalence of *P. falciparum* infection was nearly twice greater than in the previous year, gametocyte carriage rate was more than doubled (9.6% vs. 3.7%; P < 0.001), high parasitemias >20,000/µL were four times more prevalent (13% vs. 3%; P < 0.0001), and anemia (Hb <8.0) had increased in both parasitemic (45.5% vs 10.3%; P < 0.0001) and parasite-free (15% vs. 5.2%; P < 0.0001) children. Records showed that 38% of children with parasitemia were brought to clinic for treatment within seven days of the home visit. Among 259 children with malaria who did not report there were 64 parasitemias >10,000/µL, 27 with fever, and 6 with severe anemia. It appears that the benefit of ITNs was offset by heightened immunological susceptibility of these young children.

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PERFORMANCE OF A RAPID DIAGNOSTIC CARD TEST FOR DETECTION OF SINGLE- OR MULTI-SPECIES *PLASMODIUM* INFECTIONS AMONG RESIDENTS OF SOUTHERN COAST PROVINCE, KENYA

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As part of a study of polyparasitism, we examined the utility of rapid diagnostic testing for classification of malaria infection status. Diagnostic cards are readily deployable but may fail to accurately identify all prevalent cases and may miss non-*falciparum* or multi-species *Plasmodium* infections. In our study, adult and pediatric samples were collected in two villages in Msambweni District, Kenya, and tested for malaria both by ICT card and multiplex PCR-Ligase Detection Reaction (LDR). LDR is an accurate and sensitive method to detect malaria that can discriminate among single or concurrent *P. falciparum*, *P. malariae*, *P. vivax* and *P. ovale* infection. By LDR, 38% of Milalani samples (267/704) and 19% of Nganja samples (92/481) were positive for at least one *Plasmodium* species. Of these positives, 44% and 48% were positive for *P. falciparum* alone in Milalani and Nganja respectively, 20% and 17% positive for *P. malariae* alone, 7% and 2% positive for *P. vivax*, and 7% and 4% for *P. ovale* alone. Pediatric cases were more common in Milalani (32%) than Nganja (23%, p=0.014), however Milalani sampling occurred in July 2009 (early dry season) and Nganja collection in April 2009 (mid wet season). The difference between villages in adult cases was not significant. As a screening tool, ICT cards (designed for detection of *P. falciparum*) had sensitivity of 43% for *falciparum*, 10% for *vivax* and 0% for ovale and malariae. Specificity was 99% for all four species. Specificity remained the same for *falciparum* in single *vs.* multi-species infections (99%), but sensitivity lowered to 29% when non-*falciparum* species were present. ICT positive predictive value (PPV) for *P. falciparum* was 86% and negative predictive value (NPV) was 90%. For single-species *P. malariae* infection, the PPV was 0% and NPV was 92%. PPV for isolated *P. vivax* was 15% and NPV was 98%, and PPV for *P. ovale* was 0% and NPV was 97%. We conclude that accurate attribution of infection-associated morbidities will require the more sensitive and comprehensive PCR approach to molecular detection of infection.

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INTERVENTION IS NOT ENOUGH: FUNCTIONAL SOCIAL NETWORKS ARE CRITICAL TO THE SUCCESS OF COMMUNITY LEVEL MALARIA CONTROL

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Despite recent trends showing a decrease in the malaria burden in Senegal, there is still substantial regional variation. In the Tambacounda region malaria accounts for 13.5% of all outpatient consultations and 22.4% of mortality; parasite prevalence in children under five is 23.4% compared to the national figure of 5.7%. Coverage with key interventions, including access to prompt and effective treatment with artemisinin combination therapy (ACT), is low. The population of Tambacounda is widely dispersed and access to health care is limited. The health system in Senegal provides community outreach services at the village level through health huts staffed by community health workers (CHW) and managed by village health committees (VHC). However, in Tambacounda most community level services are no longer functional. The Mobilise Against Malaria programme rehabilitated 24 non-functional health huts and implemented an intervention to improve access to prompt and effective malaria treatment in these health huts. Midterm household survey data found limited overall improvement in prompt and effective treatment of febrile children under five. Contextual data suggest several reasons for these results but monitoring data indicate that some health huts perform better than others despite sharing many contextual factors. We conducted case studies of 3 well performing and 3 poorly performing health huts to investigate the facilitating factors and barriers to their effective operation. Health huts were identified using a combination of objective and subjective functionality criteria (including treatment indicators, utilisation rates, ACT stock, completeness of reporting). In-depth interviews were conducted with the CHW and VHC of the 6 health huts as well as their district supervisors. The health hut system is centred on the CHW; however the CHWs are themselves at the centre of a complex network of social relationships that function to influence the success of the health hut as a community-level service provider. These critical relationships and the broader implications of the findings are discussed.

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MALARIA INFECTION IN KENYAN PREGNANT WOMEN IS ASSOCIATED WITH LOW FETAL AND NEONATAL BIRTH WEIGHTS

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Low birth weight is associated with malaria exposure in pregnancy; however, little is known about fetal growth *in utero* among malaria-exposed fetuses, which has implications for outcomes. We sought to evaluate fetal growth, birth weight and anthropometrics associated with malaria exposure among infants born to Kenyan pregnant women. From 2005 to 2007, pregnant women in Kenya were recruited at antenatal care (ANC) and those with term live births delivering at the study hospital who consented enrolled. Women received IPTp and were tested for malaria by microscopy at presentation and delivery. At least 1 fetal ultrasound (US) exam which measured fetal head circumference (HC), biparietal diameter, abdominal circumference, and femur length to generate fetal weight was performed. Fetal growth measures stratified by gestational age at first US were compared using t-tests between fetus/infants with or without evidence of maternal malaria infection at first ANC. At birth, neonatal weight, length and HC were obtained and tested between those with and without malaria infection at first ANC. 485 women were enrolled and 29 were malaria-positive at first ANC. Fetal weights were lower in fetuses of mothers with malaria infection in early pregnancy (<22 wks) compared to <22 wks fetuses of mothers without malaria infections (324 vs 395 g, respectively, p=0.04,). At > 23wks, while the estimated fetal weights were lower for malaria-exposed, no statistically significant differences were found. Birth weight of neonates born to mothers with vs. without evidence of malaria infection at first ANC was significantly lower, 2836 g (SD 397) vs 3019 g (SD 432), respectively, p=0.03. Neonatal HC and length were not significantly different between malaria exposed and malaria not exposed neonates. Our results suggest that infants exposed to malaria *in utero* had lower fetal and birth weights compared to infants born to mothers with no evidence of malaria infection. Given the small sample size, further research is needed.

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MALARIA AND ANEMIA PREVALENCE AND LONG LASTING INSECTICIDAL NET (LLIN) OWNERSHIP AND USE MEASURED IN REPRESENTATIVE HOUSEHOLD SURVEYS IN PLATEAU AND ABIA STATES, NIGERIA IN 2010

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There have been few recent surveys of malaria prevalence and net coverage in Nigeria. In September 2010, The Carter Center worked with the ministries of health of Abia (south east Nigeria) and Plateau (north central Nigeria) states to conduct a modified Malaria Indicator Survey. The survey was completed prior to mass LLIN distribution campaigns in these states. In 60 systematically selected clusters (census enumeration areas or segments thereof) of 25 households each per state, the average household size was 4.4 persons in Abia (1429 households, 5764 persons) and 6.2 in Plateau (1379 households, 8331 persons). Children <10 years of age in all households were tested for malaria and anemia, and all persons in every third household were tested for malaria. The percentage of households owning at least one net was much lower in Abia (7.2%) than Plateau (35.1%). The majority of nets observed were LLIN: 90.2% (N=147) in Abia and 93.2% (N=811) in Plateau. The percentage of persons using nets the previous night were: Abia: 2.9% of all ages, 5.5% of children under 5 years and 4.7% of pregnant women; Plateau: 15.5% of all ages, 20.7% of children under 5 years, and 24.7% of pregnant women. Despite lower net use, the overall crude malaria prevalence (by CareStart PAN/Pf RDT) was lower in Abia (36.2%, 95% CI 34.3-38.0%, N=2614) than in Plateau (45.2%, 95% CI 43.8-46.8%, N=4212). Age-adjusted prevalence was 29.7% in Abia and 36.9% in Plateau. Age specific prevalence peaked in the 5 to 9 year age group at 52% (95%CI 48.0-55.9%) in Abia and 61% (95% CI 58.3-63.8%) in Plateau, with second highest prevalence in the 10-14 year age group (Abia 48.9%,95% CI 42.2-55.6% ; Plateau 54.9%, 95% CI 50.0-59.8).The percentage of children <5 with anemia (hemoglobin < 8 g/dl) was higher in Abia (20.5%, 95% CI 17.7-23.5%, N=785) than Plateau (9.9%, 95% CI 8.3-11.6%, N=1367). The results indicate that malaria is highly prevalent in these two states, and that LLIN ownership is low. The national campaign now underway to provide 2 LLIN to every household in Nigeria is a welcome development.

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NOVEL STRATEGIES LEAD TO NEAR ELIMINATION OF MALARIA IN PREVIOUSLY HIGH-RISK AREAS IN SURINAME, SOUTH AMERICA

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Suriname was a high malaria risk country before the introduction of a new 5-year malaria control program in 2005, the Medical Mission Malaria Program (MM-MP). Malaria was endemic in the forested interior, where the stabile village communities were most affected. The interventions of the MM-MP included new strategies for prevention, case management, behavioral change communication (BCC)/ information, education and communication (IEC), and strengthening of the health system (surveillance, monitoring and evaluation and epidemic detection system). The interventions of the MM-MP are reviewed and related to the Performance Indicators established by the donor. The changes in the national malaria situation during the years of the MM-MP, based on analysis of the national databases, are discussed. After a slow first year with non-satisfying scores for the performance indicators, the MM-MP truly engaged in its intervention activities in 2006 and kept its performance up until the end of 2009. A total of 69.994 long-lasting insecticide treated nets were distributed and more than 15.000 nets re-/impregnated. Residual spraying was performed in high risk areas and over 10.000 people were screened with Active Case Detection in outbreak or high risk areas. Additional notification points were established and the national health system was strengthened. Malaria vector populations, monitored in sentinel sites, collapsed after 2006 and the number of national malaria cases decreased from 8618 in 2005 to 1509 in 2009. Malaria transmission risk has shifted from the stabile village communities to the mobile gold mining communities, especially those along the French Guiana border. The novel strategies for malaria control introduced in Suriname have led to a significant decrease in the national malaria burden. The challenge is to further reduce malaria using the available strategies as appropriate in the affected areas and populations. Elimination of malaria in the country will require a thorough understanding of transmission dynamics and a dedicated investment in key effective interventions.

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MALARIA FORECASTING: PAST WORK AND FUTURE DIRECTIONS

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Since 1911, when Christophers quantified the strong correlation between malaria incidence and rainfall, researchers have sought to discover other sources of spatial and temporal variability of malaria. The field of malaria incidence forecasting has incorporated predictors responsible for this variability although the approaches are quite diverse. We conducted a scoping review to summarize the heterogeneous field of malaria forecasting and describe the modeling approaches and methods of model evaluation. Two reviewers identified articles by using medical subject headings and key terms to search electronic databases and grey literature, including articles that presented models predicting human *Plasmodium falciparum* malaria incidence or prevalence. The initial search captured 213 different citations, 46 (22%) of which were reviewed. Most models predicted malaria incidence in Africa (72%), with 10 studies (23%) conducted in Kenya. Models mostly included two predictors (52%), which were the previous month's cases of malaria and rainfall. Typically, researchers used ARIMA-based models, which performed optimally within the first 3 months and evaluated using magnitude of correlation between predicted and observed incidence or prevalence. Nearly all malaria prediction models have narrowly focused on a small number of environmental predictors despite the importance of other malaria risk factors (land use, bednets, indoor residual spraying, drug resistance). To advance malaria prediction forecasting, we need more comprehensive models as coarse models will not provide the precision necessary to guide targeted intervention efforts.

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MONITORING MALARIA IMPLEMENTATION COST - THE CASE OF ARTEMETHER-LUMEFANTRINE

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Tanzania has adopted Artemether-Lumefantrine (Alu) as the first line treatment for malaria. INESS has introduced a series of studies in Rufiji and Kilombero/Ulanga Health and Demographic Surveillance System (HDSS) sites to evaluate safety and effectiveness of this drug and their related factors. The collection of treatment related costs has provided an opportunity to analyse the household level costs for getting malaria treatment. Here we report findings of assessment of treatment costs in Rufiji HDSS. Data were collected using household survey conducted in Rufiji DSS site. Members with recent fever from the sampled households were asked questions about professions, economic activities, treatment seeking, costs involved and day lost due to illness. 29.6% of patients interviewed reported to be actively engaged in income generating activities. Farming is the main economic activity in the area accounting for 76.3%. A daily median income in the study area was USD.1.4. However, 22.7% of patients and 59% of accompanying persons reported to have lost their income due to illness or escorting patients respectively. The number of days lost due to a single malaria episode ranged 1-14 days for patients. The direct medical costs (drugs, laboratory tests and consultation) were paid by 81% of patients with median payment of USD0.8. Equally, 11.5% of patients paid non medical cost (including food, lodging, telephone and gifts) with median payment of USD0.4. Only 10.4% of patients reported paying transport cost to get to health providers with median payment of USD1.0. A single malaria episode was observed to cost households more than their income. Alternative payment mechanism in the form of insurance should be considered. Strengthening and expanding coverage of community funds in rural areas could offer protection to rural households from paying more than they earn and when they are not able to work.

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FORECASTING AND MONITORING MALARIA RISK IN THE AMHARA REGION, ETHIOPIA

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Malaria is a major health problem in most sub-Saharan Africa countries, including Ethiopia. Mosquito populations and malaria risk are affected by environmental triggers, including rainfall, temperature, and humidity. The main objectives of this study are to compare alternative statistical forecasting models and quantify the lead time of satellite derived environmental variables with malaria outbreaks. Daily rainfall data were acquired from the Tropical Rainfall Measuring Mission (TRMM) with 0.25 degree spatial resolution. Land surface temperature (LST), normalized difference vegetation index (NDVI), and enhanced vegetation index (EVI) were derived from MODIS 8 day and 16 day composites with a 1 km spatial resolution. Actual evapotranspiration (ETa) was estimated by using the simplified surface energy balance method. Monthly malaria case data for the Amhara region were acquired from the Anti Malaria Association (AMA), Ethiopia. Satellite derived indices were aggregated at a monthly resolution to match malaria cases. Using the historical satellite and case data, we explored a variety of time series modeling approaches with combinations of the different variables. We used environmental variables with lags ranging from 1 to 6 months and examined the temporal cross correlations between outpatient malaria cases and environmental variables. The results showed that there were significant correlations based on a two standard error limit with rainfall at a lag of 3 months; nighttime LST and ETa at 1 month; and NDVI and EVI at 2 months. We found that rainfall and nighttime LST were the strongest predictors for malaria risk in the Amhara region of Ethiopia. Forecasts were validated using data withheld from the model fitting. The results showed that the models provide indications of future outbreaks with a lead time of 1 to 3 months. The findings can be used to enhance future operational Malaria Early Warning Systems in the Amhara region, Ethiopia.

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THE DYNAMICS OF NATURAL *PLASMODIUM FALCIPARUM* INFECTIONS

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Natural immunity to *Plasmodium falciparum* malaria has been widely studied, but its effects on parasite dynamics are poorly understood. Acquisition and clearance rates of untreated infections are key elements of the dynamics of malaria, but estimates of these quantities for endemic areas is challenging because of frequent super-infection and imperfect detectibility of parasites. Consequently, information on the effects of host immune status or age on these parameters is fragmentary. An age-stratified cohort of 349 individuals from Northern Ghana were sampled six times at 2 month intervals. High-throughput capillary electrophoresis (CE) was used to genotype the msp-2 locus of all detectible *P. falciparum* infections. Force of infection (FOI) and duration were estimated for each age group using an immigration-death model that allows for imperfect detection. Effects of naturally acquired immunity on the FOI and duration should be reflected in age dependence in these indices, but FOI tends to increase with age, and persistence and chronicity of individual parasite clones is characteristic of all age-groups. Duration peaked in 5-9 year old children, (average duration 319 days, 95% confidence interval 318;320).

FOI tended to increase with age. The estimated multiplicity of infection (MOI) was considerably higher than the observed number of clones, especially in older ages. The main age-dependence is on parasite densities, and acquired immunity therefore appears to control transmission mainly by limiting parasite densities in the human circulation.

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MALARIA PARASITE GAMETOCYTEMIA IN A MIXED MALARIA SPECIES HYPOENDEMIC IN PERU: ENVIRONMENTAL FACTORS AND GENETIC MARKERS THAT PREDICT EFFICIENT *PLASMODIUM FALCIPARUM* TRANSMISSION

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Malaria parasites must convert to its sexual stage for transmission from human to mosquito. Factors regulating if asexual stage trophozoites switch to the sexual stage gametocytes are not known, but in *Plasmodium falciparum* (PF) it is clearly not a linear function of within host trophozoite density. The rate of conversion might be attributable to environmental factors and/or a heritable genetic trait. We have a study in Peru where a high proportion of infections have gametocytes despite overall low transmission, enabling us to study the density and the proportion of gametocytes to trophozoites. In active and passive case detection of c.1,900 individuals from 2003-2007, we detected 456 PF and 956 *P. vivax* (PV) infections. This study focus was PF. We surveyed for PV by microscopy to determine mixed species infections. We also considered febrile illness, age (>14.5 considered adult), and hematocrit levels as they relate to gametocytaemia. We genetically characterized PF parasites using 14 microsatellite (MS) markers. The proportion of infections with gametocytaemia ranged from 18% (year-2004) to 38% (2007). In the adults not in children, there was a stepwise increase in proportion with gametocytes each year. Gametocyte densities were higher in mixed species infections. Using the program Structure, we found six families of related parasites based on the MS markers. One of the six clusters included 56% of infections with gametocytemia. Moreover, with principle component analysis we found that PF infections with a ratio of trophozoite to gametocyte density was >25% were caused by a genetically distinct group of PF parasites. Therefore, in addition to environmental factors for conversion there appears a genetic signature for parasites with high gametocyte conversion in our study population. We are determining if there is a genetic association with PF gametocytemia independent of environment and antibody responses. Our work provides insight into the transmission of PF in Peru and suggests that eradication campaigns could create a reservoir of more transmissible PF parasites.

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SOCIAL DETERMINANTS OF ASYMPTOMATIC MALARIA ANTIGENEMIA IN TROPICAL AFRICA

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In the context of intensifying efforts toward global malaria eradication, understanding social patterns of malaria transmission from asymptomatic populations in endemic zones will become increasingly important. Social determinants of health have previously been linked with child and maternal mortality, but not to asymptomatic malaria carriage. We conducted a cross-sectional study of afebrile, healthy children aged 2 months to 14 years attending well-child and/or immunization visits in the North Kivu province in eastern Democratic Republic of Congo. A total of 656 children across three villages were tested for malaria antigenemia by rapid diagnostic test and parents simultaneously completed survey questionnaires related to demographics, socio-economic status, maternal education, as well as bednet use and recent febrile illness. 19% of children were parasitemic (11%, 22% and 23% in Goma, Butembo and Beni, respectively; p=0.009). Increasing levels of maternal education were associated with a lower risk of malaria antigenemia in their children (p=0.001). Children from households with higher numbers of children under 5 years of age were also more likely to be parasitemic (p=0.035). On the other hand, socio-economic index was not statistically associated with malaria antigenemia (p=0.27). In a multivariable model adjusting for age, recent febrile illness, and bednet use, maternal education and number of children under five in the household remained significant predictors of malaria antigenemia. In summary, children of mothers with low education level and living in households with large numbers of young children appear to be at higher risk of asymptomatic malaria carriage. Social determinants of malaria parasitemia may influence transmission patterns and may be useful tools for targeted control efforts.

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LARGE DECLINE IN *PLASMODIUM FALCIPARUM* AND *P. MALARIAE* INFECTION RATES AMONG WOMEN AND THEIR OFFSPRING IN A COMMUNITY ON THE SOUTH KENYAN COAST FROM 1997-2010

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Expanded malaria control initiatives in Kenya include intermittent preventive treatment during pregnancy, insecticide treated bed-net usage, and availability of artemisinin combination therapies for case management of malaria illness has resulted in dramatic decreases in reported hospital admission for severe malaria. The overall malaria infection rates have presumably decreased as well, however no studies have reported longitudinal malaria infection rates in the same population of women and young children, using sensitive and specific molecular diagnostic methods over the past decade of malaria control interventions in Kenya. Randomly selected archived blood samples obtained from four birth cohorts of pregnant women and their children (starting in 1997 [n=54], 2000 [n=174], 2006 [n=444], 2009 [n=76]) enrolled from the Msambweni District hospital located in Coast Province, Kenya were examined by the same PCR- based molecular diagnostic assays for infection with the four human malaria parasites. Maternal *Plasmodium falciparum (Pf)* infections at delivery (1997 and 2000 cohorts where malaria chemoprophylaxis was infrequently used) and at first antenatal clinic visit (2006 and 2009) were 37%, 40%, 15% and 17% respectively. *P. malariae (Pm)* infections in mothers also declined from a peak of 12% in 2000 to 8% in 2009. Peak *Pf* and *Pm* malaria infection rates in children occurred at 30-36 months of age in all cohorts. At this age infections rates with *Pf* declined from 58% in 2000 to 10% in 2006. Similarly *Pm* declined from 9% to 3% over the same period. Peak *P. ovale* infections remained at ~2% and *P. vivax* was not observed in the cohorts. These results show a profound reduction in malaria transmission consistent with an overall reduction in the burden of malaria in the coastal regions of Kenya.

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PLASMODIUM SPECIES UNDETECTED BY *PLASMODIUM FALCIPARUM* SPECIFIC RAPID DIAGNOSTIC TESTS AMONG FEVER PATIENTS IN ZANZIBAR

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Malaria incidence has decreased markedly in Zanzibar, and the island is considering elimination. However, non-*falciparum* malaria may be particularly difficult to eliminate. Importantly, the routine diagnostic standard in Zanzibar has been a rapid diagnostic test (RDT) based on histidine-rich protein-2, which is produced by *Plasmodium falciparum* but not other malaria species. The use of *P. falciparum* specific RDTs therefore may prevent comprehensive malaria case finding in Zanzibar. We report species data on RDT negative, PCR positive malaria cases in Zanzibar during the 2010 rainy season. We collected blood on filter paper from a random sample of febrile patients who tested negative for malaria by RDT in 6 primary health care facilities in North A and Micheweni District, respectively (N = 595). DNA was extracted with Chelex from dried blood spots in pools of 10, and nested PCR targeting the *cytochrome b* gene was performed. Samples from positive pools were reextracted individually. An *AluI* restriction digest and gel electrophoresis were then performed on positive individual samples for species identification. Of the 595 RDT negative samples, 13 (2%) were positive by PCR. Nine out of the 13 positives (69%) were identified as *P. falciparum*, 3/13 (23%) as *P. malariae*, and 1/13 (8%) as *P. vivax.* Three of 4 subjects with non-*falciparum* infections were from Micheweni and the remaining subject was from North A. This study is the first, to our knowledge, to report *P. vivax* in Zanzibar. With improved control of *P. falciparum* infection on the island and with challenges to control of other species, infection with non-*falciparum* species may make up an increasing proportion of malaria cases on Zanzibar. In this context RDTs including both *P. falciparum* specific and pan-species specific antigens should be considered for improved overall malaria case detection and to ensure that elimination efforts are comprehensive.

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RAPID DEVELOPMENT OF *PLASMODIUM FALCIPARUM* STERILE, INFECTION BLOCKING IMMUNITY OVER SUCCESSIVE INFECTIONS IN PERU SELECTING FOR PARASITE GENETIC DIVERSITY

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The malaria paradigm is that resisting symptomatic and high-density *Plasmodium falciparum* (PF) infections requires years of frequent exposure to develop and continued exposure to maintain. In contrast, in a setting of recent and low transmission in Peru, we see a rapid onset of clinical and anti-parasite immunity. Immunity might be related to the PF genetic diversity. We followed 456 individuals who had a PF infection in one year between 2003 and 2007 and were in our active and passive case surveillance in the following year. We genotyped parasites using the Merozoite Surface Protein-1 (MSP-1 B2) and using 14 MS markers scattered across the 14 PF chromosomes. Considering the 175 individuals who had two infections in these two years, the probability of febrile illness was 85% in the first and 60% in the second infection (p=0.012). The probability of not detecting an infection in the second year (despite having at least 6 active detection visits to find even asymptomatic infection) ranged from 21 to 62%, increasing with number of infections prior to these two successive infections. Febrile illness was associated with having a different MSP1-B2 genotype in the second infection (p=0.041). To further consider genetic diversity and immunity, we genetically characterized 303 PF infections using the MS markers. The overall genetic diversity increased over time, with parasites in the later years having markedly different MS haplotypes. We calculated the number of genetic differences between the first and second infection in each individual. MS markers are not considered under immune selection, but in low transmission they would indicate potential allelic differences in antigens in linkage disequilibrium with (nearby) the MS markers. Infections spaced by <18 months (mos) had an average of 5.3 (se: 0.3) differences. Infections spaced by >=18 mos had an average of 4.0 (se: 0.3) differences. The significant association with time separating infections suggested that parasites causing reinfection within 18 mos had to be more genetically different than those occurring after the immune response might no longer exert a selective pressure against a similar parasite reinfecting. Other tests of genetic differences in reinfections versus differences expected by chance indicated selection for parasite diversity. Our results suggest immunity develops and at least some of this immunity is directed to antigens that have different allelic forms in this population.

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INCUBATION PERIOD OF *PLASMODIUM FALCIPARUM* MALARIA IN ADULT TRAVELERS IN THE UNITED KINGDOM

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The incubation period (from infection to onset of symptoms) for *Plasmodium falciparum* malaria has been shown to vary widely from days to months in adults. Estimation of the incubation period distribution informs both diagnosis criteria in non-endemic areas and the design of passive surveillance in areas where local elimination is been achieved. Data on the duration of holiday, date of arrival in the UK and date of onset of symptoms for 413 adults (over 16 years old) with *P. falciparum* malaria reporting to the Infectious Diseases Unit at Northwick Park Hospital, London, UK between April 1991 to May 2006 were analysed. The mean incubation period was estimated using interval censored survival analysis assuming a Gamma distributional form. The role of self-reported previous malaria, antimalarial use, severity of disease, age and ethnic origin (a key determinant of severity in this population) was investigated. 17% of cases had onset of symptoms prior to arriving in the UK, 40% became ill within the first week of arrival and 5% reported first symptoms more than a month after arrival. The mean incubation period was 20 days (95% confidence interval 11-41days). Important determinants of the incubation period ($p<0.05$) were self reporting of previous malaria (mean 17 days for no previous malaria, 23 days with previous malaria) and severity of disease (mean 16 days in patients with severe disease, 21 days in other patients). This analysis has been performed in a group of adults resident in the UK and abroad who had severe enough symptoms to report to hospital, and these estimates may therefore be biased. Severity is associated with a short incubation period in these patients, and therefore incubation periods may be much longer for mild cases and in immune populations. The degree of variability in incubation periods has only rarely been reported and therefore this study gives useful insights for both the UK and international context.

1395

HIGHER MALARIA PREVALENCE IN CHILDREN FIVE YEARS AND ABOVE IN LAGOS, NIGERIA

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Malaria is almost invariably ranked as the leading cause of morbidity and mortality in Africa. There is growing evidence of a decline in malaria transmission, morbidity and mortality over the last decades especially in Africa. Reports on malaria prevalence in children in Nigeria are divergent in the literatures. This study therefore reports the trend in malaria prevalence in febrile children in Lagos, Nigeria. This study was conducted at the St. Kizito Primary Health Centre, Lekki, and Massey Street Children's Hospital Lagos State, Southwestern Nigeria. This was a cross-sectional study with a total of 1,211 children, aged 0-12years who presented with fever or history of fever in the last 24 hours at the Outpatient's Department of the Clinics. Among the children tested, 658(54.4%) were males and 553(45.6%) were females. Of the total children tested microscopically, 251 (20.7%) were positive for malaria parasites. Children >1-12years in Age Group III had a malaria prevalence of 25.8%, 11.0% in the Age Group I (0-≤1year) (p=0.001), 16.9% in 0-≤5years and 42.1% in >5-12years in Age Group II (p=0.001). The highest mean parasite density (43,097.6 p/µl) was reported in Age Group III (>1-12years). Most of the malaria positive children (33.9%) had parasite density between 1-500p/µl. There was no significant association in monthly malaria prevalence in the studied children. This study reported a decline in malaria prevalence, which may be attributed to large-scale implementation of malaria interventions. There was a shift in malaria prevalence from the well reported prevalence in 0-<5years to >5-12years. The shift in malaria prevalence to >5-12 year olds may reflect successful implementation of malaria control interventions in under-fives, and underscored the need to extend such interventions to older children and indeed implement universal target in malaria control.

1396

OCCURRENCE AND PATTERN OF IMPORTED MALARIA CASES IN RIO DE JANEIRO, A NON-ENDEMIC STATE IN THE BRAZILIAN EXTRA-AMAZONIAN REGION

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In Brazil, almost all (99.4%) of malaria reported cases are seen in the Amazon region where *P.vivax* accounts for 82.2% of them. The low specificity of initial clinical presentation, overlapped with other acute febrile diseases and the potential risk of severe malaria represents an extra challenge for unaffected regions. The high lethality rates of malaria in the extra-Amazon (70.8 times higher than in the Amazon region) drew the attention of authorities. A retrospective study was carried out from Jan/07 to Apr/11 in a Reference Center that handles 23% of the malaria cases seen in Rio de Janeiro. From the total of 291 admitted malaria suspect patients, 83(28.5%) had the confirmed diagnosis of malaria. The distribution according to *Plasmodium* species was 58 (69.9%) *P. vivax*, 18(21.7%) *P. falciparum*, 3(3.6%) *P. malariae* or *P. ovale* and 4 (4.8%) *P. vivax/P. falciparum* co-infection. Most of suspected cases were primo-infected men over 25 years old and the total lethality rate was 2.4%. Africa or Asia countries were the likely source of infection for 33(37.5%) between 2007-08 and 77(38.1%) between 2009-11 (OR:1.04; IC:0.59-1.82%). This pattern of imported malaria in Rio de Janeiro is different from the one recorded in the Amazon region and may result from the recent Brazilian economic growth that has increased the presence of construction, mining and oil companies in endemic areas as well as the presence of Brazilian enterprises in major international project, especially in Africa. The authors note the recent increase in the absolute number of suspect cases and in the percentage of *P. falciparum* infections, with annual increment ranging from 15-44%, in the this non endemic region. Although the total number is modest, when compared with those recorded in endemic areas in Brazil, two challenges are set for this scenario to avoid increase in morbidity and mortality: the difficulty of rapid and accurate diagnosis; and the training on the management of potentially severe malaria including multidrug resistant *P. falciparum*, which is extremely different from the observed in the Amazon region.

1397

CHARACTERISTICS OF THE INFECTIONS BY *PLASMODIUM* SPP. DETECTED BY ACTIVE SEARCH OF CASES IN FEBRILE AND NON-FEBRILE INDIVIDUALS OF THREE ENDEMIC COMMUNITIES IN OLANCHO, HONDURAS, SEPTEMBER 2010

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Public health agencies in Honduras implement malaria control activities by responding to reports of active cases. Previous investigations suggest that subclinical cases of malaria exist in communities throughout the country, but these studies were limited in scope. Failure of health authorities to include subclinical cases in planning malaria interdiction efforts minimizes the effectiveness of control programs. In this study, investigators performed malariometeric surveys to estimate the frequency of subclinical cases in populations of three semi-rural communities located in the Department of Olancho, Honduras. Medical teams visited 30 homes in the communities of Sosa Lobo, Chacon and Villa Antonia, Olancho, to perform the survey. The visits took place from August 29 - September 2, 2010. Participants were interviewed and a cursory physical exam was carried out. Blood samples were taken using finger sticks and a rapid diagnostic tests (RDT) was performed during home visits. Additionally, thick film slides were prepared for microscope evacuation and dried blood filter paper collections were used for evaluating malaria parasites for polymorphic molecular markers. Seventy-one individuals participate in the survey with 19 participants experiencing an episode of febrile illness (26.7%) within 30 days period prior to the home visit. Technicians detected one *Plasmodium vivax* (1.4%) positive when evaluating specimens with RDT. Subsequent microscopic evaluation of the thick films resulted in the detection of four additional cases of *P. vivax* (7.0%). Two of the participants that were thick film positive (2.8%) did not have a clinical history suggestive of malarial infection and could contribute to the persistence of transmission in their communities. The molecular analysis of parasite genomic material from the five positive specimens detected one genotype based on DNA fragment size: markers MSP1 5/6 (~380bp), CSP (~600bp) and GAM1 (~500bp). The analyzed samples demonstrated genetic homogeneity and an absence of polyclonal infections.

1398

ECONOMICAL RABIES POST-EXPOSURE PROPHYLAXIS: A SIMPLIFIED 4-SITE INTRADERMAL 3-VISIT REGIMEN ON DAYS 0, 7 AND 28

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Human encephalitis caused by a dog rabies virus remains 100% fatal, although on rare occasions, patients have recovered from infection with less pathogenic American bat rabies viruses. Post-exposure prophylaxis is often unaffordable or unavailable in Asia and Africa. The intradermal (ID)

route of vaccination has immunological and economical advantages over IM. Two multiple-site ID regimens (8-site and 2-site) requiring <40% of the standard vaccine have been recommended by WHO for 14 years. They are used successfully in a few places in Asia, but rarely in rural areas where 80% of rabies occurs. ID vaccination is practically unknown in Africa. Pharmaceutical and practical difficulties inhibit ID use together with failure to explain and promote the regimens. A new simplified 4-site ID vaccine regimen could overcome many of these problems (Warrell MJ et al. PLoS NTD 2008; 2(4):e224). The regimen employs the same doses of vaccine and the same schedule as the 8-site method, but can be used with rabies vaccines reconstituted to volumes of 1 ml or 0.5 ml per ampoule. The 4-site regimen consists of a whole ampoule of vaccine divided between 4 ID sites, one on each limb on day 0. Two ID injections are given on day 7 and one on day 28. The ID dose is 0.2 ml or 0.1 ml for vaccines of 1 ml or 0.5 ml per ampoule respectively. The 4-site regimen is as immunogenic as the 'gold standard' 5 dose IM course of vaccine. It requires a total of less than 2 ampoules, only 3 clinic visits and does not rely on expert ID injection technique and so is safer in inexperienced hands. It meets all the WHO requirements and is very economical for both the clinic and patients compared with all other regimens (Hampson K et al. PLoS NTD 2011; 5(3):e982 Note extra data in 'Comments'). Rabies vaccines do not contain a preservative and are not licensed as multi-dose ampoules unless authorised by a national regulatory body. If ampoules of vaccine are shared, ID rabies vaccination is given 'on the doctor's responsibility' in most countries. However without any sharing of ampoules, the 4-site regimen is economical even if only one person is treated: using a total of 3 doses with some wastage. The method is applicable globally wherever financial resources or vaccine supplies are limited or if the number of clinic visits is critical.

1399

DETECTION AND GENOTYPING OF HUMAN ASTROVIRUS IN NEPAL USING REAL-TIME REVERSE TRANSCRIPTASE POLYMERASE CHAIN REACTION

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Human astroviruses (HuAstVs) cause gastrointestinal disease; eight (1-8) classic human astrovirus (AstV-canonical) serotypes are responsible for acute, nonbacterial diarrhea in children. Recently, two new human astrovirus variants (MLB and VA) have been described in humans. We detected HuAstVs by a real-time reverse transcription-polymerase chain reaction (RT-PCR). One step TaqMan multiplex real-time RT-PCR assays were developed to broadly detect HuAstVs. The most conserved sequence between ORF1 coding for the non-structural protein and ORF2 encoding for capsid protein junction was chosen as the assay target. Three sets of primer and probe were designed with specificity for AstV 1-8, AstV-MLB1- 2, and AstV-VA1-3. Primers were optimized with known positive specimens identified by RT-PCR from a previous study using SYBR Green based real-time RT-PCR. TaqMan multiplex RT-PCR assay was applied to screen stool samples with no previously identified enteric pathogens by standard microbiology for enteric bacteria; EIA for rotavirus, adenovirus, astrovirus, giardia and cryptosporidium; or RT-PCR for norovirus and rotavirus. These stool samples were collected from children aged 3 months to 5 years with diarrhea and non-diarrhea controls in a hospital based study in Nepal. All positive samples were tested for HuAstV typing by individual sets of primers and probe. A total of 634 stool samples, 284 cases and 350 controls, were screened. HuAstVs were detected in 9/284 (3.2%) of cases and 9/350 (2.6%) of controls. All 9 samples from cases were identified as AstV-canonical. On further testing of the samples from controls, seven were identified as AstV-canonical, one as AstV-MLB and one as AstV-VA, respectively. Our results indicated that a real-time RT-PCR assays can be used to detect and genotype human astroviruses.

1400

KINETICS OF CHIKUNGUNYA INFECTION DURING AN OUTBREAK IN SOUTHERN THAILAND, 2009

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The Indian Ocean Chikunguna epidemic reemerged in Southern Thailand in September of 2009. We enrolled forty-five adults with laboratory confirmed chikungunya. Serial blood collections and clinical assessments were performed every two-three days through the acute and convalescent phase of the disease until day 30. Patient symptoms were recorded and antibody responses with viral kinetics were evaluated using PCR and serological assays. The patients mean age was 49 years with a male to female ratio of 1:1.4. Thirty-five (77.8 %) patients were rubber planters. All patients experienced joint pain and 42 (93%) of them involved more than one joint. Interphalangeal joints were the most common affected in 41 (91%) patients. The mean duration of severe joint pain was 5.8 days with 11 (25%) experiencing discomfort through the duration of the study. Rash was observed in 37 (82%) patients, a mean 3.5 days after the onset of symptoms. Patients were positive by PCR for a mean of 5.9 days and the peak viremia was at day 5 with 6.24 log PFU/ml. IgM antibodies appeared on day 4 and peaked at day 7. IgG antibodies first appeared at day 5 and rose steadily through day 24. The understanding of chikungunya disease clinical manifestation, antibody responses and viral kinetics are important for diagnosis and treatment of the disease.

1401

PRE-CLINICAL AND CLINICAL DEVELOPMENT OF A VACCINE FOR THE PREVENTION OF HAND FOOT AND MOUTH DISEASE CAUSED BY ENTEROVIRUS 71

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Hand Foot and Mouth Disease (HFMD) is caused by viral pathogens of the enterovirus genus such as enterovirus 71 (EV71) or Coxsackie A16 (CA16). HFMD is generally a self-limiting disease characterised by fever, small blisters in the mouth, and a rash with blisters. However in a small number of cases, HFMD caused by EV71 can lead to viral meningitis, encephalitis, interstitial pneumonitis or poliomyelitis-like paralysis, and may be fatal. The disease can infect any age group, but is rare in children over the age of 10. EV71 and HFMD are endemic in the Asia Pacific region causing millions of cases in recent years. We are developing a well characterized, multiple dose, highly purified, inactivated EV71 vaccine formulated with alum adjuvant. Pre-clinical studies have shown that this vaccine is highly immunogenic and generates strong neutralizing antibody responses in mice, rats and rabbits. We also have demonstrated that these antibodies are capable of cross neutralizing EV71 sub-genogroups currently circulating in Asia. A double blind, placebo controlled Phase 1 clinical study is being conducted in Singapore to assess the safety and immunogenicity of two different dose levels of the inactivated EV71 vaccine in healthy adults. An update of the clinical trial data will be presented.

1402

SENTINEL SURVEILLANCE FOR INFLUENZA IN PHRAMONGKUTKLAO HOSPITAL IN BANGKOK THAILAND

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Phramongkutklao (PMK) Hospital with the Armed Forces Research Institute of Medical Sciences conducted surveillance to identify, characterize and determine the prevalence of influenza and other respiratory pathogens in Bangkok. Patients ≥6 months who meet the criteria of influenza-like illness (fever ≤38ºC and cough or sore throat within 3 days of onset), without tuberculosis and who were not immunocompromised were eligible. History, physical examination, and nasal swabs for rapid kits were performed. Throat swabs were sent for PCR and viral isolation/characterization. 919 subjects were enrolled, 688 (75%) were ≤ 18 years old; 319 (35%) subjects tested positive for influenza by PCR (72% for influenza A). The pandemic strain was the most prevalent (197 cases). The first peak of influenza occurred in Jan/Feb 2010 with >40% of the cases testing positive, the majority were the pandemic strain. The second peak occurred in Aug 2010 with 56% testing positive; the pandemic strain remained dominant, but influenza B accounted for nearly 40% of the cases. There were 106 admissions; 19% were influenza and none had received influenza vaccine. Of 863 subjects reporting vaccination status only 113 had received it within the previous 12 months, and 27 were influenza positive (17 with the pandemic strain). HA gene sequencing on selective samples revealed that the strains in circulation were similar to the 2009 southern hemisphere vaccine strains. In conclusion, influenza is a significant cause of morbidity at PMK Hospital. The population was largely unvaccinated and the majority of influenza cases were caused by the pandemic strain. Vaccination would likely significantly reduce morbidity.

1403

A CHIKUNGUNYA VIRUS HIGH FIDELITY VARIANT LOSES FITNESS IN MOSQUITOES AND MICE

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The error rate of RNA dependent RNA polymerases (RdRp) strongly affects the mutation frequency in a population of viral RNAs. Previously, we used a high fidelity variant of an RNA virus (poliovirus) to illustrate the importance that mutation frequency plays in virus fitness and adaptability *in vivo*. Since arboviruses replicate within very different hosts, the need to generate such genetic diversity may be even more significant than for single ost RNA viruses. Using chikungunya virus (CHIKV), we describe an arbovirus fidelity variant isolated in mutagen treatment with a single C483Y amino acid change in the NSP4 RdRp gene that increases replication fidelity. The increase in fidelity does not have significant costs in terms of replication and RNA synthesis, but shows significant fitness costs *in vivo*. Compared to wild type CHIKV, the higher fidelity population presents reduced infection and dissemination in mosquitoes. Furthermore, viremias in newborn mice are truncated and organ titers are significantly lower. These results indicate that increased replication fidelity and reduced genetic diversity negatively impact arbovirus fitness in invertebrate and vertebrate hosts, a factor that may explain why RNA viruses maintain error-prone RdRp genes.

1404

SMALL MOLECULE INHIBITORS OF VENEZUELAN EQUINE ENCEPHALITIS VIRUS INFECTION IDENTIFIED USING HIGH THROUGHPUT PHENOTYPIC SCREENING OF THE VACCINE STRAIN TC83

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Currently, there is no approved vaccine or therapeutic for prevention or treatment of infection by Venezuelan equine encephalitis virus (VEEV) in humans. Antiviral therapeutics are highly desired for treatment of VEEV infection. We have developed a screening assay in 96- and 1536-well formats to identify antiviral compounds against TC-83, a BSL-2 live attenuated vaccine strain (IND stage) of VEEV. Briefly, primate kidney Vero cells are infected with TC-83 and after 48 hours, cell viability is assessed by measuring cellular ATP levels. The assay was miniaturized to 1536-well plate format and screened against 7,243 unique bioactive small molecules to identify 33 initial actives. The activities of a subset of compounds have been tested in orthogonal *in vitro* assays against VEEV TC-83, VEEV Trinidad (TrD) strains and additionally EEEV and WEEV. We have demonstrated that the approach of using authentic virus, even a surrogate vaccine strain can identify inhibitory compounds against VEEV. Furthermore, the approach can identify molecular targets critical for VEEV infection that could be further developed for human-use antiviral drugs.

1405

BURDEN AND EPIDEMIOLOGY OF ROTAVIRUS DIARRHEA: RESULTS OF A PREVALENCE STUDY IN NIGER

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Diarrhea is still the second leading cause of death in children under 5 years of age in developing countries, representing nearly one in five child deaths. Rotavirus is the most common etiologic agent of severe diarrhea and vaccines are readily available. However, knowledge about the disease burden as well the circulating strains is still lacking in many countries to make informed decisions about vaccine introduction. The regional hospital and ten health centers of Maradi region, and the three main hospitals in Niamey, Niger, were selected for a one-year prevalence study. Stool samples were collected from all children under 5 having diarrhea with moderate or severe dehydration and tested for the presence of rotavirus using a rapid diagnostic test. Genotyping was performed on a subset of rotavirus positive stools. In addition, a sample of the collected stool was used for bacteriology analyses in Maradi. From December 2009 until December 2010, 5247 children were included in the study. The median age was 9 months [IQR: 7-11 months]. Overall, the proportion of rotavirus positive diarrhea was 26.8% (95% CI: 25.6-28.0) and varied monthly from 10.6% (95% CI: 7.6-13.6) in May to 48.5% (95% CI: 44.7-52.4) in November. Around 65% of the rotavirus cases were children 6 to 12 months old. The most frequent genotypes were G2P[6], G2P[4] and G1P[8]. Coprocultures performed on stools from 1988 children showed *Salmonella* spp. in 10.5% of the cases, *Campylobacter jejuni* in 8.1%, *Shigella* spp. in 3.0%. More than 10% of the *Salmonella* spp. identified carried an extended-spectrum beta-lactamase. This study points out that rotavirus is a major cause of diarrhea with dehydration in Niger, particularly in young children under 1 year of age, and during the cool and dry season. The variety of circulating genotypes in Africa should

be taken into consideration for the development of better adapted vaccines. Bacterial pathogens are also responsible for an important part of diarrhea, and point to the emergence of resistance to third generation cephalosporin.

1406

SYNERGISTIC ACTION OF ROTAVIRUS AND COINFECTING PATHOGENS: EVIDENCE FROM A COMMUNITY-BASED CASE CONTROL STUDY IN NORTHWESTERN ECUADOR

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Diarrheal disease is a leading cause of morbidity in children under five. In developing countries, where diarrheal disease burden is greatest, enteric coinfection is common. There is little understanding, however, of the biological interaction between coinfecting pathogens. We investigated the potential for synergistic action by coinfecting pathogens on diarrhea pathogenesis using an epidemiological framework. We conducted a community-based case control study in 22 villages in northwestern Ecuador. Risk ratios of diarrhea associated with single and coinfections were estimated. Biological interaction of coinfecting pathogens was assessed through the interaction contrast ratio (ICR) and departure of the risk ratios from multiplicativity (MD). After adjusting for age, we found both departure from risk difference additivity and departure from risk ratio multiplicativity in the effects of rotavirus coinfections on acute diarrhea. The ICR was 11.9 (95% CI = 4.7-29.9) for rotavirus-*Giardia* coinfections, and 22.9 (95% CI = 10.5-46.8) for rotavirus-*E. coli/Shigella* coinfections. The MD for these coinfections was 11.8 (95% CI = 3.9-29.4) and 20.1 (95% CI = 2.2-44.2), respectively. This research provides epidemiological evidence for biological synergism between rotavirus and other enteric pathogens. During coinfection, the pathogenic potential of each organism appears to be enhanced.

1407

CREATING A PIPELINE FOR NEXT GEN SEQUENCING OF EBOLAVIRUS ZAIRE

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With Next Generation sequencing becoming a more cost-effective method of sequencing full-length microbial genomes, established pipelines for sequencing pathogens of high impact are greatly needed. These validated pipelines will exponentially increase sequence data available for studies in population dynamics, viral evolution and genetics, and the identification of novel targets for therapeutics, vaccines and rapid diagnostics. Here we developed two methods for full-length sequencing of ebolavirus Zaire using Roche 454 pyrosequencing and Illumina RNA-seq technologies. For 454 sequencing, a "demi-hemi" approach, originally developed at the Broad Institute, was used to design 5-prime amine-modified primers to create long-range PCR amplicons along the entire ebolavirus genome. Amplicons were designed with approximately 500bp overlapping regions to aid in downstream assembly. Illumina sequencing technology was also used to confirm sequence data and further refine primer sequences to capture potential divergent populations within the Zaire strain. While Illumina sequencing provides unbiased sequencing data invaluable to identifying divergent populations, 454 technology can cheaply sequence isolates at a high-throughput capacity with longer sequencing reads that aid *de novo* assembly. Together, these methods have produced a validated 454 sequencing pipeline that has been used to successfully sequence full-length ebola genomes from prepared viral seed stocks and time-course infections of nonhuman primates, with as little as 0.1ng cDNA and over 200x mean coverage depth of the entire genome. Assisted assembly methods using reference genomes, as well as *de novo* assemblies have been created with high success. This method can be used and adapted to sequence many known pathogens for high- and low-throughput sequencing initiatives.

1408

SURVEILLANCE OF MEASLES AND RUBELLA INFECTIONS IN RWANDA: 2003-2011

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Measles and Rubella viruses are still important viral infections in tropical countries including Rwanda. Since 2003, the National Reference Laboratory of Rwanda is accredited by WHO/AFRO as a national Measles laboratory, has been involved in the surveillance of Measles and Rubella infection throughout the country. Cumulative data show that of the approximately 1,894 samples suspected of Measles, 163 were positively identified by ELISA (8.61%). In Rwanda, geographical data indicates that the Rubavu district in the West province, which is at the border with Democratic Republic of Congo is particularly prone to Measles infection. Since 2003, Rwanda has been hit by 3 major epidemics of Measles. The first 2 epidemics, in 2005 and 2006, were limited to the Rubavu district while in 2010, infections were found in various locations, including Kigali city. In collaboration with Uganda Virus Research Institutes, circulating strains were genotyped. It has been observed that in 2005 and 2006, epidemics strains were mostly of the type B2 which is a strain characterized as Congolese. However, during 2010 epidemics, most of the cases were of the type B3, an indigenous strain predominantly found in Burundi. In addition to Measles testing, negative samples were tested for Rubella infection. Of these 1,731 samples tested, 282 were found to be positive (16.29%) and were distributed equally throughout the country.

1409

IMMUNOLOGICAL FEATURES AND PROVIRAL LOAD IN PATIENTS WITH OVERACTIVE BLADDER ASSOCIATED WITH HTLV-1 ARE INDICATORS OF AN EARLY STAGE OF HTLV-1 ASSOCIATED MYELOPATHY

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The majority of HTLV-1 infected subjects are considered as carriers but a high frequency of urinary manifestations of overactive bladder (OB) has been documented in these individuals. The aim of this study was to determine if viral and immunological factors that are associated with development of HTLV-1 associated myelopathy/tropical spastic paraparesis (HAM/TSP) are also observed in patients with overactive bladder associated with HTLV-1. Participants (n=135) were classified as HTLV-1 carriers, HTLV-1 associated overactive bladder (HTLV-1 OB) defined by the criteria of International Continence Society (ICS) and HAM/TSP patients. We demonstrated that peripheral blood mononuclear cells from HTLV-1 OB patients produce higher spontaneous levels of proinflammatory cytokines (IFN-γ, TNF-α and IL-17) than HTLV-1 carriers and similar levels of TNF-α and IL-17 to patients with HAM/TSP. Proviral load was higher in HTLV-1 OB and HAM/TSP than HTLV-1 carriers and correlated positively with production of proinflammatory cytokines. In contrast to HAM/TSP, patients with overactive bladder had serum levels of Th1 chemokines (CXCL-9 and CXCL-10) similar to HTLV-1 carriers and exogenous addition of regulatory cytokines (IL-10 and TGF-β) decreased IFN-γ production in cell cultures from patients with HTLV-1 OB. We conclude that HTLV-1 infected patients with overactive bladder have some immunological features and proviral load profiles in common with HAM/TSP patients. However as they are

still able to down modulate the inflammatory immune response and the recruitment of activated T cells to the central nervous system (CNS) is not enhanced, they present overactive bladder, an oligosymptomatic form of HAM/TSP.

1410

NOVEL VACCINE CANDIDATE PROTECTS MACAQUES AGAINST CHIKUNGUNYA FEVER

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Chikungunya virus (CHIKV) is a mosquito-borne alphavirus that causes an acute febrile illness typically accompanied by rash and severe, often persistent arthralgia. The virus has emerged since 2004 to cause major epidemics in the Indian Ocean, India and Southeast Asia, involving millions of persons. Autochthonous transmission in Italy and France after CHIKV introductions via travelers also underscored the risk that CHIKV poses to the U.S. Because no licensed vaccine exists to protect against chikungunya fever, we used an alphavirus attenuation approach involving a picornavirus internal ribosome entry site (IRES), which replaces the subgenomic promoter, to produce a live vaccine strain capable of inducing protective immunity after a single administration. This vaccine provides robust immunity and protection in murine models, and is incapable of infecting mosquito vectors, an important safety feature for use in nonendemic locations. For further preclinical testing, we subcutaneously or intradermally vaccinated 3 cohorts of 4 cynomolgus macaques, monitored their responses telemetrically to evaluate safety, then challenged them with wild-type CHIKV to assess efficacy. None of the 12 animals developed fever or detectable changes in respiratory or cardiac function after vaccination, and all developed robust neutralizing antibody responses. After challenge, most sham-vaccinated animals developed fever followed by hypothermia, acute viremia, and many also exhibited changes in respiratory and cardiac function. In contrast, all vaccinated animals remained completely normal in all physiological and clinical parameters, and showed no development of viremia from challenge. These results indicate that these new IRES-based vaccine candidates show great promise for use in humans to control chikungunya fever in endemic locations, as well as to reduce the risk of further spread, including into the Americas.

1411

MUTATIONS IN THE E2 GLYCOPROTEIN GENE OF CHIKUNGUNYA VIRUS ASSOCIATED WITH VIRAL-INDUCED ARTHRITIS IN MOUSE MODELS

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Chikungunya virus (CHIKV) is an emerging arbovirus associated with explosive outbreaks of febrile illness often accompanied by rash and arthralgia. The United States Army Medical Research Institute of Infectious Diseases (USAMRIID) developed a live attenuated vaccine virus, 181/clone25 (hereafter 181/25), which despite producing transient arthralgia in a small subset of patients, was shown to be highly efficacious and well tolerated in phase 1 and 2 clinical trials. CHIKV 181/25 was produced by 18 plaque-to-plaque passages of CHIKV strain 15561 in human lung cells (MRC-5). The resulting virus contains 9 nucleotide substitutions, 5 of which result in amino acid changes. In order to probe the specific genetic effects of the mutations, single nucleotide mutation-containing viruses were made that correspond to the amino acid substitutions present in CHIKV 181/25. Two viruses, 7005 and 7014, had amino acid substitutions in the E2 glycoprotein while the other two, 7004 and 7006 had amino acid substitutions in the NSP1 and E1 proteins, respectively. In alpha/beta interferon receptor deficient mice (A129) the two E2 mutation viruses showed a delay in mortality by three days compared to the other two mutants, which showed similar mortality kinetics as wild-type CHIKV, indicating a lack of attenuation. Reduced viremia, pro-inflammatory cytokines, and footpad swelling were also seen in the E2 mutants. In C57bl/6 mice, an immunocompetent arthritis model, the E2 mutants again proved to be more attenuated, behaving similarly to CHIKV 181/25 with less footpad swelling and lower viremias than the E1 and NSP1 mutants. While the single mutations in the E2 gene were not sufficient to create the attenuation seen in CHIKV 181/25, it is clear they resulted in significant attenuation to the parental CHIK virus compared to the mutations in the E1 and NSP1 genes, which resulted in a wild-type-like phenotype. Further studies with viruses containing multiple mutations should be performed to probe the genetic cause of the attenuating phenotype observed in CHIKV 181/25 vaccine virus.

1412

CHALLENGES AND BREAKTHROUGHS IN THE DEVELOPMENT OF SEQUENCING TECHNOLOGY FOR CLINICAL ISOLATES OF LASSA FEVER VIRUS

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Lassa Virus (LV) is the causal agent of Lassa fever, a severe hemorrhagic fever endemic to West Africa. It is responsible for thousands of deaths each year, and evidence suggests that LV acted as a selective agent in recent human evolution. This, combined with observations that it can infect and replicate in its natural host, the rodent *Mastomys natelensis*, without causing severe disease, make it a desirable topic for studies of host-pathogen evolution. In an effort to compile a large dataset of full-length LV genomes, we have applied 454 and Illumina next-generation sequencing technology on clinical samples collected from patients and rodents in West Africa. While we have successfully used both to generate full-length sequences, each platform presents unique challenges and benefits for viral sequencing. 454 technology can generate data from small input volumes, ideal for clinical samples of low viral titers. 454 also produces longer sequence reads, allowing for a wider range of downstream analyses than Illumina-generated sequences. A downside of 454 is that it requires high quality, undegraded starting material - a problem frequently encountered on samples stored under sub-optimal conditions in the field. Illumina sequencing does not require as high quality material but can only be used when other input requirements are met (i.e., low host-contamination, high viral titer). Here we discuss the pros and cons associated with each platform as well as the technical developments that have contributed to their successful application. Our initial results from sequencing have allowed us to make conclusions about new outbreaks of novel LV strains in Northern Sierra Leone, as well as better catalogue circulating strains. Such knowledge will aid in our understanding of viral evolution, allow us to better predict patterns of disease spread, and lay the foundation for better diagnostic development.

1413

MOLECULAR CHARACTERIZATION BY DEEP SEQUENCING OF DIVERSE MEMBERS OF THE GENUS *ORTHOBUNYAVIRUS* FROM MOSQUITOES COLLECTED IN THE AMAZON BASIN OF PERU

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Members of the genus *Orthobunyavirus* (family *Bunyaviridae*) are segmented, negative-sense, single-stranded RNA viruses that are responsible for mild to severe disease in humans. As part of a long-term study of arbovirus ecology in the Amazon basin of Peru, more than 160 viral isolates were made from mosquitoes captured near Iquitos, Peru. Preliminary analysis using immunofluorescent antibody assays (IFA) identified many of these viruses as members of the Group C, Guama, and Bunyamwera serogroups within the genus *Orthobunyavirus*. Follow-up IFAs using complex or virus-specific antisera in complement fixation and hemagglutination-inhibition assays identified some of the viruses as Caraparu, Guama, Itaqui, Mirim, Murututucu, Oriboca, and Wyeomyia viruses; others remain uncharacterized. Additionally, basic knowledge is lacking in regard to the potential reassortment of segments among these viruses in nature. Therefore, we determined the whole genome nucleic acid sequences of these viruses in relation to the viral quasispecies to investigate the extent of intra- and inter-reassortment among RNA segments (S, M, and L) of these viruses. Implications of the reassorted genomes will be discussed.

1414

DEVELOPMENT OF A MULTIPLEX PCR/LDR ASSAY TO DETECT AND GENOTYPE ROTAVIRUS

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Rotavirus and enteric caliciviruses are the most common etiologic agents of diarrhea in children and adults worldwide. The implementation of rotavirus vaccines holds the promise of significantly reducing the associated morbidity and mortality in children. However the vaccines has shown variable efficacy in Africa and Asia attributed to different circulating genotypes than those targeted and/or viral co-infection, which is common in these areas. The WHO has therefore recommended continued surveillance post vaccine implementation. We report the development of a comprehensive molecular assay that can simultaneously detect and genotype the enteric viruses. One-step reverse transcriptase PCR amplifies virus specific targets. Ligase detection reaction and subsequent hybridization of the fluorescent products to beads then detects sapovirus and identifies genotypes of rotavirus, and norovirus. The assay was optimized using previously characterized viral culture supernates and stool specimens and then used to analyze 296 clinical specimens obtained in the US and Ghana. The assay was found to be 97% sensitive and 100% specific with a 100% concordance for genotype determination of norovirus. The rotavirus G- and P-type were determined in 98.6% and 92.3% of the samples, respectively. Mixed genotypes were found in 11.8% of the samples. A significant finding was the identification of two isolates of rare genotypes G6P[6] and G6P[8], both genotypes being reported for the first time in Ghana. The PCR/LDR assay is a sensitive, specific and high-throughput method that can detect rotavirus, sapovirus and norovirus as well as determine the genotype of rotavirus and norovirus. It may therefore be of great utility in epidemiologic surveillance post rotavirus vaccine implementation

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HUMAN CELLULAR RESPONSES TO RIFT VALLEY FEVER VIRUS

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Rift Valley Fever (RVF) virus is an arbovirus in the *Bunyaviridae* family that was first isolated in the Rift Valley region of Kenya in the 1930's. The virus is a significant cause of morbidity and mortality in humans associated with devastating epizootic outbreaks in livestock. While endemic to the Rift Valley region, the virus has shown an ability to spread to virgin territory such as Egypt and the Arabian Peninsula. Furthermore, RVFV has emerged as a target for bioterrorism in recent years. RVFV can cause diverse pathology in humans from non-specific viral illness to severe hemorrhagic disease, encephalitis and death. While it is known that RVFV can cause a range of diseases, the pathogenesis is still not well understood. A number of factors such as route of transmission and host immune response are thought to play a role. Animal studies have shown that a type 1 interferon response in the infected individual affects viral clearance. In addition, a delayed IFN response is associated with worse morbidity and mortality. In this study we infect human peripheral blood mononuclear cells (PBMC) with attenuated MP-12 and MP-12 Nss knockout strains of RVFV to determine time to viral uptake and to monitor inflammatory production. Time to viral uptake was determined using plaque assays and qPCR for the L segment of the viral genome and showed that viral uptake happens occurs as early as two hours post infection. The production of various inflammatory mediators, such as TNF alpha and IFN alpha was monitored using ELISA. We show using naïve North American donors that inflammatory responses begin as early as 6 hours after introduction of the virus. Additional studies will elucidate which cells are primarily responsible for the inflammatory response and define innate receptor utilization.

1416

ECOLOGY OF VENEZUELAN EQUINE ENCEPHALITIS IN THE GULF COAST REGION OF MEXICO

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To characterize the ecology of Venezuelan equine encephalitis virus (VEEV) in endemic regions of the Mexican Gulf Coast, serosurveys in humans and equids, vector incrimination studies, studies of natural infection in equine hosts, and phylogenetic characterization of isolates were conducted in 2008-2010. Human serosurveys from suspected dengue patients (N=237) were VEEV positive in 32 individuals (13.5% seroprevalence), including 5 IgM positives. Equine serosurveys of unvaccinated animals revealed widespread endemic VEEV from the southernmost region in Tabasco State to a northern municipality in Tamaulipas State, located adjacent to the Texas border. Using rodent serosurveys, we identified putative reservoir species of the genera *Sigmodon* and *Oryzomys* in Minatitlan, Veracruz. Using hamster-baited traps in Minatitlan, high-titered mosquito pools (4.9-6.4 $\log_{10}$ PFU/pool) of *Culex (Melanoconion) taeniopus* were identified

in at least 3 transmission events as the likely principal vector of enzootic subtype IE VEEV. As previously observed on the Pacific Coast of Mexico, naturally infected horses developed neurologic disease while producing high-titered viremia (2.4-3.6 $\log_{10}$ PFU). Phylogenetic analysis was performed on isolates from horses, sentinel hamsters, and mosquitoes in Veracruz State. Based on the glycoprotein precursor sequence, all isolates grouped within the Gulf/Caribbean IE genotype and were temporally distinct from the 1963-1969 isolates from the same region. These findings suggest that endemic subtype IE VEEV is currently circulating in widespread regions of the Mexican Gulf Coast, including areas located near the Texas border. Although we implicated *Cx. taeniopus* as the main subtype IE VEEV vector in the Gulf Coast region, *Aedes (Ochlerotatus) taeniorhynchus* mosquitoes were also abundant in this region and have been associated with equine outbreaks on the Pacific Coast of Mexico. Thus, the continuous circulation of endemic VEEV in Mexico has the potential for developing into an outbreak that could rapidly spread into the US via equine amplification.

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RSV, SEASONAL INFLUENZA AND H1N1: CLINICAL MANIFESTATIONS AND CO-INFECTIONS IN 2009

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Respiratory Syncytial Virus (RSV) and Influenza primordially affect children. RSV is a major cause of Bronchiolitis and Pneumonia in children <1 yo, Influenza is characterized by abrupt onset of constitutional and respiratory signs and symptoms. Both manifest in colder months of the year in temperate climates. In April 2009 influenza virus, H1N1 (swine flu) created an outbreak reaching pandemic status in 6 weeks. United States experienced first wave on May 2009 and a second wave, that Puerto Rico also experienced, peaking by end of October. Due to similar clinical presentations of RSV, Seasonal Influenza and Influenza H1N1, physicians differentiated them by Influenza rapid test and RSV nasopharyngeal test. Aim of study: describe clinical and epidemiologic characteristics of RSV, Seasonal Influenza and H1N1. After IRB approval, records of bacteriology and epidemiology from Hospital Episcopal San Lucas were reviewed for positive results of RSV, Influenza, and H1N1 in patients 0y/o - 4y/o, admitted to Pediatrics from October-December 2009 (peak of H1N1 epidemic). Patients with respiratory problems at admission were included, bacterial sepsis was excluded. Records were reviewed for: age, gender, past medical history, symptoms prior to hospitalization, admission or transfer to PICU, supplemental oxygen and days of hospitalization. Forty seven patients tested positive for RSV and/or Influenza. RSV was the most frequently reported (29) with majority of admissions to PICU (5). Seventeen patients had Influenza, 9 H1N1. Most patients with RSV had term birth history while those with Influenza were mostly pre-term. Of 9 patients positive for H1N1 past medical history revealed pre-term birth in 6, and conditions where asthma predominated. One case was co-infected (RSV/Influenza A), admitted with no serious morbidity. Epidemic H1N1 (2009) caused difficulties in differentiation of respiratory illnesses requiring admission. In our experience most children under 4 years had RSV. RSV was associated with most morbidity.

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INTERNAL RIBOSOME ENTRY SITE (IRES)-DRIVEN EXPRESSION OF THE CAPSID PROTEIN IN VENEZUELAN EQUINE ENCEPHALITIS VIRUS INCREASES THE ATTENUATION AND SAFETY OF THE TC-83 VACCINE STRAIN

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The live-attenuated TC-83 strain is the only licensed veterinary vaccine available to protect equids against Venezuelan Equine Encephalitis Virus (VEEV) infection and to protect humans indirectly by preventing equine amplification. VEEV is a mosquito-borne virus endemic to several areas of Central and South America, where both endemic disease and periodic epidemic outbreaks affect hundreds-of-thousands of humans. However, TC-83 vaccine has previously been isolated from mosquitoes collected in the wild. Because it relies on only two point mutations for its attenuation, transmission of revertants represents a major risk to initiate an epidemic or to circulate enzootically. To improve its attenuation and stability, and to prevent infection of mosquitoes, recombinant TC-83 was previously engineered by placing the expression of the viral structural proteins under the control of the Internal Ribosome Entry Site (IRES) of encephalomyocarditis virus (EMCV), which drives translation inefficiently in mosquito cells. However, this vaccine candidate was poorly immunogenic. Here we describe the second generation TC-83 recombinant in which only the capsid protein gene is translated from the IRES, while the viral surface envelope glycoproteins are expressed from a subgenomic message in a cap-dependent manner. This TC-83/IRES/C vaccine does not infect mosquitoes, is stable in its attenuation phenotype after serial passages *in vivo*, and is more attenuated in newborn mice but still protective as well against VEEV challenge. Thus, by using the IRES to modulate TC-83 capsid protein expression, we generated a vaccine candidate that combines efficient immunogenicity and efficacy with lower virulence and a reduced potential for spreading in nature.

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EPIDEMIOLOGICAL MODELING AND RISK ANALYSIS OF VENEZUELAN EQUINE ENCEPHALITIS IN THE HUMAN POPULATION OF COASTAL CHIAPAS, MEXICO IN 2007-2009

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Analysis of 101 febrile illness patients seropositive for Venezuelan equine encephalitis (VEEV) was carried out in a retrospective study along 18 municipalities and endemic VEEV pacific coastal regions of the State of Chiapas in southern Mexico. Geographic information systems (GIS), satellite imagery and a detailed questionnaire were used in the analysis. Using ESRI ArcGIS 10.1 software and spatial statistics tools we measured the geographic distribution of VEEV cases along coastal Chiapas. The distribution of VEE cases were principally located along the Pacific coastal plain with the mean center of positive cases to be in the municipality of Huixtla. The directional distribution of cases around the mean were dispersed in a pattern between the Pacific coastline and coastal mountain range. Temporal and spatial dynamics showed clear separation between cases in the southern and northern regions during the dry season with a peak of positive cases for both regions during the wet season. The analysis was based on Euclidian distances identifying spatially significant clusters

as hot spots. All the spatial analysis was complemented with relative risk (OR) bivariate and multivariate statistical analytical models showing neck stiffness (OR=6.03; 1.2318-29.5801, p=0.027) multivariate; (OR=13.87, IC 3.1861-60.4217, p=0.000), bivariate, muscle weakness (OR=10.12; IC 2.1633-47.4002, p=0.003) multivariate; (OR=23.05, IC5.3134-100.0692, p=0.000) bivariate, taste dysfunction (OR=3.54; IC 1.1380-11.0234, p=0.029) multivariate; (OR=6.40; IC 2.3449-17.5045, p=0.000) bivariate, and photophobia (OR=16.98, IC 2.2321-129.2713, p=0.006) bivariate and conjunctivitis (OR=3.25, IC 1.3273-7.9948, p=0.010) bivariate, as the most important clinical manifestations associated with VEEV for individuals of coastal Chiapas. Overall, our results indicate a coastal band of endemic VEE extending from the Guatemalan border through the State of Chiapas to the adjacent State of Oaxaca.

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THE ROLE OF THE INTERFERONS VERSUS THE ADAPTIVE IMMUNE RESPONSE IN A MOUSE MODEL OF O'NYONG-NYONG VIRUS INFECTION

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O'nyong-nyong virus (ONNV) is an alphavirus transmitted by mosquitoes that shares 90 percent nucleotide sequence identity with Chikungunya virus (CHIKV) and has been the cause of two major epidemics in Africa during the past 20 years. These viruses produce very similar acute febrile illnesses characterized by rash and debilitating arthralgia. While there are many studies into the pathogenesis of CHIKV, little is known about the pathogenesis of ONNV. To determine which portions of the immune system are important in protection during initial infection with ONNV we inoculated several different strains of mice with two different strains of ONNV (SG650 and MP30). These mice included wild type C57BL/6J and mice knocked out for the following genes: STAT1 (STAT1 KO; defective in both type I and type II interferon signaling), Type I interferon receptor (A129), interferon g receptor (IFNgR KO), recombinase activation gene 1 (RAG1 KO). Mice were inoculated subcutaneously with 100-10000 PFU of ONNV or sham inoculated with phosphate buffered saline. Mice were bled to assess viremia, weighed for at least 14 days unless infection was lethal, and observed for illness daily. Tissue samples were collected to test for viral load and for histopathologic evaluation. The C57BL/6J, RAG KO and IFNgR KO mice showed no statistical difference in weight compared to control mice, and never generated detectable viremia. A129 mice demonstrated morbidity but survived. STAT1 KO mice demonstrated an age dependent mortality with 6-week-old mice succumbing to illness by day 12, while 8-14 week old mice developed morbidity as evidenced by weight loss and clinical signs of illness but survived. Tissues of STAT1 KO mice demonstrated a monocytic infiltrate in all major organs and infectious virus was detected in brain and skeletal muscle. We conclude that the adaptive immune system is not necessary for protection against initial ONNV infection in the mouse model while the type I interferon response demonstrates an age-dependent phenotype. These results will be valuable for designing animal models for testing candidate vaccines or therapeutics against ONNV infection.

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SEASONALITY, TIMING, AND CLIMATE DRIVERS OF INFLUENZA ACTIVITY WORLDWIDE

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Influenza is a vaccine preventable disease which annually causes substantial morbidity and mortality, but data on influenza virus activity in tropical countries are limited. We analyzed publicly available influenza data to better understand the global circulation of influenza viruses. We searched for laboratory-confirmed influenza surveillance data in FluNet, Google™, and PubMed using the key words: "influenza," "epidemiology," "season," and "surveillance" to abstract data on the percent of samples testing positive for influenza during each epidemiologic week. The start of influenza season was defined as the first week when the proportion of samples that tested positive remained above the annual median for at least 6 weeks. We assessed changes in the relationship between percent of samples testing positive and average monthly temperature using linear regression models.We identified data on laboratory-confirmed influenza virus infection from 84 countries comprising 5.4 billion (83%) of the world's population. While (44 [94%] of 47) temperate and all four subtropical countries had one annual epidemic, 24 (72%) of 33 tropical countries had one annual influenza epidemic, seven (21%) had biannual epidemics, and 2 (6%) had insufficient data to analyze. Influenza was identified every week in 4 (9%) of 47 temperate, 0 subtropical countries versus, and 10 (30%) of 33 tropical countries (p=0.04). Peak influenza activity occurred within two months after the lowest temperatures in 36 (82%) of 44 temperate, 1 (25%) of 4 subtropical, and 6 (27%) of 22 tropical countries with available data (p<0.001). Influenza activity peaked in Southeast Asia and Oceania during June-July; Australia and China during August; Middle East, North Africa and Mexico during December, Europe and North American during February-March and South America and South Africa during May-June. In conclusion, annual influenza epidemics occur in consistent temporal patterns depending upon climate. Local influenza surveillance and climate data may best inform influenza prevention activities and focus efforts during periods of highest local activity.

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CHARACTERIZATION OF THE NATURAL HISTORY OF LASSA FEVER VIRUS DISEASE IN CYNOMOLGUS MACAQUES FOLLOWING AEROSOL EXPOSURE

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Viral hemorrhagic fever (VHF) in humans is caused by members of four families of enveloped, negative-sense or ambisense RNA viruses; *Arenaviridae*, *Bunyaviridae*, *Filoviridae* and *Flaviviridae*. These viruses are considered possible biothreats due to their potential for airborne and person-to-person transmission, making characterization of the aerosol route of infection paramount. First described in Nigeria in 1969, LASV is endemic to the West African countries of Sierra Leone, Liberia and Guinea. In Africa, members of the *Mastomys* genus of mulimammate rats are persistently infected with LASV from birth. Humans become infected via the respiratory route through exposure to virus in rat excreta, as well as by preparing and eating infected animals. It is estimated LASV may cause 5,000-10,000 fatalities annually; however, there are currently no FDA-approved therapeutics or vaccines. Examining the natural history of

a disease, from exposure through resolution, permits the description of stages of disease course, the immune responses, as well as mechanisms of pathogenesis or correlates of survival. The objectives of this study were to 1) identify early clinical signs that can be used to indicate or predict infection, 2) identify markers that indicate disease progression as well as development of severe disease and 3) determine the impact of anesthesia on clinical disease progression. The natural history of LASV infection following aerosol exposure was examined in the cynomolgus macaque model. Animals were surgically implanted with telemetry providing simultaneous real-time monitoring of pressures, ECG, and temperature as well as daily blood sampling occurred via central venous catheters beginning from Dy -3. Based on these results, a sequential sampling study was designed to collect presymptomatic, early, intermediate and late stages of disease. Summary results will be presented, including hematologic changes (complete blood counts), blood chemistry analysis (Piccolo and iSTAT), coagulation assays, and plasma cytokine responses, as well as pathology findings.

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SUSCEPTIBILITY OF MARMOSETS (*CALLITRIX JACCHUS*) TO MONKEYPOX VIRUS

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Although current nonhuman primate models of monkeypox and smallpox diseases provide some insight into disease pathogenesis, they require a high titer innoculm, use an unnatural route of infection, and/or do not accurately represent the entire disease course. In our studies, we altered half of the test system by using a New World primate species, the common marmoset. Adult male marmosets were intravenously infected with $2.4x10^7$, $9.5x10^5$, and $7.8x10^4$, $5.0x10^3$, 510, and 48 PFU. Clinical, hematological, and viral load data were assessed. Animals were euthanized or succumbed to disease between 6 and 15 days post-infection, in a dose dependent manner. The animals exhibited signs of hemorrhage, had high genome viremia, and altered hematological parameters. At the lower doses, rash was more demarcated and some short-lived macules were observed. As is, our model is 6 logs lower than the current intravenous cynomalgus model and 4-6 logs lower than respiratory models. The aggressive nature of the disease manifested in these animals implicates an even lower dose and warrants exploration of other infection routes. Also, these data should invoke consideration for variola experimentation in marmosets.

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SURVIVAL AND EXPANSION OF HTLV-1-INFECTED CELLS WITH HIGH DNA DAMAGE: RELATIONSHIP BETWEEN SOD1 AND GENOMIC STABILITY

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HTLV1 may exert an initial pro-apoptotic stimulus via the oncoprotein Tax-induced DNA damage. On the other hand, the viral protein Hbz antagonizes some Tax effects and recent studies show that hbz is always expressed in leukemic cells, suggesting its involvement in the maintenance of malignity. Tax has been implicated in the initiation of cellular transformation, chromosomal instability, and induction of cellular DNA damage by reactive oxygen species (ROS). Superoxide dismutase 1 (SOD1) is an antioxidant enzyme present in the cytoplasm, nucleus, and intermembrane space of mitochondria; SOD1 catalyzes the dismutation of superoxide to hydrogen peroxide and molecular oxygen, thus playing an important role in genomic stability. We hypothesize that SOD1 is diminished in cells from HTLV-1-infected subjects with high DNA damage, and that hbz expression is associated with the expansion of these cells. To test this hypothesis we measured Proviral Load (PVL), hbz mRNA, and SOD1 protein levels in cells from HTLV-1-infected subjects with high (HD) and low (LD) DNA damage. Peripheral blood mononuclear cells (PBMCs) were isolated to estimate DNA damage by alkaline comet assay. Two groups of HTLV-1-infected subjects were defined: HD (≥ 51 comets/100 nucleoids; n=18); and LD (≤ 50 comets/100 nucleoids; n=9). We measured PVL and hbz mRNA levels by real time PCR, and plasma SOD1 levels by a sandwich Enzyme-Linked Immunosorbent Assay (ELISA). PVL was expressed as HTLV-1 tax copy number /104 PBMCs. Samples were classified as hbz+ (detectable) or hbz (undetectable). Statistical analyses were based on non-parametric tests. We did not find differences in PVL (p=0.959) or SOD1 (p=0.064) between HD and LD. However, all hbz+ samples came from a cluster of HD subjects with high PVL (3168 ±980), who also showed lower levels of SOD1 than the remaining HD group (p=0.039) and the LD group (p=0.01758). In conclusion, DNA damage is not always associated with low SOD1 levels in HTLV-1-positive subjects. Our results suggest that hbz expression supports survival and expansion of HTLV-1-infected cells bearing DNA damage associated with low SOD1 levels.

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OLIGONUCLEOTIDE MICROARRAYS FOR THE DETECTION AND CONFIRMATION OF ARBOVIRAL PATHOGENS IN THE FIELD

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With the emergence and re-emergence of arthropod-borne diseases throughout the world, it is critical to be able to detect arthropod-transmitted pathogens in a timely manner. Standardized field-diagnostic protocols for identifying arthropod-borne pathogens within any given region of the world are indispensable tools for obtaining real-time information for health-care providers and preventive medicine specialists in field settings. Arboviruses are responsible for major outbreaks of acute, febrile disease throughout most areas of the world. Dengue (DEN), Japanese encephalitis (JE), yellow fever, Chikungunya, and tick-borne encephalitis complex viruses are but a few of the viruses that account for a majority of the arboviral infections that cause morbidity and mortality in humans. Here we report the development and field-testing of an oligonucleotide microarray for the detection and confirmation of arboviruses in pools of field collected mosquitoes. During the field evaluation, mosquito pools were screened by using generic PCR assays and then by using virus specific real-time PCR assays. The phylogenetic relationships among these viruses, their mosquito hosts, and their possible role in causing human disease is also presented.

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MOLECULAR DIAGNOSIS AND ANALYSIS OF IMPORTED CHIKUNGUNYA VIRUS STRAINS, JAPAN, 2006-2010

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Chikungunya (CHIK) virus has re-emerged as an important mosquito-borne pathogen causing epidemics in several parts of the world. The CHIK virus belongs to the Alphavirus genus in the family Togaviridae.

A large-scale epidemic of CHIK fever started in Kenya in 2004 and spread to Reunion Island, other Indian Ocean islands, India, Sri Lanka, Singapore, Thailand and Malaysia. One of the main vectors responsible for transmission between humans is *Aedes albopictus*, which is widely distributed in urban areas of Europe, the USA and East Asia. This fact raises concern that the virus could be introduced and become established in these areas. During 2007-2010, 19 imported CHIK cases were detected in Japan from South and Southeast Asia. The samples were tested for dengue virus as well as CHIK virus by IgM-capture ELISA, real time RT-PCR, virus isolation with Vero and C6/36 cell, and plaque reduction neutralization tests. In this study, we report two cases of imported infection in patients who had returned to Japan from Malaysia and Indonesia. Both viruses were successfully isolated from the cases by using a plaque purification technique. Phylogenetic analysis showed that the strain from Indonesia was grouped into the Asian genotype. However, the isolate from Malaysia was identical to the Central/East/South African genotype and was clustered with currently reported Indian isolates. The strain from Malaysia also had the E1-A226V mutation. These data suggest that the CHIK virus circulating in Malaysia is related to that currently epidemic in South and Southeast Asia. Further characterization of these isolates is in progress.

1427

THE APPLICATION OF PYROSEQUENCING TO IDENTIFY MULTISPECIES *PLASMODIUM* INFECTIONS IN HUMANS AND APES IN WEST CENTRAL AFRICA

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Recent studies have identified several new species of *Plasmodium* in wild-living chimpanzee and gorilla populations. Using single genome amplification (SGA), we have shown that most apes are co-infected with multiple divergent strains and traced the origin of human *P. falciparum* to western gorillas. This has raised the question whether wild-living apes serve as a recurring source of human infection. While SGA methods are useful in precluding *in vitro* recombination, this approach is not designed to identify low abundance strains in multi-species infections. Here, we describe a novel method of addressing this question by using pyrosequencing technologies. We developed a set of 454 FLX Titanium pan-*Plasmodium* primers that amplify a 510 bp fragment of mitochondrial DNA containing sufficient diversity to differentiate all previously identified ape and human *Plasmodium* species. These primers, in conjunction with the GS FLX System, generate over 1 million reads in a single run, providing both depth of sequencing and high sample throughput. Primers are tagged with sample-specific 12-mer barcodes allowing us to identify the sample origin of each read. To process our data, we have developed a method of rapidly classifying reads by alignment to multiple reference sequences from all known primate *Plasmodium* lineages. Amplicon sequencing by this method has an error rate of $1.5x10^{-3}$ mismatches/nucleotide, low enough to accurately distinguish even the most closely related lineages. In a pilot run of 90 human buffy coat samples from Cameroon, 59 were positive for *P. falciparum* alone, while the remainder represented multiple species infections with *P. falciparum* and *P. malariae* (n=23), *P. falciparum* and *P. ovale* (n=5), or all three species (n=3). Read ratios give an indication of the relative abundance of each *Plasmodium* species in a sample. These data demonstrate that deep sequencing technology can be used to identify mixed-species infections, even when one or more species is present at ratios below 1:2000.

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THE POPULATION STRUCTURE OF AMAZONIAN *PLASMODIUM FALCIPARUM*

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We previously showed that population structure of Peruvian *Plasmodium falciparum* during the peak expansion of malaria in 1990s, following almost two decades of very low malaria transmission, was confined to five major clonal lineages that we referred to as clonets. We have also shown that Venezuelan *P. falciparum* parasites appeared to have somewhat more diverse clonets. Given the shared borders between Brazil and these two countries, we compared the population structure of *P. falciparum* parasites in the Amazon basin, and coastal Peru, using neutral microsatellite markers. The microsatellite data was also combined with drug resistance genotypes and microsatellite markers flanking genes associated with drug resistance (*pfcrt*, *dhfr*, *dhps* and *pfmdr1*) in an aggregate analysis. A total of 190 samples from Brazil were examined from three states (Amapá, Pará, and Rondônia), representing multiple sites and time periods since the 1980s in this study. We analyzed our data using network diagrams, estimates of heterozygosity, and tests for bottlenecks and recent population expansions. Like Peru and Venezuela, Brazil exhibited low parasite diversity and the distance between collection sites was not significantly correlated with increasing genetic differentiation, but unlike these countries there were no obvious clonets. We interpreted the apparent Brazilian *P. falciparum* population structure to be the logical outgrowth of multiple waves of internal migration, admixture, and sexual recombination over past decades. We described the evidence for population bottlenecks at various sites within Brazil. Furthermore, we related the parasite populations from Peru and Venezuela to the Brazilian Amazon basin. At least two of the clonets in the Pervian Amazon were linked with Brazilian Amazon isolates, while the Peruvian coastal lineages were only related by way of samples collected in the Peruvian Amazon. While Venezuelan isolates generally seemed to have more in common with each other, they were also directly linked to Brazilian isolates. This underscored the remarkable clonality of Peru and the intermediate clonality of Venezuela. Our findings suggest that, if Brazilian patterns of internal migration continue in the future, future drug resistance might rapidly spread throughout the country.

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POPULATION GENETICS OF COPY NUMBER VARIATION IN *PLASMODIUM FALCIPARUM*

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Genome rearrangements, such as copy number variation (CNV), are ubiquitous in eukaryotic genomes. Gene dosage changes resulting from gene duplications or deletions may play an important role in adaptive evolution. However, the role of DNA rearrangments has been largely ignored in malaria biology despite the fact that the *Plasmodium* karyotype is highly variable, many rearrangements have been reported in laboratory lines and CNVs are known to influence drug resistance. This project examines extent and functionality of genome rearrangements in the malaria parasite *Plasmodium falciparum*, as well as their origins and evolutionary dynamics. We detected genome-wide CNV in more than 100 parasites from SE Asia, (Cambodia, Lao PDR and Thailand) and Africa (Malawi and Gambia) using comparative genomic hybridization (CGH) on

a custom Nimblegen microarray (the CNV-SNP array) that assays both CNV and SNP variation at high resolution genome-wide. The parasites examined were prescreened to exclude multiple clone infections, and patient derived material was used to avoid artifacts caused by laboratory adaptation. We determined the size, gene content, and population frequency of genome rearrangments within and between parasite populations and evaluate the roles of drift and selection (positive and purifying) in shaping the CNV distributions. Geographical variation in SNPs and CNVs and linkage disequilibrium in regions flanking CNVs were used to better understand the evolution of genome rearrangements in *P. falciparum*.

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HIGH COVERAGE GENOME SEQUENCING OF FIELD ISOLATES PROVIDES UNIQUE INSIGHTS ON *PLASMODIUM VIVAX* BIOLOGY

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The biological diversity of *Plasmodium vivax* is poorly understood, partly because the parasite cannot be easily propagated *in vitro*. As an alternative it is becoming increasingly feasible to characterize the genetic diversity of *P. vivax* isolates across their genomes and to associate genetic variation with biological traits. We present here whole genome sequences generated directly from the blood of three patients and show how robust characterization of the genomic diversity can provide unique insights about *P. vivax* biology. We analyzed blood samples from one Cambodian and two Malagasy patients harboring parasitemias between 0.1 to 0.35% and confirmed *P. vivax* mono-species infection by *Plasmodium* species PCR-based diagnosis. After leukocyte depletion of whole blood (5 ml; using CF11-packed columns), we extracted *P. vivax* DNA from parasitized red cells and prepared libraries from each individual sample after fragmentation of the DNA into 250-300 bp. We sequenced each library on individual lanes of an Illumina HiSeq 2000. We were able to map 20-60 % of the 80 million 100 bp paired-end sequences generated from each sample to the SalI *P. vivax* genome sequence while the remaining reads (40-80%) mapped to the human genome. The very high coverage (100-300X) generated by our sequencing effort provides both a robust description of the genetic diversity across the entire *P. vivax* genome, and allows us to identify and differentiate multiple *P. vivax* strains within each infected patient. In addition, we show that we can analyze variations in sequence coverage along each genome to identify gene duplications and deletions. Finally, we describe multiple DNA sequences shared among the newly sequenced genomes that are missing (for either technical or biological reasons) from the SalI reference genome. Overall, our analyses confirm that sequencing of *P. vivax* genomes from field isolates is very feasible following leukocyte depletion and illustrates that substantial information can be generated regarding genome structure, sequence polymorphism, and complexity of infection.

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GENOME SEQUENCING ASSOCIATION STUDIES: A NEW APPROACH FOR UNDERSTANDING ANTIMALARIAL RESISTANCE IN *PLASMODIUM FALCIPARUM*

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Plasmodium falciparum malaria's rapid adaptation to new drugs allows it to remain one of the most devastating infectious diseases of humans. Understanding the genetic basis of these adaptations is critical to successful intervention. Using next-generation sequencing and drug-sensitivity testing, we performed genome sequencing association studies (GSAS) on 25 recently isolated parasites from Senegal against and 13 antimalarial drugs_including amodiaquine, artemisinin, atovaquone, chloroquine, dihydroartemisnin, halofuganone, halofantrine, lumefantrine, mefloquine, piperaquine, primaquine, pyrimethamine, and quinine_and 20 synthetic compounds. This novel use of whole genome sequence data greatly expands our power to detect associations in low LD populations, as it assays nearly a thousand-fold more positions in the genome than our previous 17,582 SNP array (average coverage of 17.2 Mbp per parasite). However, it comes with some new analysis challenges, including the reliable characterization of marker genotypes from read data (127,362 SNPs and 7,919 microsatellites are polymorphic in this population), handling missing data and more LD in the denser marker set. We adapted recent mixed-model GWAS tools, such as EMMA and GCTA, and selection tools, such as HLR and XP-EHH, to study the heritability of drug response phenotypes and identify known and novel loci associated with drug resistance at genome-wide significance. This demonstrates improvements of the GSAS approach over traditional, array-based GWAS for understanding the genetic basis for antimalarial drug resistance in the wild, potentially identifying important biomarkers for surveillance as elimination and eradication efforts are pursued.

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GENETIC ANALYSIS OF *PLASMODIUM FALCIPARUM* GAMETOCYTOGENESIS

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Within the mammalian host, the *Plasmodium* parasite has two developmental fates: cyclic asexual replication or terminal sexual differentiation (gametocytogenesis). The sexual forms of the parasite (gametocytes) are the only form that is able to survive and propagate in the mosquito vector. Therefore, gametocytes are absolutely essential for parasite transmission. Very little is known about the mechanisms involved in the commitment of *Plasmodium* to sexual differentiation. To gain insight into these mechanisms, we conducted a *piggyBac* transposon-mediated insertional mutagenesis and screened for parasites that no longer formed mature gametocytes. Of 736 parasites (clones) screened in 3 independent transfection experiments, 29 clones did not form gametocytes. For each clone, insertion of *piggyBac* was verified by Southern blot analysis and the disrupted genes were identified by inverse PCR. This led to the identification of 16 putative gametocytogenesis-disrupting genes. Genetic complementation for 4 of the 16 genes was successfully carried out showing that these genes are essential for gametocytogenesis. To epistatically order the 16 genes, we measured their expression pattern along with the expression pattern of other known gametocyte-specific

genes in each of the gametocyte-minus mutants using RT-PCR. We found a subset of the genes that are likely to act very early in commitment to gametocyte differentiation, another subset likely to act just after the committed merozoite invades the red blood cell, and a third set likely to act early (stage I) gametocytes. Thus, we have carried out a comprehensive screen for genes essential to commitment and early differentiation of the *Plasmodium* gametocyte. This line of investigation may lead to novel strategies to reduce parasite transmission and disease burden.

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VARIANT ANTIGEN EXPRESSION IN PEDIATRIC MALARIA

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The ability of *Plasmodium falciparum*-infected erythrocytes to sequester from the circulation into organ microvasculature is associated with much of the lethality of this species. We have investigated genetic variation and antigen expression of parasites in organ biopsies from 25 Malawian paediatric malaria patients. Patients were autopsy-confirmed cerebral malaria cases or parasitaemic controls with an incidental or mild *P. falciparum* infection and another identified cause of death. Cerebral malaria cases had low multiplicity of infection with often a single genetic variant dominating the infection throughout the organs. Expression of the variant surface antigen, *P. falciparum* erythrocyte membrane protein-1, was investigated by quantitative PCR and analysis of expressed sequence tags. Cerebral malaria infections and parasitaemic controls showed similar patterns of antigen expression in host tissues, with particular antigens often being expressed at dominant levels (>33%) by parasites in the brain, heart or gut. These dominant antigens can vary between organ populations within a single host. There was high overlap in the antigens observed in different patients, with 22% of the 644 antigens being detected in multiple patients, and 30% of antigens in the brain also observed in brain biopsies from other patients. This finding was unexpected given the negligible overlap in antigen diversity seen globally and in endemic sites. Our findings suggest that a restricted number of antigens are implicated in sequestration in the paediatric host.

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AN EVALUATION OF THE IMPACT OF INTEGRATED INTERVENTIONS TO IMPROVE ACCESS TO MALARIA TREATMENT IN TANZANIA - THE ACCESS PROGRAM

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The ACCESS Programme was implemented between 2004 and 2008 in two Tanzanian districts to improve access to malaria treatment with a set of integrated interventions at three levels: 1) community level; 2) public heath facilities; and 3) commercial drug sector. The study period saw the switch from Sulphadoxine-Pyrimethamine (SP) to Arthemeter Lumefantrine (ALu) as first line treatment for malaria in 2006. This study aims at evaluating the ACCESS Programme's interventions. We conducted yearly censuses in all health facilities and drug shops between 2004 and 2008 and treatment seeking surveys on approximately 150 individuals in 2004, 2006 and 2008 in the Ifakara Demographic Surveillance Site (DSS). The DSS provided yearly estimates of under-five mortality between 1997 and 2009. Results: We observed improvements in the availability (from 0.24 shops per 1,000 people in 2004 to 0.39 in 2008) and accessibility (from 71% of households within 5 km of a shop in 2004 to 87% in 2008) of drug shops. After the introduction of ALu stock levels of the drug were relatively high in public health facilities (over 80% months in stock), but the drug could only be found in 30% of drug shops. The proportion of children treated with an antimalarial within 24hrs of onset of fever increased from 66% to 89% between 2004 and 2008. However, only 51% were treated with the newly introduced ALu in 2008. Under-five mortality decreased from 28.4 cases per 1000 person years (c/1000py) in the years before 2004 to 18.5 c/1000py in 2008 and 2009. The ACCESS interventions were independently associated with decreases in mortality, controlling for other malaria interventions and contextual factors (incidence rate ratio comparing before 2004 vs. after 2008=0.84, 95%CI=0.72 to 0.99). In conclusion, an integrated approach which tackles both users and providers, recognising the important role of the private retail sector, can lead to improvements in terms of access to malaria treatment and can contribute to decreases in mortality in rural African settings.

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STRENGTHENING MALARIA INFORMATION SYSTEMS IN SOUTH AFRICA: MOVING TOWARDS ELIMINATION

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South Africa has made significant progress in controlling malaria during the past decade. Between the years 2000 to 2010 malaria incidence has been reduced from 8.79 to 0.63 local cases per 1000 population at risk, respectively. Total locally transmitted malaria cases have declined by 92% and malaria deaths have declined by 82% in 2010 as compared to 2000. As a result, South Africa is progressing towards achieving malaria elimination and will need to develop and manage a robust malaria information system (MIS) that will form the basis for effective management by enabling evidence-based decision-making on appropriate use of human, technical and financial resources. To monitor and evaluate progress towards elimination, a comprehensive MIS will need to capture epidemiological information on cases and deaths, GIS mapping of malaria foci and breeding sites and entomological and parasitological data. The purpose of this review is to determine the 2010 baseline for intervention coverage rates stratified among targeted municipalities as well as to determine reporting breakdowns within the current MIS. This paper describes South Africa's National MIS and reviews challenges and best practices in developing an integrated rapid notification malaria database that is unified and standardised across provinces. This standardisation is essential in order to increase information flow and inform programmes of potential outbreaks, allowing for prompt response and investigation. As countries in sub-Saharan Africa move towards elimination, a vigorous surveillance program is necessary to rapidly identify new and reignited foci of transmission, as well as track the movement of vectors, parasites and parasite carriers in this fluid environment. Within the context of South Africa the lack of a functional, standardised information system, and the absence of timely notification of changes in the epidemiological landscape, has led to an inability to inform targeting of interventions due to undetermined foci of transmission. The obstacles encountered and overcome in the restructuring of South Africa's MIS provides necessary guidance to other countries who seek to strengthen control programmes and lay a strong foundation for the eventual development of elimination strategies.

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MALARIA BURDEN AND COVERAGE ESTIMATES FROM THE 2010-2011 MONTHLY 'ROLLING' MALARIA INDICATOR SURVEY (RMIS) IN CHIKHWAWA DISTRICT, MALAWI: A POTENTIAL DISTRICT-LEVEL MALARIA MONITORING AND EVALUATION (M&E) TOOL FOR PROGRAM MANAGERS

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Novel malaria M&E tools are urgently needed to complement the current 'gold standard' Malaria Indicator Surveys (MIS). Rapid up-scaling of malaria control efforts is resulting in substantial reductions in malaria burden across sub-Saharan Africa. As transmission goes down, timely, accurate, sub-national and district level burden estimates are needed to guide increasingly targeted, sub-national control efforts in remaining hotspot areas. To test a novel district level M&E tool, we started a monthly 'rolling' MIS (rMIS) in May 2010 covering 51 villages in Chikhwawa district. This is one of the National Malaria Control Programme (NMCP) focus districts with high insecticide treated net coverage and annual indoor residual spraying with round 1 in January-February 2011. During the first year, approximately 1,200 households were randomly selected using a probability proportional to (village) size. Approximately 100 households were visited each month, and approximately 60 under-fives were tested for anaemia and parasitaemia. Parasitaemic children (by RDT in the field) were treated as per national guidelines. Each month, data was collected in a one-week period by two teams of two people. Data was collected using Personal Digital Assistants, and uploaded daily for quality checking. Results were usually available within two weeks from completion of data collection. Data quality was maintained throughout the whole period. Standard malaria impact indicators (moderate anaemia (Hb<8g/dL) and malaria prevalence in under-fives) as well as intervention coverage indicators will be presented for the first 12 months of the study and by season. The strengths and weaknesses of burden estimates from monthly data collections will be discussed. There will be a particular focus on whether short-term changes in malaria indicators can be detected following district-wide indoor residual spraying after accounting for seasonality. Small-scale, rolling sub-national MIS surveys could be a viable complementary malaria M&E approach for district level program managers and control efforts.

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EVIDENCE FOR LOCAL MALARIA TRANSMISSION IN THE WET SEASON AND IMPORTED MALARIA IN THE DRY SEASON IN ZANZIBAR

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Zanzibar is considering malaria elimination. A critical component of this strategy is better understanding transmission. To investigate malaria transmission in Zanzibar, we combined molecular tools with passive surveillance at all 47 public health facilities in three contiguous districts of Zanzibar from May 2010 to April 2011. Subjects presenting to these facilities with fever who tested positive for malaria by rapid diagnostic test (RDT) or microscopy were enrolled and provided travel history, location of residence, and fingerprick blood samples. We performed genotyping of 9 *P. falciparum* microsatellites to see if genetic information would provide insight into transmission patterns. We enrolled 906 patients, 712 (79%) in the wet season (May-July). 35% of patients presenting in the dry season reported travel outside of Zanzibar in the past month versus 3% of those in the wet season (RR 11, 95%CI 7-17, p <.001), suggesting that a higher fraction of dry season cases may be imported. Thus far, we have 511 complete multilocus genotypes from 384 patients. Closely related parasites (100%, 80-99%, or 50-79% identity) were more likely to come from patients living in the same shehia (smallest administrative unit) than less related parasites, indicating fine-scale spatial clustering of related parasites (RR: 18, 15, and 3 respectively, vs. <50% identity, p<.001 for all). 91 clusters of parasites ≥80% identical were identified, with cluster size ranging from 2 to 19 parasites. In the wet season, 57% of patients were infected with a clustered parasite versus 13% in the dry season (p<.001), demonstrating that local transmission occurs and likely dominates in the wet season. Exploratory visualization of clusters of parasites on a map suggested specific patterns of local transmission. Local transmission appears to account for a high proportion of malaria cases during the wet season in Zanzibar, but imported malaria may play a significant role during the dry season. Analysis of parasite genetics may provide insight into malaria control strategies in low-endemic settings.

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MALARIA ACTIVE INFECTION DETECTION IN AN AREA OF LOW PARASITEMIA PREVALENCE: LUSAKA DISTRICT, ZAMBIA

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Malaria surveillance in Zambia has been via passive case detection. The 2010 Malaria Indicator Survey reported a very low prevalence of malaria in Lusaka and it is suspected that cases of malaria in the urban parts of this district are primarily imported. As transmission rates continue to decline as this area progresses towards malaria elimination, it is necessary to find and treat asymptomatic malaria cases implying the need for cost-effective, focal intervention and surveillance strategies. To address this need, the National Malaria Control Centre, Lusaka District Health Office and partners began malaria active infection detection (AID) where laboratory-confirmed, passively-detected malaria cases are followed-up in the community through testing and treating of households (n=9) immediately surrounding the house of each index case. Since March 2011, community-based teams have led weekly AID responses in 5 health facility catchment areas within Lusaka District with expansion to additional clinics forthcoming. Thus far, a total of 21 index cases have received AID response, with a total of 678 individuals tested for malaria during these responses. Of the 678 tested, 20 (2.9%) were found to be positive. Of the 20, 14 reported a recent (within 1 month) history of malaria indicating their rapid diagnostic test (RDT) results were potential false positives. Remaining positives were likely to be imported as they reported recent travel to malarious areas outside Lusaka. Of the index cases reporting no travel history that were followed-up, there was no indication of ongoing transmission within their household or among sampled surrounding households. Initial results indicate that as low-prevalence areas in Zambia progress towards malaria elimination, it is important to collect patient travel history and to provide public health messaging encouraging individuals to practice

malaria prevention especially when traveling to malaria endemic areas. Furthermore, results indicate AID may be a reasonable, focal intervention for application in low prevalence areas such as urban Lusaka District.

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VALIDATING LAMP FOR IDENTIFYING TRANSMISSION 'HOT SPOTS' FOR MALARIA ELIMINATION AND ERADICATION

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Recent trends in reducing malaria transmission in sub-Saharan Africa and an interest in the steps to reach pre-elimination levels of malaria control mean a paradigm shift in surveillance for malaria is needed. Surveillance and monitoring of all infections including asymptomatic infections is required to find foci of ongoing transmission and to detect when reintroduction of malaria has occurred in an area of local elimination. For this purpose it is likely that a more sensitive test is needed than microscopy or currently available Rapid Diagnostic Tests (RDTs) can deliver. In this study we compare the use of 3 different molecular methods for the purpose of screening blood samples from a large cross sectional survey in a moderate to low transmission setting in north western Tanzania. This study was carried out in Misungwi District, Mwanza, Tanzania, to identify potential *P. falciparum* transmission hotspots by mapping parasite prevalence within house holds. Participants were recruited from 4 villages in Kanyelele namely Mwakalima, Kanyelele, Gambajiga and Budutu. The villages have a total of 33 sub-villages with 1,600 households and a population of about 11,000 people. These villages were characterized for transmission intensity as low transmission with less than 5% parasitaemia and high transmission with more than 10% parasitaemia, using RDTs. A pilot study was conducted of 180 finger prick blood samples collected from participants on Whatman® filter paper, representing 90 randomly selected from each of two villages, one low and one high transmission. Three molecular methods were performed on chelex-extracted DNA samples: LAMP using a mitochondrial target sequence, qPCR with SybrGreen detection, and nested PCR. Taking the nested PCR as the established gold standard, the sensitivity and specificity of LAMP were 85.7% and 91.5%, respectively while the qPCR gave a sensitivity and specificity of 70% and 90%, respectively. In this context of asymptomatic individuals LAMP has performed better than qPCR in terms of sensitivity. The difference in detecting parasite prevalence between LAMP and qPCR was highly significant p=0.0000 (OR 29.7; 95% CI [9.07 - 123.11].These results coupled with the rapidity of obtaining results (<1hour) and the potential ability to perform LAMP in field conditions by direct boiling of blood spots renders this technique a useful tool for identifying hot spots of parasite transmission for malaria elimination.

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MALARIA TOOLS: A USER-FRIENDLY SOFTWARE PACKAGE FOR EXPLORING THE IMPACT OF COMBINATIONS OF INTERVENTIONS IN AFRICAN COUNTRIES

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In the current era of intensified efforts to control or eliminate malaria, individual countries need to prioritise which combinations of interventions they will introduce to reduce transmission and to set realistic expectations about their likely impact. We describe a tool developed for African countries to help guide such decision-making. Underlying the tool is an individual-based dynamic malaria transmission model incorporating current interventions, namely long-lasting insecticide treated nets (LLINs), indoor residual spraying, mass drug administration, IPTi, IPTc and a pre-erythrocytic vaccine. This model has been parameterised by an extensive literature review and Bayesian fitting to multiple data sources. The tool is a downloadable user-friendly interface to the model which can be run on any standard Windows PC (each model scenario running in under a minute). The model includes pre-computed estimates of current parasite prevalence (as a marker of transmission intensity), estimates of LLIN use in the past 10 years, a seasonal pattern of transmission determined by rainfall data and expert-maps of malaria vectors (*An.gambiae* s.s., *An.funestus, An.arabiensis*) at the first administrative level. The user can modify these inputs based on local knowledge and data: if they do, the program will recalculate the natural transmission potential taking account of the fact that conditions are changing both from one year to another and seasonally The user can run scenarios with any combination of interventions, different coverage levels and staggered timings for their introduction. Outputs include projected EIR, parasite prevalence and incidence of clinical malaria over time, which are plotted visually and can be output for further analysis. Thus users can quickly and easily visualise and compare the possible impacts of various control strategies. Future updates will include the ability to enter data on individual program costs so that the cost-effectiveness of different scenarios can be compared.

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EXPULSION OF THE DENGUE VIRUS GENOME BY A PEPTIDE INHIBITOR

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A 33 amino acid peptide (DN59) of identical sequence to the highly conserved amphipathic stem region of the E protein of the dengue 2 virus is able to inhibit DENV entry into multiple cells types with independent entry pathways and at similar low micromolar concentrations. DN59 inhibits entry of all representative flaviviruses tested and is non-toxic to cells. Cryoelectron microscopic images of cell-free, peptide-treated dengue virions suggest that the virions are empty. Three-dimensional reconstructions of these images reveal hollow particles with holes at the five-fold vertices. Peptide treatment of dengue virions in the absence of cellular receptors renders their genomes sensitive to RNase digestion over a wide range of input virus. Western blot analysis suggests that the capsid is still associated with treated virions. Thus, a peptide mimicking a conserved sequence in the membrane-associated stem region of the flavivirus E protein induces expulsion of the viral RNA genome resulting in the inhibition of infection.

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GLUCOSIDASE INHIBITOR AS BROAD-SPECTRUM ANTIVIRAL AGAINST MULTIPLE HEMORRHAGIC FEVER VIRUSES

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Viral Hemorrhagic fever (VHF) designates a group of diseases, caused by enveloped, single-stranded RNA viruses from four different virus families: Arenaviridae, Bunyaviridae, Filoviridae and Flaviviridae. Because many VHF initially do not present with distinguishing symptoms and are difficult to clinically diagnose at early stages, it is important to develop a drug that is universally active against all or most of these agents. Although viruses causing VHF differ in certain features, from the virology point of view, they all have enveloped virions, with viral glycoprotein(s) as envelope. The host ER α-glucosidases are considered to be essential for the maturation, secretion, and function of viral envelope glycoproteins. In this study, we provided genetic evidence that both α-glucosidase I and II are essential host factors for dengue virus and tacaribe virus. Consistent with this notion, we have demonstrated that, imino sugar, the known inhibitor of glucosidases, inhibited multiple hemorrhagic fever viruses from four families (Junin, Dengue, Rift valley fever and Ebola), in tissue culture. Furthermore, treatment with one of our lead imino sugar significantly protected animal death in two mouse models with lethal dengue virus infection. Currently, our extensive SAR study has led to the discovery of several more potent imino sugar derivatives in tissue culture. The *in vivo* efficacy test of these compounds in multiple VHF animal models is underway.

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ECONOMIC IMPACT OF DENGUE ILLNESS AND THE COST-EFFECTIVENESS OF FUTURE VACCINATION PROGRAMS IN SINGAPORE

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Dengue fever and dengue hemorrhagic fever cause 50-100 million cases worldwide and threaten 2.5 billion people in the tropical and subtropical regions. Little is known about the disease burden and economic impact of dengue in resourced countries and the cost-effectiveness of potential dengue vaccines. We estimate the direct and indirect costs of dengue from hospitalized and ambulatory cases in Singapore. We consider *inter alia* the impacts of dengue on the economy using the human-capital and the friction cost methods. Disease burden was estimated using disability-adjusted life years (DALYs) and the cost-effectiveness of a potential vaccine program was evaluated. The average economic impact of dengue illness in Singapore from 2000 to 2009 ranged between US 2010 $0.95 billion and 1.25 billion, with 59-63% corresponding to control costs. We estimated an annual average disease burden of 16-27 DALYs per 100000 habitants, making it comparable to diseases such as meningitis or tuberculosis (22 and 36 DALYs per 100000 habitants, respectively). The rate of symptomatic dengue cases detected by the national surveillance system was estimated to be low, decreasing with age (e.g. ratio of infected symptomatic individuals per detected individual of 1.7-3.8 for 0-24 years, 12.2-50 for >55 years). Potential vaccines are estimated to be highly cost-effective (net savings per DALY averted) for very conservative scenarios, even if vaccination does not reduce the need for vector control. Paediatric vaccination is preferred to mass vaccination for prices per dose greater than $187 and below $365. Mass vaccination, however, presents greater potential of avoided costs and will be preferred for vaccine prices below $187 per dose. If the price of the vaccine is above $45 per dose and below $187, a serology test to administer the vaccine only to non-exposed individuals should complement the mass vaccination program.

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DIFFERENTIAL EFFECT IN MONKEYS AND MAN OF PARTIAL IMMUNITY TO THE CYD DENGUE VACCINE, AND IMPLICATIONS FOR THE SAFETY OF TETRAVALENT CYD VACCINATION

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The sanofi pasteur tetravalent dengue vaccine (TDV) candidate is composed of four recombinant, live, attenuated viruses (CYD-1-4). This vaccine, given in a 3-dose, 0-6-12-month regimen, is currently being investigated in clinical phase 3 trials. In a monkey model we previously observed that pre-existing immunity conferred by bivalent CYD vaccination enhances immune responses to subsequent bivalent CYD vaccination performed 2 months later against the remaining two serotypes, without increasing viremia, an indirect indicator of safety. We explored such a complementary, bivalent vaccination regimen as part of a phase 2 clinical trial (Clinicaltrials.gov: NCT00740155): flavivirus-naïve adult volunteers were vaccinated with a bivalent mixture of CYD-1 and -3, and 3.5 months later with a complementary bivalent mixture of CYD-2 and CYD-4. In contrast to findings in monkeys, immunity conferred by CYD-1 and -3 vaccination neutralized the subsequent CYD-2 and CYD-4 vaccine viruses (absent/lower viremia compared to that observed after tetravalent CYD vaccination) and dampened the serotype 2 and 4 specific immune responses. This inhibition was less marked at the serotype-specific cellular immunity level. From these findings we draw two important conclusions. Firstly, as the first bivalent vaccination provided 'protection' against the second (rather than enhancing viremia and immunogenicity) we propose that in the case of natural infection before the completion of the 3-dose TDV vaccination regimen, the partial immunity elicited by the first one or two doses will not enhance disease, even if antibody responses to the infecting serotype are low; furthermore, these findings suggest that heterologous immunity induced by CYD vaccination may be broadly cross-protective between immunizations. Secondly, the interval of time during which cross-neutralization occurs is longer in humans than in monkeys, further highlighting the importance of interspecies differences in terms of immune responses and immunization regimens.

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SAFETY EVALUATION AND IMMUNOGENICITY OF FIVE TETRAVALENT ADMIXTURES OF THE NIH LIVE ATTENUATED DENGUE VACCINE CANDIDATES

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Dengue virus (DENV) has become the most important arbovirus worldwide with approximately 36 million cases of dengue fever and more than 2 million cases of severe dengue occurring annually. Because a secondary DENV infection with a serotype different from that which caused the

primary infection is a significant risk factor for DHF/DSS, a DENV vaccine must induce a long-lived immune response to all four DENV serotypes. The goal of the National Institutes of Health (NIH) DENV vaccine program is to produce a minimally reactogenic, highly immunogenic, genetically stable, live attenuated DEN vaccine that is cost-effective and safe for the community. Over the past 10 years, the NIH has tested 8 monovalent vaccines in 15 Phase I clinical trials to identify DENV-1, DENV-2, DENV-3, and DENV-4 candidate vaccine viruses that are safe and maintain the optimal infectivity and immunogenicity profiles for inclusion in a tetravalent formulation. Each monovalent candidate was well tolerated by volunteers with no volunteer experiencing a dengue-like illness. Six monovalent DENV candidate vaccines (a DENV-1 candidate, a DENV-2 candidate, two DENV-3 candidates, and two DENV-4 candidates) were evaluated in five different tetravalent admixtures in healthy adult flavivirus-naïve subjects to identify those admixtures with the most favorable safety and immunogenicity profiles. Safety, infectivity, and immunogenicity data of the five admixtures following administration of a single subcutaneous dose will be presented. Up to 90% of subjects had at least a trivalent antibody response following a single vaccination, with an excellent safety profile similar to that observed in the monovalent trials. Preliminary data from a second dose administered 6 months after the first dose will also be presented. Factors contributing to the immunogenicity profiles of the different admixtures will be discussed.

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A RECOMBINANT LIVE ATTENUATED TETRAVALENT DENGUE VACCINE INDUCES NEUTRALIZING ANTIBODIES TO ALL FOUR DENGUE VIRUSES IN HEALTHY ADULT VOLUNTEERS

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The tetravalent live attenuated dengue vaccine (DENVax) is based on the DEN-2 PDK-53 virus. Recombinant DENVax-1, DENVax-3 and DENVax-4 strains were generated in which the prM and E genes of PDK-53 were substituted with those of DEN- 1, -3 or -4 viruses. This approach retains the genetic attenuation markers present in PDK-53. A randomized, placebo-controlled phase 1 clinical trial was performed to evaluate the safety and immunogenicity of tetravalent DENVax formulations in healthy, flavivirus negative adults. The study was completed in Rionegro, Colombia, a high altitude area with no *Aedes aegypti* and no dengue exposure. Low or high dose formulations of DENVax were administered at 0 and 3 months by either intradermal or subcutaneous injection. The vaccine was well-tolerated with mostly mild and transient local or systemic reactions. In addition, DENVax induced significant neutralizing antibody responses to all four dengue viruses after one or two administrations. This study highlights the safety and immunogenicity of the tetravalent DENVax formulations; the vaccine warrants further evaluation in clinical trials in dengue endemic areas.

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PRECLINICAL AND CLINICAL TESTING OF A RECOMBINANT SUBUNIT VACCINE FOR DENGUE

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Dengue viruses are a major cause of morbidity and mortality throughout the tropics and subtropics with an estimated 50-100 million infections annually. To date no specific vaccine or therapy has been licensed to combat this important disease. Live attenuated vaccines for dengue have faced issues with interference between the four viral components. To overcome this issue Merck and Co. is evaluating a tetravalent recombinant subunit vaccine to protect individuals against dengue virus-induced disease. Preclinical studies conducted in mice and non-human primates have demonstrated the immunogenicity and efficacy of both monovalent and tetravalent formulations adjuvanted with alum or ISCOMATRIX™ adjuvant. These studies have shown the capacity of the recombinant proteins to induce balanced tetravalent responses without evidence of interference. Formal preclinical safety assessment studies have demonstrated the acceptable safety of the antigens in various formulations in rats and rabbits. A Phase 1 clinical study of monovalent recombinant protein (DEN1-80E) adjuvanted with alum has been conducted in healthy volunteers and the data from this study will be presented.

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MICRODAM CONTRIBUTION TO THE PRODUCTION OF *ANOPHELES* SPP. IN WESTERN, LOWLAND KENYA

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Anopheles funestus is an important vector of human malaria in Africa yet the production of adults of this species from larval habitats is poorly understood. In western Kenya, this species remains an important malaria vector and has been increasing recently in the Asembo Bay region, where insecticide-treated bed nets are in widespread use. One hypothesis for this resurgence is the increased larval habitat provided for *A. funestus* by small dams ("microdams") built in the area. Constructed simply with earthen dikes, the microdams create small reservoirs (mean surface area ~ 0.2 ha) by impounding stream water and rain run-off from upslope in the water sheds of this gently rolling landscape. They are important sources of water for both humans and livestock in the local communities and conserve soil. Naturally growing vegetation, water-logged hoof prints from livestock, and pools of standing water near the microdams create ideal habitats for anopheline larvae. We georeferenced the microdams and used a combination of larval and adult mosquito collections to quantify the contribution of these microdams to the population of malaria vectors in this region. Microdam architecture revealed an up-slope mud plane providing hoof print habitat for *A. gambiae* and *A. arabiensis* larvae, as did the muddy edges of the dams; while vegetated zones provided habitat for *A. funestus* and *A. coustani* larvae. Collections of adult *Anopheles* indoors using the pyrethrum knockdown method showed that houses closer to the microdams had more *A. funestus* than did houses farther away. While the microdams are important sources of water for the communities, they also contribute to the production of malaria vectors in the area, creating a conflict in this rural landscape.

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ARE TRENDS IN HOUSING AFFECTING VECTORIAL CAPACITY AND MALARIA TRANSMISSION IN AFRICA?

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Changes in housing design and structure are believed to have made a significant contribution to the elimination of malaria from the USA and Northern Europe. Could something similar happen in Africa? Housing changes are occurring, and not only in urban areas: even in villages that a few years ago contained only mud-and-thatch houses, brick walls and metal or tile roofs are now becoming more and more common. Numerous studies have reported an association between such structural features of house design (screening, ceilings, metal roof) and a reduction in mosquito entry and/or the risk of malaria for the human occupants. Here we review these studies, and discuss the hypothesis that housing improvements may cause a reduction not only in house entry by malaria vectors, but also in vector survival and longevity, and hence vectorial capacity. Such effects could have considerable public health value, because they represent a means by which national authorities could plan to reduce malaria receptivity, as part of a very-long-term elimination strategy. Further studies of such effects are needed in order to give evidence-based advice to householders, local authorities and governments about how best to "build out" malaria.

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IMPACTS OF INSECTICIDE-TREATED BEDNETS (ITNS) AND INDOOR RESIDUAL SPRAYING (IRS) ON HOST SELECTION PATTERN BY *ANOPHELES GAMBIAE* S.S., *AN. ARABIENSIS* AND *AN. FUNESTUS* IN WESTERN KENYA

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Malaria continues to be a global public health priority and control interventions are undergoing scale-up of historical proportions. Emphasis has been placed on vector control with insecticides due to its effectiveness, especially when used in nets as insecticide-treated nets (ITNs) and indoor residual spraying (IRS). The WHO has approved vector control using of ITNs and IRS as one of the key malaria control strategies. The use of ITNs and implementation of IRS in selected districts in western Kenya is on the rise, however, mosquitoes are still able to feed and continue to transmit malaria. These interventions may result in marked changes in the vector population structure and behavior, manifested in alteration of biting time and host preference. Knowledge of the biology and behavioral changes of *Anopheles* mosquitoes is important in enhancing understanding of ways of malarial transmission and can further aid in evaluation and designing of appropriate control interventions. A study aimed at determining the actual blood feeding and host preference by *An. gambiae* s.s*., An. arabiensis* and *An. funestus* in the presence of ITNs, IRS or both interventions in western Kenya was conducted in Nyando, Rarieda, Busia and Bungoma districts in western Kenya. Molecular techniques involving Polymerase Chain Reaction (PCR), sequencing and a BLAST search in the GeneBank database were used to test for host blood. In an initial analysis of host blood type in 196 mosquitoes from Asembo in Rarieda district, all samples being *An. arabiensis*, 13.3% had cow blood, 25% human blood, 5% goat blood and 0.5 % rat blood. A number of the analyzed mosquitoes failed either PCR or sequencing, however, the percentages of host blood show a shift in the host selection by *An. arabiensis* with more selections for human blood than cattle in a region with high ITN coverage (above 75%) and high nightly use. Such a shift in the feeding of *An. arabiensis* is of interest since the vector is reported to be highly zoophagic and endophilic. A total of 2000 samples of the three vector species from the remaining study areas are under investigation and results of the study will be presented and discussed.

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EVIDENCE FOR NEW MALARIA VECTOR SPECIES IN THE WESTERN KENYAN HIGHLANDS

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In Africa current malaria control interventions such as indoor residual spraying and long-lasting insecticidal bednets rely heavily on targeting the endophilic and late biting nature of a few key mosquito vectors. The presence of vectors that do not conform to these behaviours may limit the effectiveness of such interventions. In a recent entomological study carried out in Kisii, an area prone to malaria epidemics in Western Kenya, the majority of *Anopheles* caught were morphologically distinct from previously described vectors. For 81% of 475 samples caught, no amplification product was obtained following an *An. gambiae* complex species diagnostic PCR. The ITS2 region of ribosomal DNA was successfully sequenced for 395 samples, of which 73% (n= 289) could not be matched to known sequences. The samples were grouped according to similarity of sequences and phylogenetic trees were made. The most abundant group of samples could not be matched to known sequences (173 of 395 samples sequenced) and could not be identified to species level using conventional morphological keys. Sequencing of the mitochondrial DNA CO1 gene also indicated that the majority had no published sequence, and the resulting phylogenetic tree groupings were similar to the rDNA ITS2 trees for the same samples. Of all samples sequenced, 5 were found to be sporozoite positive for *Plasmodium falciparum* by ELISA, all of which were caught outdoors. These 5 had no previously published ITS2 or CO1 region sequence and 3 fell into the most abundant sequence group. 86% of this group (149/173) were caught outdoors and the majority (77%) were caught before 22:30, prior to when people enter their houses. Preliminary morphological identification of house spray catches and larval collections from 2009 and 2011 in 6 other villages across highland Nyanza Province, also revealed the presence of this species. These results indicate the presence of a novel malaria vector in the highlands of Nyanza with early, outdoor biting behaviour. The implications of these findings in relation to malaria control in the area will be discussed.

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COLLAPSE OF *ANOPHELES DARLINGI* POPULATIONS IN SURINAME AFTER INTRODUCTION OF INSECTICIDE-TREATED NETS (ITNS); MALARIA DOWN TO NEAR ELIMINATION LEVEL

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A longitudinal study of malaria vectors, aiming to study *Anopheles darlingi* population dynamics, man-biting and sporozoite rates, was carried out in three villages in the Interior of Suriname between January 2006 and April 2010. During 13,392 man hours of human landing collections, a total of 3,180 female mosquitoes were collected of which 33.7 % were anophelines. *An. darlingi* and *An. nuneztovari* accounted for 99.2 % of the total anophelines collected. The highest mean human biting rate (HBR) observed per survey for *An. darlingi* was 1.43 bites/man/hour outdoors and 1.09 bites/man/hour indoors. Individual ELISA assays of 683 anophelines yielded two *An. darlingi* females infected with *Plasmodium falciparum*. The anopheline HBR decreased to zero in all sites after the onset of malaria intervention activities in 2006, which included the mass

distribution of ITNs. Malaria transmission decreased significantly and Suriname reached the Millennium Development Goal for malaria in 2007. It is concluded that the combination of ITN introduction and climatic events have led to the disappearance of malaria vectors in the study sites in the interior of the country.

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DIFFERENCES IN *ANOPHELES GAMBIAE* GENE REGULATION IN RESPONSE TO INGESTION OF LOCAL AND GEOGRAPHICALLY DISTANT ISOLATES OF *PLASMODIUM FALCIPARUM*

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On ingestion of malaria parasites, *Anopheles gambiae* is known to regulate a suite of genes and launch an immune response. Most research on this host-parasite interaction has been done using either model systems or mosquito and parasite strains colonised many years ago. With little research using natural malaria systems the details of host-parasite interactions in the wild remain poorly understood. This study aimed to determine *An. gambiae* gene regulation in recently colonised strains after ingestion of wild *Plasmodium falciparum* isolates. Mosquito strains from Cameroon and Burkina Faso in Central and West Africa were experimentally infected in parallel with their local and distant parasite isolates for comparison. Whole genome microarrays were completed on the mosquitoes 24 hours after infected blood meal ingestion. Eight biological replicates were completed with both the local and distant parasite. Gene regulation was found to be highly diverse across all infections, suggesting that the natural genetic polymorphism present in the samples used here but absent from most previous studies has a great effect on host-parasite interactions, leading to specific gene regulation. Previous work comparing these local and distant infections showed that local mosquito-parasite combinations produce lower infection intensities. The current study highlights a small number of mosquito genes potentially involved in these interactions that are consistently regulated in local infections but not in distant ones. It also shows that the most important factor in determining gene regulation patterns is the parasite population rather than the mosquito strain or local/distant pair. These findings have great impacts on future malaria control methods via the mosquito, especially those which aim to interfere with host-parasite interactions. The fact that these interactions may be differentially evolving over space and or time means that any such control method must be vigorously tested before implementation.

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PLASMODIUM FALCIPARUM DOES AFFECT SURVIVAL OF ITS NATURAL VECTOR *ANOPHELES GAMBIAE*

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The question of the effect of malaria parasites on vector survival has been raised for decades but is still not properly resolved. Several studies addressed the subject but most of them used unnatural *Plasmodium-Anopheles* combinations and therefore disregarded the coevolution between the parasite and the vector. In the most relevant couple for human malaria, *A. gambiae- P. falciparum*, the number of studies is very limited and results are contrasted due to difficulties to obtain large samples of wild-caught infected mosquitoes and to assess their age. In our study, we exposed *A. gambiae* females of a colony from Burkina Faso to *P. falciparum* isolates from naturally infected patients of the same area. An adequate negative control was determined to obtain a corresponding pool of non infected mosquitoes fed on the blood of the same donor. Females exposed and non-exposed to the parasites were maintained until death and dissected immediately after to measure the level of infection. In optimal environment for the mosquitoes, we observed an effect of infection on mosquito survival in interaction with the origin of the blood. This interaction could be the result of variation in parasites (more or less virulent genotypes; single vs. multiple infections), human patients, or less probably in mosquitoes. In conditions of limited nutritional resources after the blood meal, the infected mosquitoes had significantly shorter lifespan than non-infected mosquitoes. This is the first clear evidence of a negative effect of *P. falciparum* sporogony on the survival of its natural vector *A. gambiae*. In malaria transmission, longevity is the most important factor of vectorial capacity. The results have important implications in understanding evolutionary forces that maintain susceptible and resistance alleles to parasite infections in natural mosquito populations. Moreover, the effect of the parasite on mosquito survival might impact malaria control strategies that would aim at increasing mosquito vector refractoriness or specifically target infected mosquitoes.

1455

CHARACTERIZATION OF THE VIRAL FITNESS OF NORTH AMERICAN WEST NILE VIRUS ISOLATES BY *IN VIVO* COMPETITION IN BIRDS AND MOSQUITOES

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West Nile virus (WNV) is a mosquito-transmitted flavivirus of global public, veterinary and wildlife disease importance. Genetic changes in the genome of the invading WNV strain (NY99) have given rise to new seemingly better mosquito-adapted genotypes (WN02) that may represent a key evolutionary mechanism enabling the persistence of WNV. Although some newly emerged WNV genotypes have been genetically characterized, no in-depth study has compared the phenotypic performance and *in vivo* competition fitness of these genotypes in biologically relevant avian and mosquito hosts. Our study addresses the viral fitness of several North American WNV strains utilizing an *in vivo* competition fitness assay in House finches and *Culex tarsalis* mosquitoes. The founding 2003 California WNV isolate belonging to the current dominant WNV genotype (WN02) was genetically marked by site-directed mutagenesis and used as the reference strain for competition against the original invading strain (NY99) and post invasion California isolates. Starting with a 1:1 virus ratio for inoculation, the outcome of competition between the two virus populations was analyzed based on individual replication data resulting from a novel RT-PCR approach, specifically designed for genetic distinction and quantification of mixed virus competition samples. Fitness in birds was determined by examining sera obtained throughout the viremia period and from tissues collected post mortem. In mosquitoes, fitness was evaluated based on the ability of each virus to infect, disseminate and be transmitted after extrinsic incubation. Here, we report a novel diagnostic approach for the quantitative detection of nucleotide polymorphisms, facilitating viral competition studies. Furthermore, we present data on the fitness of North American WNV isolates in their natural hosts and discuss our findings in the context of WNV evolution and persistence.

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TEMPORAL AND SPATIAL FLUCTUATIONS IN ARBOVIRUS MUTANT SWARMS

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Arboviruses often exist within hosts as a swarm of closely related minority genotypes. Although the size and composition of these mutant swarms can have direct phenotypic consequences, the specifics of how stochastic and selective pressures shape arboviral swarms is not fully defined. Although host-specific differences in mutant swarm breadth have been identified with arboviruses, the influence of genetic bottlenecks in mosquitoes during initial virus infection of midgut cells, egress from midgut tissue, salivary gland infection and, ultimately, host transmission remain uncharacterized. In addition to these spatial influences, temporal changes are also important in shaping swarm dynamics both within hosts and throughout seasons. In order to better define swarm dynamics, we've combined experimental studies with genetic analyses of natural isolates and evaluated changes to West Nile virus (WNV) and St. Louis encephalitis virus (SLEV) through time and space. Specifically, studies with WNV in *Cx. pipiens* following feeding on artificial swarms have begun to define within-host bottlenecks. Results indicate that when infecting with equal proportions and high input titers (>8.0 $\log_{10}$ pfu/ml) mutant swarm breadth is maintained spatially throughout the mosquito, yet a significant decline in diversity occurs over time. Results from feeding on different variant ratios indicate also that proportions of variants are maintained in early midgut infection and replication, yet stochastic pressures may result in the dominance of rare minority variants (<2.5%). In addition, analyses of primary mosquito isolates of SLEV from TX and CA indicate a decline in intrahost diversity over time, possibly reflecting the role of seasonal bottlenecks in limiting swarm breath. Studies with WNV isolates from NY are also providing insight into the genetic consequences of local maintenance. These data significantly advance our understanding of how intra- and interhost dynamics alter viral swarms over time and space and how such fluctuations could impact virus evolution and adaptation.

1457

NATURALLY OCCURRING WEST NILE VIRUS DELETION MUTANTS: ROLE AS DEFECTIVE INTERFERING PARTICLES AND INFLUENCE ON PATHOGENESIS *IN VIVO*

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Due to error-prone replication, RNA viruses such as West Nile virus (WNV; Flaviviridae, Flavivirus) exist in individual hosts as heterogeneous populations of related genomes. Genomes that contain deletions are frequently overlooked components of these populations because they are inefficiently detected by conventional approaches to sequencing the virus genome and they are widely considered to be replication-incompetent, dead-end byproducts of infection. We have identified three WNV isolates from birds that have as a portion of their population mutants with large (~2.5 kb), in-frame, internal deletions to their structural coding sequences. To determine whether these mutants function as defective-interfering (DI) genomes, we infected Vero cells at a range of multiplicities of infection (MOI) and evaluated virus output and whether the deletions were maintained through subsequent passage. At high MOIs, deletion mutants persist through several passages and reduced production of full length virus, relative to an infectious clone derived control. Therefore, the mutants appear to act as DI genomes *in vitro*. To determine whether deletion mutants can influence pathogenesis and/or WNV persistence *in vivo*, we infected C3H and C57 Bl/6 mice and day old chickens with virus containing deletion mutant or a full length virus purified from the same isolate, and evaluated morbidity, mortality and virus persistence. These studies allow a clearer understanding of how genomes that contain large in-frame deletions can influence virus replication and pathogenesis, and may shed light on mechanisms for WNV persistence in vertebrates, an emerging health concern in particular regions where the virus is enzootic.

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GENETIC DIVERSIFICATION AND DYNAMICS OF WEST NILE VIRUS IN A NORTHERN TEMPERATE REGION: CONNECTICUT 1999-2008

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West Nile virus (WNV) has become firmly established in northeastern U.S., reemerging every summer since its introduction into North America in 1999. To determine whether WNV overwinters locally or is reseeded annually, we examined the patterns of viral lineage persistence and replacement in Connecticut over 10 consecutive transmission seasons by phylogenetic analysis. In addition, we compared the full protein coding sequence among WNV isolates to search for evidence of convergent and adaptive evolution. Viruses sampled from Connecticut segregated into a number of well-supported subclades by year of isolation with few clades persisting ≥2 years. Similar viral strains were dispersed in different locations across the state and divergent strains appeared within a single location during a single transmission season, implying widespread movement and rapid colonization of virus. Numerous amino acid substitutions arose in the population but only one change, V to A at position 159 of the envelope protein, became permanently fixed. Several instances of parallel evolution were identified in independent lineages, including one amino acid change in the NS4A protein that appears to be positively selected. Our results suggest that annual reemergence of WNV is driven by both reintroduction and local-overwintering of virus. Despite ongoing diversification of WNV, most amino acid variants occurred at low frequencies and were transient in the virus population.

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CHARACTERIZATION OF WEST NILE VIRUSES ISOLATED FROM CAPTIVE AMERICAN FLAMINGOES (*PHOENICOPTERUS RUBER*) IN MEDELLIN, COLOMBIA

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Serum samples were collected from captive otherwise healthy wild birds in the summer of 2008 at the zoological collection in Medellin (Colombia) and tested for the presence of flaviviruses. Total RNA was extracted from sera and tested by reverse transcription-polymerase chain reaction (RT-PCR) using both universal flavivirus and West Nile virus (WNV) specific primers. Eight serum and swab pools from groups of 12-14 wild birds and containing 20 species were evaluated. Interestingly, eighteen samples of twenty five tested from American Flamingoes (Phoenicopterus ruber) were positive for WNV. Selected samples were then inoculated onto subconfluent monolayers of *Aedes albopictus* C3/36 cells and three serially blind passages were conducted before confirmation of virus presence by immunofluorescence. Four isolates (524, 739, 928, and 9835) were further selected for full sequence analysis as well as *in vitro* and *in vivo*

phenotypic characterization. All RT-PCR products revealed West Nile viruses. In addition, sequence analysis showed a total of 15 nucleotide changes resulting in 6 amino acid substitutions in comparison to the WNV New York 1999 strain. Further analysis has confirmed that these viruses are more closely related to Louisiana isolates from 2002. All viruses were highly cytopathic on BHK21 cells while no cytopathic effect was observed on Vero cells. Viruses were highly pathogenic in embryonated chicken eggs and newborn mice. Surprisingly, these isolates diverged in their pathogenicity in 4 week-old Balb/c mice. The epidemiological implications of this new West Nile virus in Colombia and the potential effect on wild and domestic animals as well as human populations are currently being investigated.

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URBAN ECO-EPIDEMIOLOGY OF WEST NILE VIRUS IN ATLANTA, GEORGIA

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Since its introduction in 1999, West Nile Virus (WNV) has become the most important mosquito-borne disease in the USA. WNV activity in the mosquito vectors and reservoir hosts (birds) is clustered in space and time, with transmission focused in certain urban centers (in the East and Midwest) during the summer. However, not all urban areas with intensive enzootic activity see corresponding human cases of disease. In Georgia, substantial WNV presence in the vector and host species has not translated into a large number of human cases, reflecting a similar pattern seen throughout the Southeast, one that is in sharp contrast to some urban areas in the Northeast and Midwest. In a study conducted in Atlanta, Georgia's major urban center, we are addressing the question: in the face of abundant reservoir hosts, disease vectors, and viral presence, why is spillover transmission of WNV (beyond the enzootic) suppressed? We perform comprehensive avian and mosquito sampling in a variety of urban microhabitats, over multiple seasons, to determine the distribution, density, and prevalence of WNV infection in the host and vector species of Atlanta. We focus on sampling in four habitat types within the urban center: mixed-use parks, old-growth forest patches, residential areas, and outdoor animal-holding facilities. Fine-resolution aerial imagery is used to characterize habitat types, percent tree cover, and height of the tree canopy. Avian point counts are conducted at each site to estimate bird species richness and abundance. Using these data, we evaluate the role of Atlanta's diverse urban habitats in disease transmission, focusing on differences in percent tree cover and height of the tree canopy in constraining WNV transmission in time and space. We also explore the extent to which the diversity of avian host species in Atlanta contributes to a WNV "dilution effect." This study targets some of the complex ecological factors governing vector-borne disease transmission in urban settings, combining ecological, epidemiological, and general public health approaches.

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WEST NILE VIRUS (WNV) EPIDEMIOLOGY IN NEW YORK STATE: DO HYDROGEOGRAPHY AND CLIMATE VARIABLES INTERACT IN THE OCCURRENCE OF WNV IN HUMAN CASES AND MOSQUITO POOLS?

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The complex ecology that determines West Nile virus (WNV) epidemiology is not completely understood. The interaction between climate and hydrogeography (HG) in WNV occurrence has not been identified. This study examines the distribution of mosquito pools positive for WNV (WNVm) and human WNV cases (WNVh) across the 62 counties of New York State (NYS) during the 2006 WNV season (May through October). Climate data were obtained from the National Climate Data Center, HG data came from the USGS National Hydrology Dataset, and WNVh and WNVm surveillance data were obtained from the NYS Department of Health. The distributions of total precipitation (PR) during the WNV season, mean July temperature (TM) adjusted for mean temperature standard deviation for May through October, HG, and WNVh and WNVm were mapped in ArcGIS, and specific county clustering was identified using the local Moran's Index (LMI). Poisson regression was used to model the associations between each of the 2 outcomes, WNVh per county population and WNVm per square mile, and the climate and HG variables. WNVh and WNVm were concentrated in the coastal counties corresponding to the Atlantic Ocean/Long Island Sound and Lake Ontario Tributaries watersheds in the southeastern and western counties, respectively. The LMI indicated a high degree of local WNV clustering. The HG parameter, total surface water area (SWA), was an effect-modifier of the association between the presence of WNV and the amount of PR per county. A significant inverse relationship was observed between total PR and WNVh (IRR=0.99; $p<0.0001$) among counties with low SWA (< the median SWA), whereas high SWA (≥ the median SWA) counties showed no association between PR and WNVh (IRR=1.0; $p=0.5$). The same effect-modified associations between PR and WNVm were observed for low (IRR=0.99; $p<0.0001$) and high (IRR=1.0; $p=0.1$) SWA. The associations between TM and WNVh and WNVm were modified in the same way by SWA. These findings suggest the possibility that associations between precipitation and WNV may depend on the surface water present.

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NITRIC OXIDE MEDIATES EXPERIMENTAL HOOKWORM INFECTION

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Hookworm infection is a major cause of anemia, malnutrition, and growth delay in resource poor countries, where more than 500 million people are infected. Human and animal studies confirm that infection with these intestinal nematodes is associated with suppression of the host immune response. We have previously reported higher levels of nitric oxide (NO) from supernatants of spleen cells harvested from *Ancylostoma ceylanicum* infected hamsters compared to uninfected animals. In order to further characterize the role of NO in hookworm pathogenesis and pathology, *in vivo* experiments involving inhibition of NO secretion using N-Monomethyl-L-Arginine (L-NMMA) were conducted. Ten male golden Syrian hamsters were infected with 100 third stage larvae (L3) of the hookworm *A. ceylanicum*. Five infected hamsters received a daily intraperitoneal (IP) injection of L-NMMA starting on day 0 post-infection (PI), while five others had a daily IP injection of PBS. Uninfected control animals received a daily IP injection of either L-NMMA or PBS. At day 36

PI, infected L-NMMA-treated hamsters showed reduced intestinal worm burdens (4 ± 2) compared to PBS-control treated animals (21 ± 4, p < 0.005). Flow cytometry analysis using splenocytes at day 36 PI revealed a higher proportion of $CD4^+$ T cells in infected L-NMMA-treated hamsters than in control animals (12.5 ± 1.2 % vs. 6.8 ± 0.7 %, p= 0.001); a similar difference was also observed for surface IgG^+ B cells (32.0 ± 1.4% vs. 23.1 ± 4.3 %, p= 0.03). There was no difference between uninfected PBS-treated controls and L-NMMA-treated hamsters in the proportion of CD4+ T cells (27.8 ± 1.8 % vs. 27.7 ± 1.8 %) or surface IgG^+ B cells (44.83 ± 1.0 % vs. 46.7 ± 3.1 %). Infected L-NMMA-treated hamsters also had higher blood hemoglobin levels than infected PBS-treated control animals. Together, these data demonstrate that NO modulates both hookworm infection intensity and anemia *in vivo*. Experiments are underway to identify the mechanism(s) by which NO mediates hookworm disease pathogenesis and blunts host cellular immune responses.

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HOOKWORM INFECTION AMONG SCHOOL AGE CHILDREN IN THE KINTAMPO NORTH MUNICIPALITY, GHANA: NUTRITIONAL RISK FACTORS AND RESPONSE TO SINGLE DOSE ALBENDAZOLE TREATMENT

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A cross-sectional study of hookworm infection and household nutrition was conducted in the Kintampo North Municipality, Brong Ahafo Region, Ghana. Children (N=844) between the ages of 6 and 11 years attending 16 schools were screened using anthropometry. Study participants were selected from the tails of a normal distribution of stunting (Height for Age Z-score (HAZ) <= -1.80 or HAZ >= -0.10). Hookworm prevalence at baseline was 39% (109/279), while the overall prevalence of anemia was 62% in the study population. Of children who provided fecal and blood samples (N=248), 35% were co-infected with both hookworm and malaria, 50% were infected with malaria alone, and 3% were infected with hookworm alone. Nearly all (96%) of the hookworm infections were light (<2000 eggs/gram of feces). When controlling for age, gender, household absolute wealth index, and history of recent deworming, statistically significant risk factors for baseline hookworm infection included malaria co-infection (p<0.05), access to health care (p<0.01), lower weekly consumption of protein-rich food groups (p<0.05), and household geographic location (p<0.05). The degree of stunting based on HAZ did not correlate with hookworm infection status at baseline. Hookworm-infected children (N=109) received a single oral dose of albendazole (400mg) and follow-up fecal analysis revealed an overall egg reduction rate of 88% in the study population, with a cure rate of 44%. No single variable collected in this analysis was significantly associated with remaining hookworm positive after receiving a single dose of albendazole. These results confirm prior observations of treatment efficacy for single dose albendazole against hookworm in Kintampo, and offer insight into the potential for current school based deworming programs to impact hookworm disease in areas of moderate prevalence and low infection intensity. Future studies are needed to more clearly define the role of nutritional parameters, including dietary protein intake, in mediating susceptibility to human hookworm infection.

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MATERNAL GEOHELMINTH INFECTIONS INCREASE SUSCEPTIBILITY TO INFECTION IN CHILDREN

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In utero exposure to helminth infections may affect newborn immunity and influence susceptibility to infection during childhood. To test the hypothesis that maternal geohelminth infections increase susceptibility to infection in children, we conducted a nested case-control study in an area of Ecuador where geohelminths are endemic.1004 children with infection data from between 7 months and 3 years of age were selected from an ongoing cohort study. Cases were children with *Ascaris lumbricoides* and/or *Trichuris trichiura*, controls without. Exposure was defined as the presence of maternal infection with *A. lumbricoides* or *T. trichiura* detected in a stool sample collected in the third trimester of pregnancy. To control for risk of infection, the study was restricted to households with at least one family member infected with *A. lumbricoides* and/or with T. trichiura, as determined by stool samples collected after the child's birth. Children of mothers with geohelminth infections had a significantly greater risk of infection relative to children of uninfected mothers (46.3% exposed vs. 30.7% unexposed, adjusted OR 2.62, 95% CI: 1.90-3.62, p<0.001). This effect was particularly strong in children of co-infected mothers (62.6% exposed vs. 30.7% unexposed, adjusted OR: 5.57, 95% CI: 3.46-8.98, p<0.001). No significant differences were observed between intensities of infection or between distributions of egg counts. Our data suggest that maternal geohelminth infections increase susceptibility to infection during early childhood. This may be due to *in utero* immune modulation by maternal geohelminth infections that induce greater neonatal and childhood tolerance.

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IMMUNE REGULATION BY TREG SUBSETS DURING HUMAN GEOHELMINTH INFECTION: EFFECT OF ANTI-HELMINTH TREATMENT

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Chronic helminth infections induce profound regulatory immune responses, leading to strong T cell hyporesponsiveness. The reduced immune reactivity does not only affect parasite antigens, but may also be extended to vaccines or to other coinciding pathogens, such as malaria parasites. Helminths induce regulatory T (Treg) cells, characterized by high expression of CD25 and FOXP3, which are able to downregulate effector T cell responses. The question arises whether Tregs are responsible for the immunosuppressive state during human helminth infection and might have consequences on bystander responses. To investigate Tregs in helminth infections, we set up a study in a rural area on Flores island, Indonesia where schoolchildren with or without helminth infections were selected. $CD4CD25^{hi}$ T cells were magnetically depleted from PBMC and subsequently, mock- and Treg-depleted PBMC were cultured with BCG, *Plasmodium falciparum* parasitized RBC (pRBC) or control RBC. Proliferation and cytokine responses were analyzed as function of helminth infection status. Proliferation to BCG and pRBC was reduced in geohelminth-infected compared to uninfected children. This difference was not reflected in changes in Treg frequencies in peripheral blood. However, following $CD4CD25^{hi}$ T cell depletion proliferation in the

helminth-infected group was restored to levels similar to those seen in helminth negatives. Removal of $CD4CD25^{hi}$ T cells also increased the IFN-γ production in response to BCG and pRBC only in helminth positive children. Although numbers of Treg were not increased, Tregs displayed a higher capacity to suppress proliferation and IFN- γ production to bystander antigens in geohelminth-infected compared to uninfected children. In an ongoing anti-helminth treatment trial, we are investigating Treg phenotype and function in a larger cohort at Flores island. Treg depletion assays were carried out and repeated 1 and 2 years after anti-helminth treatment, enabling us to see differences in helminth-infected, -uninfected and -treated individuals. Cytokine and proliferation data from the longitudinal Treg study are still under analysis, but this is expected to be finalized by September 2011. Treg depletion in human helminth infection is a new concept and this helminth-induced effect on the immune system may have major consequences in vaccine implementation and malaria elimination strategies.

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INCREASED LOCAL REGULATORY T CELLS AND DIMINISHED IGE EXPRESSION IN DUODENAL MUCOSA OF SS/HTLV-1 COINFECTED PATIENTS

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Strongyloides stercoralis (SS) is an intestinal nematode unique in its ability to replicate in the human host, permitting ongoing cycles of autoinfection, persisting for decades within host. Although usually asymptomatic, overwhelming infections can occur in SS and HTLV-1 co-infected individuals (SS/HTLV-1). Regulatory T cells (Treg) are able to blunt specific Th2 response necessary to control the parasite. We previously reported that peripheral blood Treg are increased in SS/HTLV-1 and correlate with low Th2 responses. We hypothesized that local Tregs are increased in duodenal mucosa of SS/HTLV-1 patients. Paraffin embedded duodenal biopsies were obtained from 10 HTLV-1/SS subjects and 3 control samples from non-parasitic chronic duodenitis (NPCD) subjects. Immunohistochemistry was performed with CD3, IgE and FoxP3 human mAbs. The numbers of cells were counted using a conventional light microscope. 400x magnification images were taken and the area was measured using the ImagePro Plus software. The numbers of CD3+, FOXP3+ and IgE positive cells per 0.35 mm^2 were assessed. Patients with HTLV-1/SS had higher T lymphocyte counts in areas non-adjacent to the parasite (NAP) compared to areas adjacent to the parasite (AP) (NAP [P50]: 24.5 IQR: 20.3-36.3, AP [P50]: 6.5 IQR: 2.8-12.3, p=0.0003 Mann-Whitney). The number of IgE expressing cells was higher in areas non-adjacent to the parasite (NAP [P50]: 35 IQR: 17.5-57.5, AP [P50]: 3 IQR: 0.5-6.0, p=0.001 Mann-Whitney). Patients with HTLV-1/SS show an increased counts of Tregs cells (FoxP3+ expressing cells) when compared with patients with non-parasitic chronic duodenitis (SS/HTLV-1 [P50]: 3.3 IQR: 0.6-8.4, NPCD [P50]: 0.0 range 0-1.2, p=0.099 Mann-Whitney). In conclusion, our data shows an increased Treg cell count in the duodenum of SS/HTLV-1 patients. In addition, T lymphocytes and IgE expressing cells were diminished in parasite adjacent areas. Altogether, this study suggests an important role for Tregs in down-regulating local parasite effector responses during SS/HTLV-1 co-infection.

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SEROPREVALENCE OF ANTIBODIES TO *STRONGYLOIDES STERCORALIS* NIE AS A TOOL TO IDENTIFY COMMUNITIES FOR ANTHELMINTIC INTERVENTIONS IN NORTHERN ARGENTINA

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Infections with *Strongyloides stercoralis* (Ss) are cosmopolitan and mostly subclinical; new diagnostic approaches are needed to define the prevalence and distribution of infections and to monitor intervention activities. The identification of Ss-specific recombinant antigens increases the availability of serologic assays for these efforts. We evaluated the performance of Ss-NIE-1 by ELISA previously shown to be sensitive and specific in the setting of a mass deworming program in a rural community of northwestern Argentina (S 22°53´60´´; W64°20´06´´) with a total population of 618 people. Prior to drug administration for community-wide treatment, a subset of individuals was randomly selected for soil transmitted helminth (STH) assessment at baseline evaluation. Of the 80 persons (mean±SD age: 28.5± 20) who participated in the Ss study, 48 (mean±SD age: 30.5± 21) were also evaluated by stool analysis that included 4 techniques (concentration-sedimentation, agar plate, Harada-Mori and Baermann with bone charcoal culture). Serum samples were analyzed with the Ss-NIE-1 ELISA. The results demonstrated Ss larvae in 16% (8/48) stool samples; in contrast, 31% (25/80) were positive by serology (mean±SD age: 32.8.5± 20.8). Thus, there was a significant difference between the stool and more sensitive serum analysis ($p<0.05$ by Fisher's exact test). Although three stool positive individuals (mean±SD age: 31.3± 22.4) were Ss-NIE seronegative, the known improved sensitivity of the SS-NIE ELISA for Ss infection with its higher throughput and ease of performance provides an epidemiologic tool to identify Ss-endemic regions of the world in need of anthelmintic therapy. Moreover, the Ss-NIE ELISA confirmed the high prevalence of this STH in the study area, which aids in region-wide strategies for community intervention for this important STH.

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STAGE-SPECIFIC GENE EXPRESSION BY *STRONGYLOIDES STERCORALIS* FOLLOWING HOST INVASION: A MICROARRAY-BASED ANALYSIS

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The key molecular factors that enable *Strongyloides stercoralis* (Ss) larvae to initiate infection have not been elucidated to date, and are critical for developing new insights into the biology of this nematode and identifying novel drug and vaccine targets. We describe here a microarray analysis of gene expression by infective third stage larvae before (L3i) and 72 hours after (L3+) host invasion. Differentially labeled cDNA obtained from RNA extracted from these larvae was hybridized to a Ss DNA microarray. Genes that were more highly expressed in either stage were identified based on a cutoff of 2 fold increased gene expression and adjusted p-value < 0.01 [false discovery rate of 1%]. Using this method, 96 differentially expressed genes were identified. Expression of genes putatively encoding extracellular matrix proteins, such as collagen, was notably increased in L3i compared to L3+ larvae (p = 0.0003). By contrast, an enzyme homologous

to *Brugia malayi* chitinase (BLAST E value 4E - 009) was significantly expressed in the L3+ stage (p = 0.001). Following invasion, genes putatively encoding components of the ubiquitin proteasome pathway were highly expressed. L3+ larvae additionally demonstrated a significant increase in the number of differentially expressed genes encoding enzymes with putative catalytic activity (such as hydrolases, ligases and transferases ; p = 0.02). Stage-specific differences in metabolism were found. A significantly higher number of differentially expressed L3i genes were putatively involved in energy metabolism (p = 0.042), while twice as many differentially expressed L3+ genes were putatively involved in amino acid, carbohydrate and lipid metabolism. These data indicate that Ss larvae downregulate expression of extracellular matrix and energy metabolism genes, and increase expression of genes encoding catalytic enzymes following host invasion, and provide important clues regarding differentiation to the L3+ stage and establishment of infection following host invasion.

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THE IMPACT OF A COMMUNICATION CAMPAIGN ON USE OF ORAL REHYDRATION SOLUTION BY MOTHERS OF CHILDREN UNDER FIVE IN MALAWI

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The use of oral rehydration solution (ORS) has stagnated at 40% in most countries since 1995. Population Services International (PSI) began a social marketing program in 2005 in Malawi to improve access to and use of *Thanzi* ORS for children under five. PSI uses targeted communications to bring about behavior change in early recognition and treatment seeking during diarrheal illness. Repeated household surveys were used to evaluate the impact of the communications campaign. Nationally representative household surveys were conducted in 2005 and 2008, and are complemented by 2010 preliminary DHS results. Guided by a conceptual framework for understanding caregiver behavior, multi-item scales were developed to measure opportunity, motivation and ability factors. Factor and reliability analysis guided scale development. At baseline, multivariate logistic regression was used to test for adjusted associations between hypothesized behavioral determinants and diarrhea treatment. At follow-up, significant changes in behavior and behavioral determinants were examined. At baseline (2005), perceived availability of ORS and knowledge of causes of diarrhea were found to be key determinants that differentiated users of ORS from non-users. Baseline results guided development of a communication campaign involving mass media and interpersonal communication. In 2008, perceived availability, knowledge, and self efficacy improved significantly. Similarly, attitudes toward the *Thanzi* brand improved. In 2005, 58.1% of children with diarrhea in the past two weeks were reported to have taken ORS. This increased to 64.3% in 2008 and to 69% in 2010. *Thanzi* ORS made up 75% and 83.7% of the ORS utilized in 2005 and 2008 respectively. Although a number of factors could have resulted in the increased use of ORS to treat diarrhea in under-five children in Malawi, *Thanzi's* proportionally large ORS market share, marketed by PSI using a social and behavior change approach, is indicative of the role that evidence-based social marketing interventions can play in improving diarrhea treatment.

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DO LIMES ACCELERATE SOLAR DISINFECTION OF WATER (SODIS)? A LAB/FIELD STUDY COMPARING EFFICACY WITH MOUSE NOROVIRUS, *E. COLI* AND MS2 BACTERIOPHAGE

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Two million people in the world die annually of diarrheal disease, mostly among the 850 million people who do not have access to sources of improved drinking water. Household water treatment (HWT) can prevent waterborne illness. Solar disinfection of water (SODIS) is a HWT method that involves exposing water bottles to sunlight for 6 hours. A potential mechanism to increase use of SODIS would be to reduce the treatment time. Psoralens and acids both interact synergistically with ultraviolet radiation to accelerate inactivation of microbes. This study replicated field-based SODIS conditions by using 2L bottles and dechlorinated tap water in solar experiments (n=8). SODIS + lime juice bottles contained approximately one-half lime in 2L water. 5-Methoxypsoralen was used as a control for naturally occurring psoralens. Treatment efficacy (n=3) was evaluated for E. coli, MS2 bacteriophage, and mouse norovirus. E. coli was ablated >6.08log by SODIS + lime slurry, >5.98log by SODIS + lemon juice, and 5.59log by SODIS + lime juice in 30-minute solar exposures, compared with 1.50log for SODIS alone. MS2 was inactivated >5.77log by SODIS + lime slurry, 3.10log by SODIS + lime juice, and 2.73log by SODIS alone in the sunniest of three 2.5-hour solar exposures. In contrast, mouse norovirus, a surrogate for human norovirus, was highly resistant to all forms of SODIS. Using simulated sunlight in laboratory ultraviolet experiments, SODIS + lime slurry and SODIS + lime juice inactivated mouse norovirus 2.03log and 1.84logs, respectively, after 6 hours lamp exposure, while conventional SODIS achieved only a 0.30log reduction. In field-based conditions, all treatment bottles (n=3) showed <2log reductions in mouse norovirus after a 6-hour solar exposure. A pH of <4 facilitated inactivation of E. coli, while psoralens were more effective than pH in inactivating viruses. SODIS + citrus dramatically reduced E. coli levels in just thirty minutes, a treatment time on par with boiling. Furthermore, familiarity with citrus juice may make this method appealing to potential users. Mouse norovirus, a human norovirus surrogate, was highly resistant to all SODIS treatments. The efficacy of SODIS against human norovirus should be investigated further.

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AN ACCEPTABILITY AND FEASIBILITY PILOT OF HOUSEHOLD LEVEL WATER TREATMENT IN URBAN BANGLADESH

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Household water treatment prevents diarrhea. However, most household water treatment interventions fail to be used consistently. We conducted a small trial in urban Bangladesh to assess the acceptability of various approaches for point of use water treatment. In May and June 2010, we used the trial of improved practices methodology in 15 low income compounds with a total of 88 individual households. Of 3 available water treatment options, we introduced and provided 1 option in each compound for a free 30 day trial. These options included liquid sodium hypochlorite from a dispenser for communal use (n=58), liquid sodium hypochlorite from a dropper bottle for household use (n=20), and a double chamber ceramic filter for household use (n=10). For the 78 households that received chlorine-based options, we provided

standardized water containers to 51households. In each of 5 follow-up visits we conducted either semi structured interviews, household observations, and/or group discussions. The double chamber ceramic filter had the most (10/10), and the chlorine dispenser had the least (10/58) sustained self reported use. Most of the participants were able to follow the operating instructions as observed during follow up visits. However, 25/27 participants without the standardized water container compared with only 5/51 participants with the standardized container had difficulty achieving the correct concentration of chlorine. Frequently mentioned benefits of using the various technologies included ease of use, the clarity of treated water and being less time consuming than boiling water. Barriers in using the technologies included the strong smell of chlorine, lack of a standardized storage container with the chlorination options, and the leaking chlorine dispenser's valve. The double chamber water filter was most acceptable because the filtered water did not have any odor, whereas, water chlorination was less acceptable due to the strong smell of chlorine. Odor minimizing techniques, including better models of dispenser that do not leak and standardized containers should be tested. Prospective users should be reassured that the odor is inconsequential to their health and well-being. In addition, the reported benefits, such as clarity of treated water compared to supply water and the short treatment time required can be used to promote sustainable water treatment behavior change interventions.

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THE RISK OF MODERATE AND SEVERE DIARRHEA IN CHILDREN LESS THAN FIVE YEARS OLD IS INCREASED AMONG FAMILIES WHO SHARE A SANITATION FACILITY

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The WHO classifies sanitation facilities shared by two or more families as "unimproved" for tracking progress towards the Millennium Development Goals. However, little is known about the health impact of shared sanitation. We examined associations between household sanitation facilities and moderate to severe diarrhea (MSD) among children <5 years old participating in the Global Enteric Multicenter Study (GEMS) in 7 developing country sites in sub-Saharan Africa and South Asia. Cases seeking care for MSD (defined as ≥3 loose stools in 24 hrs with one or more of the following: sunken eyes, skin tenting, dysentery, IV rehydration, or hospitalization) were enrolled at health facilities. Age-, gender- and community-matched controls were enrolled at home. Sanitation facilities were observed during follow-up household visits (between 60-90 days) to all GEMS participants. Among 8,977 cases and 12,278 matched controls, 5.7% practiced open defecation; the remainder used pit latrines (55.5%), VIP latrines (3.6%), pour flush toilets (32.4%) and flush toilets (1.6%). No type of sanitation facility was statistically associated with MSD overall or at any site. However, families of case children more commonly used shared sanitation facilities than control families (47.5% vs. 41.2%, mOR = 1.2; 95% CI: 1.1-1.3), overall and in Pakistan (mOR=1.7; 1.4-2.0), Mali (mOR=1.2; 1.1-1.4), India (mOR=1.3; 1.0-1.6), and Kenya (mOR=1.2; 1.0-1.5). The odds of MSD for shared sanitation were increased two-fold if feces was present (mOR=2.2; 1.6-3.2) than if was absent (mOR=1.2; 1.1-1.3). While access to unshared sanitation facilities was more common among higher-income households, shared sanitation facilities were consistently more common among case than among control households across all wealth index quintiles. Our observations indicate that shared sanitation facilities can increase the risk of diarrhea, regardless of the type of facility, and supports their classification as "unimproved". Increasing access to private sanitation facilities may reduce diarrhea incidence among young children.

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ACCESS TO WATERLESS HAND SANITIZER IMPROVES HAND CLEANING BEHAVIOR AFTER TOILET USE AT PRIMARY SCHOOLS IN KIBERA, KENYA

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Hundreds of millions of school days are estimated to be lost each year due to diarrheal illness. Handwashing is an established effective strategy to reduce diarrheal and respiratory illnesses. However, promoting handwashing is challenging in settings with limited water access. This study investigated the impact of providing waterless hand sanitizer on student hand cleaning behavior and health in six primary schools within Kibera, Kenya. Two schools received a waterless hand sanitizer (HS) intervention, two received a handwashing with soap (HW) intervention, and two received no intervention (controls). Hand cleaning behavior was monitored for 8 weeks through structured observation and by surveillance cameras placed at school toilets. Students were interviewed weekly to monitor diarrheal and respiratory illness. The average rate of hand cleaning after toileting was 82% of 2589 events observed in HS schools (OR=8.1, 95% CI=2.8-23.0), 38% of 3607 events observed in HW schools (OR=1.1, 95% CI=0.2-7.5), and 37% of 3031 events observed in control schools. At HW schools, soap was used for 97% of handwashing events, while at control schools, soap was used for only 6% of events. Water was unavailable in 39% and 30% of observations at HW and control schools, respectively. When water was available, handwashing rates were 62% at HW schools and 53% control schools. Students at HS schools were 26% less likely to report diarrhea (p=0.005), 20% less likely to report cough (p=0.013), and 30% less likely to have a runny nose observed by enumerators (p=0.009) compared to control schools. Students at HW schools were 19% less likely to report diarrhea (p=0.04) and 32% less likely to have an observed runny nose (p<0.001) compared to control schools. Among Kenyan primary schools, provision of waterless hand sanitizer markedly increased rates of hand cleaning after toilet use, while provision of soap and water tanks did not. These findings suggest that use of waterless hand sanitizer is a promising option for reducing infectious disease in schools with limited water access.

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ASSOCIATIONS WITH HANDWASHING IN THE HOME AND RESPIRATORY AND DIARRHEAL ILLNESS IN CHILDREN UNDER FIVE YEARS OLD IN RURAL WESTERN KENYA

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Diarrhea and pneumonia are leading causes of death in children worldwide. Handwashing with soap is effective in reducing childhood diarrhea and pneumonia in resource poor areas in south Asia, but data are sparse for Sub-Saharan Africa. We observed presence of a designated handwashing (HW) location, soap at a designated HW location, or soap in the home, and examined the association with longitudinal prevalence of

diarrhea and acute respiratory illness (ARI) in children <5 years old in rural Asembo, Kenya. Syndromic surveillance for diarrhea and ARI in children <5 years old is conducted biweekly in Asembo. In April 2009, we assessed households in the surveillance area for handwashing indicators. Syndrome data collected in the four months preceding the survey was used. We used generalized linear regression models to estimate differences in longitudinal prevalence of illness associated with living in households with each HW indicator, adjusted for household wealth, age, sex, and within household intraclass correlations. The sample included 2547 children in 1745 households. Overall longitudinal prevalence of diarrhea and ARI were 2.5 and 12.7 days of illness per 100 child-days, respectively. Longitudinal prevalence was 30.7% lower (95% CI 24.4%, 34.4%) for diarrhea and 20.5% lower for ARI (95% CI 12.2%, 25.8%) in children in households with observed soap compared to children in households with no observed soap. A designated HW location was identified in 3.3% of households, and 1.2% had a designated HW location with observed soap and/or water present, and neither was associated with a difference in diarrhea or ARI prevalence after adjustment. The presence of observed soap in the home, a proxy measure of handwashing behavior, is associated with reduced illness in children in rural western Kenya. A minority of households had a designated HW location, which, based on previous studies of handwashing behavior, offers substantial opportunity to increase handwashing behavior by providing a visual cue to stimulate handwashing at critical times for pathogen transmission.

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THE ASSOCIATION OF SOAP IN HOUSEHOLD ON CHILD MORTALITY FROM RESPIRATORY INFECTIONS

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Respiratory disease is the leading cause of childhood death globally. Hand washing with soap prevents respiratory disease morbidity but there is no direct evidence of its impact in preventing deaths from respiratory infection. We explored the contribution of household handwashing behavior to child mortality from respiratory diseases in rural Bangladesh. We obtained data on deaths among children <5 years of age from a large scale, sanitation, hygiene education and water supply intervention in rural Bangladesh which spanned 16 districts. To assess cause of death, we conducted verbal autopsies approximately 1 year after child death. Each death among a child <5 years (ICD-9 code 869.3-869.6) was age matched to three children from the same community who did not have respiratory symptoms at the time of interview. We interviewed family members, conducted 5 hours of household structured observation including observation for the presence of soap in the household. We constructed a socioeconomic index using principal component analysis of parental education, household assets, number of rooms, and housing material. We used logistic regression to adjust for immunization, socio-economic status, family size, and number of people sleeping in the same bed as child. Compared to 315 age matched healthy children, the 105 cases were more likely to have smaller families (p<0.001) and less educated mothers (p=0.012). Cases (29%) were more frequently assigned to the poorest wealth quintile than controls (18%). In multivariate analysis, having had any childhood vaccination [AOR: 0.26, CI: 0.11-0.58] and having any bar soap in the home [AOR: 0.53, CI: 0.29-0.98] was independently protective. Household participants washed hands in only 10 (2%) of 523 observed respiratory events such as coughing, sneezing, or nose cleaning or blowing. All 10 washed with only water. Household ownership of soap, a proxy hand washing measure was associated with deaths from respiratory disease. However, handwashing with soap was not practiced after contact with respiratory secretions. Understanding optimal strategies within households to interrupt respiratory pathogen transmission by hand washing would help decrease respiratory disease mortality as well as morbidity.

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FILARIAL-EXPANDED HUMAN NATURAL REGULATORY T CELLS DO NOT DIRECTLY MODULATE ANTIGEN PRESENTING CELL RESPONSES TO MALARIA ANTIGEN IN A FILARIA/ MALARIA CO-ENDEMIC REGION

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We have previously shown that patent filarial infection is associated with an expansion of regulatory T cells (both adaptive [aTreg] and natural [nTreg]) and the IL-10 mediated suppression of malaria-induced production of IL-12, CXCL-9 (MIG), CXCL-10 (IP-10) and IFN-γ. Because nTregs have been shown to inhibit antigen presenting cell (APC) function by altering their maturation and modulating the expression of co-stimulatory molecules, we sought to determine whether the filarial-induced expansion of nTregs contribute to the diminished APC cytokine production following malaria antigen (MalAg) stimulation in a filarial/malarial co-endemic population of Mali. From filarial-infected (Fil+; n=18) or -uninfected (Fil-; n=19) individuals, purified nTregs (CD4+CD25+CD127low) and effector T (Teff) (CD4+CD25-CD127low) cells were cultured with purified APCs in the presence or absence of MalAg, and APC-derived cytokines measured. APCs from Fil+ individuals produced significantly lower levels of CXCL-9 ($p = 0.0006$) and IL-12 ($p = 0.02$) compared to APC from Fil- subjects. Although MalAg induced CXCL-9 ($p = 0.0009$), and CXCL-10 ($p = 0.0009$) as well as IFN-γ ($p = 0.0011$) in the APC:Teff co-culture of all subjects, the addition of nTreg to APC cultures did not directly alter the APC cytokine response to MalAg ($p > 0.05$) in either group. Surface marker blockade, previously implicated in the delivery of negative signals by nTregs (e.g GITR, PD1, LAG-3, IL-10R and CTLA-4) to APC failed to alter cytokine production when nTregs were added to APCs. In contrast, blocking PD1 or IL-10R in APC:Teff cocultures markedly augmented the production of IFN-γ, IL-12p70, CXCL-9 and CXCL-10 ($P < 0.05$ for all comparisons) in Fil+ subjects. This augmented cytokine production was significantly higher in Fil+ subjects compared to Fil- subjects. Our data clearly show that nTregs do not directly inhibit APC cytokine production in response to MalAg stimulation and suggest that expansion of nTregs is not responsible for the diminished cytokine production by APCs in filarial infections.

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HISTAMINE RELEASE DURING *LITOMOSIDES SIGMONDONTIS* INFECTION ENHANCES ADULT WORM BURDEN

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Numerous studies have demonstrated that helminth antigens induce release of histamine from basophils and mast cells of infected hosts. To date, however, the role histamine plays in the immune response against helminths has not been well characterized. In this study, we evaluated the role of histamine in mice infected with *Litomosoides sigmondontis*, a tissue-invasive filarial infection of rodents that lives for months in immunocompetent Balb/c mice. Extended time-course studies revealed that histamine in plasma peaked at 8 weeks of infection (mean 30nM at 4 wks, 325nM at 8 wks, and 100nM at 12 wks compared to 20nM in uninfected age-matched controls) whereas expression of histidine decarboxylase mRNA in circulating blood cells increased throughout the course of infection (fold increase over uninfected age-matched controls = 20 at 8 wks and 35 at 12 wks). Mice vaccinated with irradiated L3 larvae demonstrated substantial increases in circulating histamine levels 30 minutes after challenge infection (mean 400nM vs 20nM in unchallenged,

p<0.001), but administration of HR1 and HR2 receptor blockers did not attenuate the protective efficacy of vaccination (82% protection in untreated groups, 82% protection in HR1 treated, 80% protection in HR2 treated). Interestingly, short time course measurements demonstrated that primary infection of unvaccinated mice with L3s also causes histamine release into the bloodstream 30 minutes following infection (mean 200nm vs 20nM in uninfected, p<0.05), indicating a non-specific mechanism of histamine release. To evaluate the role histamine may play during infection, mice were chronically administered HR1, HR2, and a combination of HR1 and HR2 blockers in their drinking water and assessed for adult worm survival after inoculation with 40 L3 larvae. Surprisingly, at 8 weeks post-infection all groups of mice treated with antihistamine antagonists had significantly reduced numbers of adult worms compared to untreated controls (mean number of adult worms = 5 in HR1 group, 12 in HR2 group, 6 in HR1/HR2 group, and 20 in untreated infected control group, p values of <0.05 when comparing each treatment group against infected controls). Taken together, these data indicate that histamine, rather than being involved in vaccine-mediated protection, may be induced by filarial parasites for their growth and/or survival *in vivo*.

1477

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MICROFILARIAE OF *BRUGIA MALAYI* INDUCE BOTH AN ALTERNATIVELY ACTIVATED AND PROINFLAMMATORY PHENOTYPE IN HUMAN MONOCYTES

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Monocyte dysfunction has been proposed as a cause of the reduced parasite-antigen specific T-cell response seen in patients with chronic filarial infections. Monocytes from these infected individuals internalize filarial antigens and express markers associated with alternatively activated macrophages (M). To understand the role of filarial antigens in monocyte differentiation, human monocytes from healthy volunteers were exposed to either live microfilariae (mf) of *Brugia malayi* or cytokines known to produce classically activated (combination of LPS and IFN-γ or MCSF) or alternatively activated (IL-4) phenotypes. The cells were then assessed for their expression of markers associated with alternative activation and their ability to phagocytose. Our data indicates that, similar to IL-4, mf significantly (p<0.05) upregulated mRNA expression of CCL15, CCL17, CCL18, CCL22, PDL1, and PDL2 but not Arg-1 in monocytes. Secreted products from mf or IL-4 significantly downregulated the monocyte mRNA expression of TLR3 and TLR7 resulting in decreased production of IL-6 following TLR ligand stimulation. Unlike IL-4, but similar to LPS/IFN-γ, mf significantly (p<0.05) upregulated the production of proinflammatory cytokines IL-1β, IL-6, neopterin, soluble ICAM-1, and TNF-α. Furthermore, both live mf and soluble factors from mf enhanced the cell surface expression of ICAM-1 on monocytes. Interestingly, mf significantly upregulated (4 fold) the mRNA expression of Indoleamine2, 3-dioxygenase (IDO) as well as its activity. Functionally both IL-4- and mf-exposed monocytes showed a significant decrease in their phagocytic ability compared to MCSF-cultured cells. Our data suggest that exposure of human monocytes to live mf of *Brugia malayi* induce monocytes to have characteristics of both alternative and classical ('proflammatory') activation. Further studies directed toward defining, at the single cell level, the multifunctionality of a given monocyte subset induced by filarial worms are ongoing.

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ONCHOCERCA VOLVULUS RECOMBINANT ANTIGENS OV-103 AND OV-RAL-2 ARE ASSOCIATED WITH PROTECTIVE IMMUNITY IN HUMANS AND INDUCE RESISTANCE TO INFECTION IN MICE

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Onchocerca volvulus (*Ov*) remains an important cause of blindness and chronic disability. While mass drug administration with ivermectin is ongoing, evidence is building for the existence of resistance to the drug. The development of an anti-larval vaccine would reduce adult worm burdens and thereby diminish microfilariae numbers in the skin resulting in reduced pathology and the interruption of transmission. Two *O. volvulus* vaccine candidates, Ov-103 and Ov-RAL-2 were chosen for study based in part on their homologues being highly protective in other nematode animal models. The antigens are expressed by the larvae in the basal layer of the cuticle and hypodermis. Ov-103 is also present in the basal lamina, channels connecting the esophagus to the cuticle and multivesicular bodies within the hypodermis. Ov-103 and Ov-RAL-2 are highly antigenic

in *Ov* exposed and infected populations with significant correlations between the IgG1 and/or IgG3 cytophilic antibodies responses and the development of protective immunity. The anti-Ov-103 IgG3 responses in the putatively immune individuals (PI) and the infected (INF) were elevated and similar while the anti-IgG1 responses were significantly higher in the INF. The anti-Ov-RAL-2 IgG3 responses in the INF are significantly increased with age, while the IgG1 is highly elevated regardless of age. To test the antigens' potential as vaccine candidates, mice were immunized with the antigens in alum. Yeast derived Ov-103 induced significant protection (31%) against larval *Ov*, while antigen from *E. coli* did not. Immunization with *E. coli* derived Ov-RAL-2 induced significant protection (44%), whereas the yeast produced antigen did not. These results suggest that the expression system has an effect of the potency on the recombinant vaccine antigens. We conclude that Ov-103 and Ov-RAL-2 are associated with protective immunity in humans and that both antigens induced significant levels of protective immunity in mice, thus making them potential candidates for a prophylactic vaccine against onchocerciasis.

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PROTECTIVE RESPONSE OF HOST NON-HOMOLOGOUS CONFORMATIONAL EPITOPES OF *WUCHERERIA BANCROFTI* THIOREDOXIN (*WB*-TRX)

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Vaccine studies in parasitic diseases have been a long term struggle due to the complex life cycles and immune evasion mechanisms. The present study explores the avenues of structural analysis on parasitic antigens to qualitatively improve protective immune response in mammalian hosts against nematode parasitic infections. In this regard, *Wuchereria bancrofti* thioredoxin (*Wb*-TRX) which protects filarial worms from radical-mediated damage of the host was selected. Accordingly, recombinant *Wb*-TRX was purified without fusion tag for structural studies. The enzyme activity was demonstrated using insulin reduction assay. The structure of *Wb*-TRX was solved by X-ray crystallography and was utilized for conformational epitope analysis. Since *Wb*-TRX shares sequence homology (~40%) with mammalian proteins, certain putative host-non-homologous regions from the protein sequence was selected and analysed by *in silico* immunoinformatic tools. These selected regions were located and analysed in the protein structure of *Wb*-TRX. Additionally, these regions were also validated against online conformational epitope databases. Based on these analyses, putative Discontinuous Epitope Peptide regions (DEP) were selected and synthesized. The activity assay performed for *Wb*-TRX in the presence of anti-sera raised in mice against these Discontinuous Epitope Peptides (DEP) characteristically reduced activity proposing a mechanism of enzyme inhibition depriving antioxidant ambience and challenging parasite survival. Further, a linear Peptide Conjugate (PC1) derivative of the DEP was evaluated for vaccine efficacy in permissive *Mastomys coucha* model. Interestingly, PC1 showed 75% greater protection compared to 63% protection of r*Wb*-TRX. Hence, the current study reports the utilization of conformational epitope enrichment through structural validation as a novel strategy for enhancing the efficacy of parasite antigens in host protection.

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FATTY ACID AND RETINOL-BINDING (FAR) PROTEINS OF FILARIAL NEMATODES: POTENTIAL VACCINE CANDIDATES FOR ONCHOCERCIASIS AND LYMPHATIC FILARIASIS

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The FAR proteins of filarial nematodes are helix-rich, fatty acid and retinol-binding (FAR) proteins that appear to be specific to nematodes and secreted into the surrounding tissues of the host. These proteins may play an important role in scavenging fatty acids and retinoids from the host and are probably essential for the survival of filarial nematodes and hence are considered as potential vaccine/drug targets. In previous studies, immunization with a fragment of the *Onchocerca volvulus* retinol-binding protein (Ov-RBD-1/Ov-FAR-1) induced significant resistance to challenge infection with third-stage larvae. Ov-FAR-2 was cloned by using serum from individuals who have developed concomitant immunity and Bm-FAR-2 was identified by bioinformatics. To further the development of recombinant vaccines against *O. volvulus* and lymphatic filariae, we focused on two members of the filarial FAR protein family. Recombinant FAR-1 and FAR-2 proteins from *O. volvulus* and *Brugia malayi* were expressed in *Escherichia coli* and *Pichia pastoris* and their ligand binding properties were compared using a fluorescence-based assay. The O. volvulus and *B. malayi* FAR-1 proteins bind to both retinol and DAUDA and can be displaced from DAUDA by structurally-related oleic acid. Surprisingly, both filarial FAR-2 proteins bind only to retinol and not to DAUDA, which suggests that FAR-2 and FAR-1 are different in structure and potentially also in function. The recombinant FAR proteins were tested for their efficacy as vaccine in the *O. volvulus* - mouse chamber model and in the *B. malayi* - jird animal model. Mice immunized with Ov-FAR-1 in alum, expressed in either *E. coli* or *P. pastoris*, killed 54% of the *O. volvulus* challenge infection. Immunization of jirds with recombinant Bm-FAR-1 expressed in E. coli and formulated with alum resulted in 41% reduction in worm count and immunization with Bm-FAR-1 formulated with the Montanide-720 adjuvant resulted in 74% reduction in worm count. Further vaccination experiments investigating the FAR-2 molecules in these two animal models are underway. Correlation between the immune responses in humans to the FAR proteins vs. those in the protected mice and jirds will be presented. In conclusion, the FAR proteins of filarial nematodes are excellent candidates for use in prophylactic vaccines against infection with *O. volvulus* and *B. malayi*.

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FILARIAL LYMPHATIC PATHOLOGY REFLECTS ELEVATED LEVELS OF CIRCULATING INFLAMMATORY BIOMARKERS OF LYMPHATIC AND IMMUNE DYSFUNCTION

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Infection with *Wuchereria bancrofti* can be associated with development of serious pathology in the form of lymphedema, hydrocele, and elephantiasis in a subset of infected patients. Dysregulated host inflammatory responses, lymphatic dysfunction, endothelial activation and extracellular matrix remodeling play central roles in filarial disease pathogenesis. To identify factors contributing to pathogenesis of disease in lymphatic filariasis, we examined the role of microbial translocation markers (LPS, LBP, EndoCAb and sCD14); acute phase proteins [α-2

Macroglobulin (α-2 m), Haptoglobulin, C-reactive proteins (CRP) and Serum Amyloid protein-A (SAA)]; angiogenic factors (VEGF - A, C, D, R1, R2 and R3 and Angiopoietin -1 and 2); pro- and/or anti-fibrotic factors (MMP - 1, 7, 8 and 9 and TIMP - 1, 2, 3 and 4) and pro-inflammatory cytokines (IFNγ, TNFα, IL-12, IL-1β, IL-6, IL-17 and GM-CSF) in chronic filarial pathology with (CP Ag+ (n=24)) or without (CP Ag- (n=65)) active infection as well as in asymptomatic, infected (INF; n=84); and uninfected, endemic normal (EN; n=64) individuals. Markers that were significantly elevated in CP Ag+ compared to INF but not in CP Ag- compared to EN individuals were considered to truly reflect biomarkers of pathogenesis. CPAg+ individuals had significantly elevated plasma levels of LPS (p=0.0001), α-2m (p=0.0003), haptoglobulin (p<0.0001) and SAA (p=0.0385) among the microbial translocation and acute phase panels. Among the angiogenic growth and fibrotic factors, we found significantly elevated levels of VEGF-A (p=0.0031) and C (p<0.0001), VEGF-R1 (p=0.0033), R2 (p<0.0001), R3 (p=0005) and Angiopoietin-1 (p=0.0481) but not the MMP/TIMP family. In addition, a variety of pro-inflammatory cytokines including IFNγ, IL-12, GM-CSF (p<0.0001 for all) and IL-1β (p=0.0073) were significantly elevated in CP Ag+ individuals. The elevated levels of these factors suggest quite strongly that the alteration of lymphatic integrity and peri-lymphatic inflammation should be implicated in the pathogenesis of lymphatic filarial pathology.

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DOES MALARIA IN PREGNANCY IMPAIR PLACENTAL DEVELOPMENT? EVIDENCE FROM AN *IN VITRO* MODEL

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In malaria endemic areas, *Plasmodium falciparum* (Pf) malaria during pregnancy is the leading preventable cause of low birth weight and neonatal mortality, often due to fetal growth restriction (FGR). The underlying pathogenic mechanisms are poorly characterized, but may include impaired placental development. The peak prevalence of maternal Pf infections between 13-18 weeks gestation coincides with the establishment of the placental circulation, when extravillous trophoblasts (EVT) invade the maternal uterus and transform maternal spiral arteries increasing placental blood supply. Adequate trophoblast invasion is essential for the establishment of appropriate placental function and successful fetal growth and impairment of this process is associated with other causes of human FGR. To address the impact of malaria infection early in pregnancy on placental development, we tested serum from Papua New Guinean women with Pf in peripheral blood at their first antenatal presentation (between 16 and 22 weeks gestation) for the ability to inhibit first trimester EVT-cell line invasion and viability *in vitro*. Compared to uninfected controls, serum from malaria-infected women significantly reduced trophoblast invasion (P <0.001). This phenomenon could not be explained by changes in trophoblast viability (P =.2). Because trophoblast invasion is enhanced by a number of hormones, cytokines and chemokines, and is inhibited by pro-inflammatory cytokines, many of which are dysregulated in malaria in pregnancy, we further compared concentrations of known modulators of trophoblast invasion in maternal blood between the groups. Serum collected from malaria-infected women had significantly lower levels of trophoblast invasion promoting factors (Insulin like growth factors -1 and -2, P =.0001, P =.01 respectively, and IL-8 P = 0.02) and higher levels of invasion inhibitory modulators (human chorionic gonadotrophin P =.002, IL-10 P =.01). Although malaria-induced elevated pro-inflammatory cytokines and reduced fetal growth hormones have been reported at delivery, this study is the first to describe altered levels of such factors early in pregnancy. These inflammatory and hormonal disturbances in early pregnancy may impair placental development. This is a significant advancement in our understanding of the temporal and pathophysiological events that may contribute to FGR due to Pf malaria in pregnancy.

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ANTENATAL CLINIC ATTENDANCE, INTERMITTENT PREVENTIVE TREATMENT USE AND PREGNANCY OUTCOME AMONG PARTURIENT WOMEN ATTENDING ADEOYO TEACHING HOSPITAL, IBADAN, OYO STATE, NIGERIA

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Intermittent Preventive Treatment in pregnancy with Sulfadoxine Pyrimethamine (IPTp-SP) is currently the recommended method for control of malaria in pregnancy. However, in Nigeria the proportion of women who take the recommended two doses during Antenatal Clinic (ANC) is low. In this study, we investigated the relationship between number of ANC visits and IPTp coverage, relationship between IPTp use and prevalence of malaria parasite at parturition in both mother and baby as well as pregnancy outcome.339 mother-baby pairs were enrolled at delivery in a secondary health care facility in Ibadan, south western Nigeria. An interviewer administered questionnaire was used to collect information on demographic details, history of index pregnancy. Thick blood films from maternal finger prick and neonatal heel prick were prepared and stained with fresh Geimsa stain. Data was summarized using frequency tables and means while differences in proportion were compared using Chi-square test. There were six twin delivery giving 339 mothers and 345 neonates. Mean age of mother was 28.1 ± 0.281 years, 126/339 (37.2%) mothers were primigravida, 80/339 (23.6%) were secundigravidae, 133/339 (39.2%) had had 3 pregnancies and above. Use of IPTp-SP was reported by 88/339 (26%) parturient women and 17/88 (19.3 %) of them received two doses before delivery. Coverage of IPTp-SP was significantly higher among women who had ≥4 ANC visits (32.2%) as compared with women who <4 visits (15.6%). There was no significant relationship between number of ANC visits and transportation cost as well as distance of residence to clinic. The prevalence of malaria parasite in the parturient women and neonates were 13.3% and 3.5% (12/345). Prevalence of malaria parasitemia was lower (0%) in those who used 2 or more doses of SP than in those who used less than 2 doses 4/71 (5.6%). There was no significant relationship between the number of doses of SP taken and maternal and neonatal haematocrit, birth weight and gestational age at parturition. Compliance with IPTp-SP use is ensured by frequent ANC visits and reduces level of parasiteamia in both mother and child. Effort at malaria control should be targeted more at encouraging women to attend antenatal clinic regularly to achieve completion of recommended dose of intermittent preventive treatment.

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PARASITOLOGIC ASSESSMENT OF TWO-DOSE AND MONTHLY INTERMITTENT PREVENTIVE TREATMENT OF MALARIA DURING PREGNANCY WITH INTERMITTENT PREVENTIVE TREATMENT WITH SULPHADOXINE-PYRIMETHAMINE (IPTP-SP) IN LAGOS, NIGERIA

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Intermittent preventive treatment of malaria with sulphadoxine-pyrimethamine (IPTp-SP) is a key strategy in the control of malaria in pregnancy from the second trimester. However, there is no data on the protective efficacy of IPTp-SP in Lagos, Nigeria. High sulphadoxine-pyrimethamine (SP) resistance reported among *Plasmodium falciparum*

isolates has been reported from clinical trials and molecular studies in children. This has necessitated the continuous monitoring of the efficacy of SP in pregnant women. Reports of malaria prevalence in Nigeria suggest that malaria is hyperendemic in most areas, thus raising concerns on the adequacy of the standard two-dose IPTp-SP strategy adopted for HIV-negative pregnant women. This study was done to determine the protective efficacy of IPTp-SP; and to assess the equivalence of monthly-dose to the standard two-dose IPTp-SP in Lagos. The study was a longitudinal study. The women were randomly allotted to two arms: two-dose IPTp-SP (Arm A) and monthly dose IPTp-SP (Arm B). A total of 259 pregnant women [Arm A=122; Arm B=137] attending antenatal clinics in two hospitals in Lagos, Nigeria were recruited. Eligibility criteria were the absence of symptomatic malaria, HIV and multiple pregnancy. Outcome measures were: absence of malaria parasites in peripheral blood; proportion of live births and low birth weight. Baseline parasitaemia (M0) in the two group was 5(4.1%) and 3(2.2%) in Arms A and B respectively. The overall protective efficacy of IPTp-SP was 98.4% (Arm A, 98.3% and Arm B, 98.5%) at M1(P=0.636). Similar result was obtained at the second month (M2) (P = 0.466). However, none of the women in the monthly IPTp-SP (Arm B), developed parasitaemia after M1; while a woman became parasitaemic at M2 in the 2-Dose IPTp-SP group (Arm A). The monthly dosing was not superior to the two-dose regime. The proportion of live births and low birthweight were similar in the two study arms (P>0.05). Intermittent preventive treatment of malaria during pregnancy with SP is effective in protecting pregnant women from malaria infection in Lagos. Monthly-dose IPTp-SP is equivalent to the standard two-dose IPTp-SP in Lagos, Nigeria.

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ACQUISITION AND MAINTENANCE OF IGG RESPONSES TO *PLASMODIUM FALCIPARUM* AND *P. VIVAX* DURING PREGNANCY

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Pregnant women are more susceptible to, and more severely affected by, malaria and other infectious diseases. In malaria endemic regions pregnant women typically develop high parasite densities, placental infection and associated complications, despite substantial immunity to malaria that may have been acquired prior to pregnancy. This has largely been attributed to both the modulation of maternal immune responses and the sequestration of *Plasmodium falciparum* parasites in the placenta. However, data on the acquisition and maintenance of antibody responses throughout pregnancy, and their relation to malaria is unclear. Furthermore, there are limited data on malarial immunity among pregnant women in low transmission settings, in Asia, and in a setting where *P. falciparum* and *P. vivax* are prevalent. In a nested case-control study of pregnant women on the Thai-Burmese border, we measured IgG levels to *P. falciparum* merozoite antigens (AMA1, EBA-175, MSP2, MSP3, schizont extract) and *P. vivax* merozoite antigens (Pv-AMA1) and the *P. falciparum* pregnancy-specific antigen VAR2CSA-DBL5 at 2-weekly intervals during pregnancy until delivery in 136 malaria cases and 124 controls (over 2000 samples in total). ELISAs were performed using novel high-throughput technology to facilitate determination of antibody levels in a large number of samples. Longitudinal analysis revealed that at the individual level, antibody responses could be grouped as dynamic or relatively stable during gestation. The most dynamic species-specific responses were seen in women who experienced active infection during pregnancy and the biggest magnitude of effect seen with VAR2CSA-DBL5 compared to merozoite responses. At the population level, antibody titres increased with gestation time in those with concurrent *P. falciparum* parasitaemia most likely reflecting boosting of responses with each successive infection. This study provides the most comprehensive analysis, to date, of antibody dynamics towards two *Plasmodium* spp. and contributes to our understanding of malaria during pregnancy and immune responses to infectious diseases during pregnancy.

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MALARIA IN EARLY PREGNANCY ADVERSELY AFFECTS *IN UTERO* FETAL GROWTH

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Maternal malaria produces adverse maternal and fetal outcomes, including maternal anemia and intrauterine growth restriction (IUGR). There is a scarcity of research regarding the effects of early malaria on pregnancy outcomes despite, typically, a lack of prevention and control measures during this critical time of placental development. We evaluated the effect of malaria parasitemia prior to 21 weeks' gestation on estimated fetal weight (EFW) and IUGR among a sample of 128 pregnant women enrolled prior to 21 weeks' gestation in a longitudinal ultrasound study from May 2005 to May 2006 in Kinshasa, DR Congo. Malaria exposure and ultrasound estimated fetal weight were measured monthly until delivery. Intermittent preventive treatment in pregnancy (IPTp) was provided twice (from 16-27 and 28-32 weeks' gestation), insecticide treated nets were provided, and slide-positive malaria cases were treated. Linear mixed models were fitted to estimate beta coefficients and 95% confidence intervals (CIs) to describe the effect of early malaria parasitemia on the mean difference in subsequent EFW; log-binomial general estimating equation (GEE) regression models were fitted to estimate risk ratios (RRs) and 95%CIs for effect of early malaria on risk of subsequent IUGR. Twenty-one percent of pregnant women had malaria parasitemia prior to 21 weeks' gestation and 43% ever experienced an IUGR episode after 21 weeks' gestation. Primigravidae with early malaria parasitemia had an approximately 80 gram decrease in EFW compared to primigravidae with no early malaria (95%CI: -116, -34). Primigravidae with early malaria had 3.6 times the risk of subsequent IUGR compared to the referent group of multigravidae with no early malaria parasitemia (95%CI: 2.1, 6.2). Early malaria was also associated with a small, non-significant increased risk of IUGR among multigravidae (RR: 1.4; 95%CI: 0.8, 2.5). Our findings indicate that appropriate malaria prevention and control efforts should begin earlier in pregnancy in order to prevent the adverse effects of malaria on fetal growth.

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EVALUATION OF SULFADOXINE-PYRIMETHAMINE FOR INTERMITTENT PREVENTIVE TREATMENT OF MALARIA IN PREGNANCY--- MANSA, ZAMBIA, 2010

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Intermittent preventive treatment of malaria in pregnancy (IPTp) with sulfadoxine-pyrimethamine (SP) decreases placental parasitemia and maternal anemia, thus improving birth outcomes, especially among primigravidae. Zambian policy recommends three SP doses, given presumptively to pregnant women, spaced one month apart after 16 weeks of gestation. Given increased parasite resistance to SP, we evaluated the effectiveness of SP for IPTp. HIV-negative pregnant women were enrolled during delivery at two facilities in an area of high malaria transmission. Women were interviewed, SP exposure was determined from antenatal records and self-report, and blood and placental samples were taken. We evaluated outcomes of maternal parasitemia, maternal anemia (Hb<11 g/dl), and placental parasitemia in women who took ≥2 (n=286) versus <2 (n=149) SP doses. The median age of participants was 23 years (range 16-44). Among all women, ≥2 SP doses provided no protection from maternal parasitemia [n=435, unadjusted odds ratio (OR) 1.0, (95% confidence interval (CI) 0.5-2.0)], anemia (OR 0.7, 95% CI 0.5-1.1), placental parasitemia (OR 0.9, 95% CI 0.4-2.0), and placental monocyte infiltration, (OR 0.9, 95% CI 0.4-2.0). We found similar, non-significant results when evaluating ≥3 SP doses versus <2 doses. However, taking ≥2 SP doses was protective against anemia among primigravidae (n=159, OR 0.4, 95% CI 0.2-0.9). Maternal age, urban living, and use of insecticide-treated nets were not significantly associated with outcomes. Controlling for age, vaginal delivery, marital status, and living in a house with indoor residual spraying, taking ≥2 SP doses remained protective for anemia among primigravidae (OR 0.4, 95% CI 0.2-0.7). SP for IPTp may confer less protection against malaria than previously observed. Limitations of this study include strata too small to analyze [e.g. severe anemia (Hb<8 g/dl), and no SP taken], insufficient power to examine birth weight as an outcome, and unverified SP quality. These findings emphasize the urgent need to evaluate alternative medications for IPTp.

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LINKING MALARIA IN PREGNANCY TO POPULATION TRANSMISSION DYNAMICS: A MODEL OF THE PROGRESSION OF PLACENTAL INFECTION AND THE ROLE OF PARITY-DEPENDENT IMMUNITY

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Malaria in pregnancy, which is associated with maternal anaemia, preterm birth, low birthweight and increased mortality for both mother and child, continues to be a major public health issue in many parts of the world. Ensuring that prospective mothers are provided with the most effective treatment or preventative measures is a priority. However, assessment of the effectiveness of any intervention is problematic because the infection status of the placenta can only be assessed at delivery. Thus, for the preceding period of gestation, the prevalence of malaria in pregnancy can only be estimated from peripheral blood which has been shown to be neither a sensitive nor a specific indicator of placental infection. To improve estimation of the prevalence of infection directly from placental prevalence, a mathematical model linking the progression of placental infection throughout pregnancy to an existing model of the prevalence of malaria within the general population was developed which includes both age and parity as factors which affect the dynamics of infection. The model was fitted to placental histology data (prevalence of acute, chronic and past infection by age and parity) from two transmission settings (Kilifi, Kenya, 1995/96, EIR≈5 and Ifakara, Tanzania, 1994/95, EIR≈365) using Bayesian MCMC methods to provide estimates of the rate of progression of infection within the placenta. Our results suggest that parity-specific immunity which results in faster clearance of placental infection is more consistent with patterns of placental infection in different parities than an immunity mechanism that decreases the probability that an acquired peripheral infection sequesters the placenta. Furthermore, we estimate that the duration of chronic infection decreases rapidly with exposure to infection in previous pregnancies with a single prior infection reducing this time by over two-thirds. From these parameters we derived the relationship between transmission intensity and age- and parity-specific prevalence of placental infection which can be used to aid the assessment of appropriate intervention strategies in areas of differing transmission intensity.

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LONGITUDINAL MOLECULAR EPIDEMIOLOGY OF PREGNANCY-ASSOCIATED MALARIA IN BLANTYRE, MALAWI

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Pregnancy-associated malaria (PAM) is an important cause of poor birth outcomes in sub-Saharan Africa, though its prevalence is decreasing. From 1997 to 2005, during which the prevalence of parasitemia at delivery decreased from 24% to 5% at a single hospital in Blantyre, Malawi, we analyzed trends in molecular markers of drug resistance and parasite diversity among 358 *Plasmodium falciparum* isolates from the peripheral blood of delivering women. The average multiplicity of infection (MOI), as determined by merozoite surface protein-2 (msp2) genotyping, decreased from 3.8 (standard deviation 1.3) to 1.8 (SD 1.2) during the study period. The prevalence of the triple-mutant dihydrofolate reductase (dhfr) haplotype increased from 33% to 100%, and that of the double-mutant dihydropteroate synthase (dhps) haplotype from 33% to 100%; concomitantly, the prevalence of the combined quintuple-mutant haplotype increased from 17% to 100%. In contrast, haplotypes of the *P. falciparum* multidrug resistance gene (pfmdr1) were unchanged over time, and mutations at codon 164 of dhfr and codon 581 of dhps failed to emerge. Accounting for parasite multiplicity, we calculated frequencies of alleles and haplotypes and used these to compute selection coefficients as a quantitative index of the strength of selection upon mutant alleles and haplotypes. The results demonstrate the molecular correlates of improved control of pregnancy-associated malaria, and serve as a model for the emergence and fixation of drug-resistance in a semi-immune population exposed to increasing sulfadoxine-pyrimethamine. Clinical correlation of these phenomena will inform drug therapy and resistance surveillance policies.

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GENOMIC DIVERSITY OF VAR2CSA DUFFY-BINDING-LIKE DOMAINS IN PREGNANCY-ASSOCIATED MALARIA EXPLORED WITH MASSIVELY-PARALLEL PYROSEQUENCING

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VAR2CSA expression on the surface of erythrocytes infected by *Plasmodium falciparum* mediates placental malaria by allowing for the sequestration of infected erythrocytes in the placenta. Because of this specific molecular pathogenesis, VAR2CSA has been advanced as a target for a vaccine against pregnancy-associated malaria. To do so, pathogenic and immunogenic motifs must be identified within var2csa, but these efforts have been bedeviled by the sequence diversity of var2csa. Genomic diversity between and within populations of parasites that comprise a single infection is not adequately described by traditional genotyping methods because they fail to capture all variants. To this end, we employed massively-parallel pyrosequencing to explore the diversity of two var2csa Duffy binding-like (DBL) domains in genomic DNA of parasites infecting the placentae and peripheral blood of delivering women in Malawi. After amplifying across hypervariable regions of DBLs 5 and 6, amplicons were sequenced on a Roche 454 System, which sequences individual amplicons up to 500bp long. We used population genetic analysis methods to compare populations of parasites between women, between study sites, and between peripheral and placental blood, and we computed DBL variant discovery curves. The combination of next-generation sequencing and population genetic analysis provides a method by which to capture and exploit the genomic diversity of *P. falciparum* within individual infections. These tools may aid the identification of motifs that are critical to the pathogenicity and immunogenicity of pregnancy-associated malaria.

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INTENSIVE SURVEILLANCE FOR MALARIA DURING PREGNANCY

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In studies of pregnancy-associated malaria, surveillance is frequently limited to examination of the placenta for evidence of active or past malaria infection. The sensitivity of placental histology for the detection of malaria during pregnancy is not well studied. We conducted a prospective observational study recruiting women in their first or second pregnancy who were less than 28 weeks gestation and attending the Ndirande Antenatal Clinic in Blantyre, Malawi for the first time. Participants had microscopy and hemoglobin measurement done monthly and whenever they were ill. All women received three doses of intermittent preventive therapy. At delivery, a maternal peripheral, placental and cord blood, and placental biopsy samples were collected. A total of 450 women were recruited; 63% (n=285) were primigravidae. Among the 342 women with evaluable birth outcomes, 38 (11%) had malaria at enrollment and an additional 22 (6%) had malaria detected on peripheral blood smear after enrollment. On histological evaluation, 62/342 (18%) had evidence of placental malaria based on the presence of parasites and/or pigment. Fifty six percent of women with positive malaria smears during pregnancy had evidence of malaria infection in the placenta and 43% without peripheral parasitemia had placental malaria. There was no association between timing of malaria infection and the likelihood of detecting placental malaria. This study did not have the power to detect the relationship between these measures of malaria exposure and low birth weight or maternal anemia. We will report the relationship between malaria and gestational age at delivery based on ultrasound results. The first half of pregnancy, before women typically seek out antenatal care, is a period of vulnerability to malaria infection. Placental histology identified approximately half of women who had detectable infection in the peripheral blood during pregnancy. Studies with large sample sizes are needed to determine if histology is an adequate surrogate for intensive surveillance in detecting the adverse outcomes associated with malaria during pregnancy.

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THERAPEUTIC EFFICACIES OF ARTEMISININ-BASED COMBINATION THERAPIES IN NIGERIAN CHILDREN WITH UNCOMPLICATED *FALCIPARUM* MALARIA DURING FIVE YEARS OF ADOPTION AS FIRST-LINE TREATMENTS

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The therapeutic efficacies of 3-day regimens of artesunate-amodiaquine and artemether-lumefantrine, during 5 years of adoption as first-line treatments, were evaluated in 811 <12 year-old malarious children. Compared with artemether-lumefantrine, amodiaquine-artesunate significantly reduced the proportion of children with fever and parasitemia 1d after treatment began (day 1) (P <0.008 for both). The proportion of parasitemic children on day 2, and gametocytemia on presentation and carriage reduced significantly over the years (P <0.000001, and P <0.03, respectively, test for trend). Overall efficacy was 96.5% (95%CI 94.5-98.6) and remained unchanged over the years (P = 0.87, test for trend). Kinetics of parasitemias following treatments was estimated by non-compartmental model. Declines of parasitemias were monoexponential with mean elimination half-life of 1.09h (95%CI 1.0-1.16). Parasitemia half lives and efficacy were similar for both regimens and in all ages. Artesunate-amodiaquine and artemether-lumefantrine remain efficacious treatments of uncomplicated *falciparum* malaria in Nigerian children 5 years after adoption.

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EVALUATION OF ADVERSE DRUG REACTIONS TO ARTEMISININ-BASED COMBINATION THERAPY IN A COMMUNITY HEALTH CLINIC IN PAPUA PROVINCE, INDONESIA

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Malaria is one of the main health problem in east of Indonesia. Since 2004, Ministry of Health Republic of Indonesia has recommended the use of orally administered Artemisinin-Based Combination Therapy (ACT) to treat uncomplicated malaria. However, some patients experienced uncomfortable adverse reactions due to ACT, leading to its discontinuation. Recognition of these adverse reactions may lead to better management. The aim of this study is to determine the prevalence of adverse reactions due to orally administered ACT and types of reactions experienced by uncomplicated malaria patients. A cross sectional study among uncomplicated malaria patients was conducted in Community Health Clinic Medical Emergency and Rescue Committe, a non-governmental health organization, in Timika, Papua, Indonesia, between September 2010 and March 2011. Sample was collected consecutively. Primary data were collected by interview using a questionnaire to assess

adverse reactions experienced by the patients, while demographic data were obtained from medical records. There were 250 uncomplicated malaria patients who fulfilled the inclusion criteria and did not meet any of the exclusion criteria. Adverse reactions of orally administered ACT were experienced by 48% respondents. Most common adverse reaction was nausea (28%). Approximately 33% respondents who experienced adverse reactions considered them intolerable. These findings are much higher compared to other studies. Adisa et al (2008) and Cairo et al (2008) found adverse reactions due to ACT in 32.9% and 8.8% respondents respectively. Nausea, the most common adverse reactions found in this study, was found only in 1,3% patients interviewed by Adisa et al and 16% of Cairo et al's subjects. This difference is probably due to different types of ACT used and dosage given to the patients. Eventhough, only 33% respondents who experienced adverse reactions considered these adverse reactions intolerable, around 22% of these respondents discontinued their ACT against medical advice. In conclusion, prevalence of adverse reactions due to orally administered ACT was quite frequent. Efforts should be made to minimize the percentage of patients discontinuing their medication. Patients should understand the adverse reactions of ACT that might occur and doctors should carefully manage these adverse reactions.

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POPULATION PHARMACOKINETICS OF ARTESUNATE AND DIHYDROARTEMISININ IN HEALTHY AND MALARIA-INFECTED SUBJECTS

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Pyramax® is a pyronaridine/artesunate combination currently under evaluation for treatmentof uncomplicated malaria in adult and pediatric patients. We analyzed the population pharmacokinetics of artesunate (AS) and its active metabolite dihydroartemisinin (DHA) in healthy and malaria-infected subjects participating in eight Pyramax® clinical trials. Pharmacokinetic data were available from 166 healthy Caucasian and Korean adults and 631 African and Asian adult and pediatric patients with mild to moderate uncomplicated malaria. Plasma AS and DHA concentrations were measured using a validated LC-MS method. Non-linear mixed effects modeling was used to obtain the pharmacokinetic and variability parameter estimates. A simultaneous parent-metabolite model was developed consisting of first-order absorption of AS, a one-compartment model for AS, and a one-compartment model for DHA. The population estimates showed that AS absorption was rapid, with an absorption rate constant (Ka) of 3.02 h^{-1}. Apparent AS clearance (CL/F) and volume of distribution (V2/F) were 1170 L/h and 1040 L, respectively. Estimates obtained for apparent clearance (CLM/F) and volume of distribution (V3/F) of DHA were 82.0 L/h and 107 L, respectively. Stepwise covariate modeling yielded four significant covariate-parameter relationships: weight on CL/F, CLM/F, and V3/F and ritonavir coadministration on CLM/F. Ritonavir coadministration was associated with a substantial increase in CLM/F of 30.5 L/hr. Malaria infection did not exert a significant influence on any pharmacokinetic parameter. Creatinine clearance, hepatic enzyme elevations, and gender also did not meet the model inclusion criteria. Estimated inter-individual variability was greatest for Ka (159%), followed by V2/F (34.6%). Evaluation of the final model using bootstrapping, visual predictive check, and condition number indicated that the model displayed satisfactory robustness, predictive power, and stability. The final model was utilized to simulate AS and DHA concentration-time profiles for representative weights in each of four Pyramax® dosing groups.

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PHARMACOKINETICS, SAFETY AND EFFICACY OF ARTEMISININ-NAPHTHOQUINE COMBINATION (ARCO™) THERAPY IN PAPUA NEW GUINEAN CHILDREN WITH UNCOMPLICATED *PLASMODIUM FALCIPARUM* AND *P. VIVAX* MALARIA

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Artemisinin-naphthoquine (AN) is available as a fixed-dose oral co-formulation marketed in Papua New Guinea (PNG) and other countries as ARCO™ (Kunming Pharmaceutical Company). It is currently recommended as single-dose treatment even though the WHO stipulates that artemisinin-based combination therapy should be given as a 3-day regimen. To date there have been limited published data on the pharmacokinetics of naphthoquine, particularly in children. We conducted a randomised study in PNG children aged 5-12 years with uncomplicated *falciparum* or *vivax* malaria comparing single-dose ARCO (A:N 15:6 mg/kg) given with water (Group A, n=15), single-dose (A:N 22:9 mg/kg) administered with full-cream milk (Group B, n=17) or as two daily doses (A:N 22:9 mg/kg) given with water (Group C, n=16). Blood samples were collected from each child at 15 time points after dose, and naphthoquine in plasma was quantified by liquid chromatography-mass spectrometric assay. Of the 48 children (46 with *falciparum* and 2 with *vivax* malaria), 2 in Group B withdrew because of inability to complete study procedures. All regimens were well tolerated with no serious adverse events. There were no significant changes in pulse, blood pressure, rate-corrected QT interval, or routine biochemistry after treatment, and fever clearance was prompt. The mean 50% parasite clearance times were 3.9, 3.9 and 4.6 h for Groups A, B and C, respectively. In Group A, 1 patient had Day 23 parasitological failure, and 2 children presented with re-infections on each of Days 28 and 42. In Group B and C there were no cases of parasitological failure but 1 Group B child had a re-infection on Day 42. Gametocyte clearance was prolonged with 20%, 27% and 9% of participants still positive on Day 14 in Groups A, B and C, respectively. Using a two-compartment model, the mean terminal elimination half-lives of naphthoquine were 420, 372 and 453 h for Group A, B and C, respectively. The maximum plasma concentration after dose was similar for all three groups. Both single and two-dose ARCO regimens were safe and well tolerated. The long half-life of naphthoquine is similar to those reported for 4-aminoquinolines and related compounds and protects against recrudescence. Milk co-administration did not appear to influence naphthoquine pharmacokinetics.

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IN VITRO "PITTING" TO DETECT ARTEMISININ-RESISTANT *PLASMODIUM FALCIPARUM*?

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Artemisinin-based therapies are currently the reference treatment for malaria worldwide. *Plasmodium falciparum* resistant to artemisinin derivatives is spreading in South-East Asia. Tools that would rapidly detect the onset of resistance are urgently needed. Conventional assessment of *P. falciparum* drug resistance *in vitro* is based on the determination of the IC_{50}, but IC_{50} of artesunate or dihydroartemisinin (DHA) only weakly correlate with parasite clearance in artemisinin-treated patients. In these patients, *P. falciparum* parasites are expulsed from their host red blood cell by the spleen, a "pitting" process that greatly accelerates parasite clearance, as reported previously. We want to use *in vitro* pitting as a test to assess parasite resistance in a new, physiologically relevant way. *In vitro*

pitting will indeed mimic the major natural parasite clearance mechanism. Using an ex-vivo human spleen model, we had obtained the pitting of red blood cells containing artemisinin-induced parasite remnants, as reported previously. We had also observed that parasite remnants are deposited along the wall of red pulp sinuses in the spleen of an artemisinin-treated malaria patient. We recently established and validated an *in vitro* red blood cell filtering device that mimics the mechanical sensing of red blood cells as they cross the wall of red pulp sinuses in the human spleen, as reported previously. Using this device we have obtained the pitting of parasitized red blood cells previously exposed to artesunate or DHA. More than 25% of artesunate-exposed parasitized red blood cells are pitted by a single passage through the filters. This filtering process requires less than 50 µl of peripheral blood and less than 5 hours of parasite exposure to the drug. We are currently identifying the major determinants of the *in vitro* pitting process, and will soon determine the pitting rate of parasite isolates from patients with fast or slow parasite clearance upon treatment with artesunate.

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DECREASING *IN VITRO* SUSCEPTIBILITY OF FRESH *PLASMODIUM FALCIPARUM* ISOLATES TO DIHIDROARTEMISININ IN COLOMBIA

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The main drawback to controlling malaria is the emergence and spread of multidrug resistant parasites. Artemether+lumefantrine therapy was implemented during 2007 in Colombia for *falciparum*-malaria, showing excellent outcomes. Recent reports from Southeast Asia show that it is likely that resistance to Artemisinin Combined Therapies-ACTs is developing. This phenomenon highlights the need to keep monitoring systems for early detection of resistance in other endemic areas. Our aim was to determine the spatial/temporal changes in the *Plasmodium falciparum* phenotypes through the first years of ACTs implementation in Colombia. From 2008 to 2010, 121 isolates from Quibdó and Tumaco, (North and South Colombian Pacific coast) were evaluated for *in vitro* susceptibility to mefloquine-MQ, lumefantrine-LUM and dihidroartemisinin-DHA, through microscopic and ELISA-HRP2 tests. For quality control, the reference strain W2 was evaluated. The IC_{50}s were calculated using NH-nonlin software and non-parametric tests were used to compare the results. In 2010, DHA showed a significant increased IC_{50}s ($p<0.05$) in Tumaco (Geometric Mean-MG in 2008: 2.02nM, 2009: 1.2nMand 2010: 4.0nM). Meanwhile, during 2008-2010, LUM and MQ IC_{50}s were stable in both regions. The parasites from Quibdó were less susceptible to MQ (MG in 2008: 36nM, 2009: 47.8nMand 2010: 30.7nM) compared to those from Tumaco (MG in 2008: 14.3nM, 2009: 25.1nM and 2010: 15.8nM) in this period ($p<0.05$). Spearman correlation test between both methodologies was 0.92 ($p<0.01$). The fact that *in vitro* DHA IC_{50}s are increasing in Tumaco highlights the need of continuing with active surveillance. Although all the isolates tested showed a high susceptibility to DHA and LUM, which is comparable to the high efficacy observed in Colombia with ACTs. Because there is cross *in vitro* susceptibility between LUM and MQ, our findings underline the importance of close monitoring of the *in vitro* response to LUM in the North Pacific region. *In vivo* studies are costly and not sensitive for early detection of resistance (due to the high efficacy of ACTs) and there are no molecular resistance markers to artemisinin derivates. Therefore *in vitro* assays are currently the main strategy for early detection of resistance to ACTs.

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THE INFLUENCE OF NEVIRAPINE ON ARTESUNATE AND DIHYDROARTEMISININ EXPOSURE IN HIV-INFECTED NIGERIAN ADULTS

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Nevirapine (NVP)-based antiretroviral therapy (ART) may result in important drug-interactions with some antimalarials. Artesunate plus amodiaquine (AS-AQ) is a common antimalarial combination used in Nigeria and the pharmacokinetic (PK) effect of combining AS-AQ with NVP is unknown. AS and AQ are converted to active metabolites via hydrolysis and cytochrome p450 pathways, respectively; the latter being most susceptible to drug-drug interactions. We conducted a two-group comparison of AS-AQ PK in HIV-infected patients receiving NVP-based ART for at least 8 weeks (n=10) to HIV-infected patients not on ART (control; n=11). AS-AQ 200/600mg was given as a daily dose for three days. Blood sample collection for the measurement of AS and AQ was commenced following the final dose at 0 (pre-dose), 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24, 48, 72 and 96h. AS and the active metabolite dihydroartemisinin (DHA) were quantified with LC-MS/MS. PK parameters [maximum concentration (C_{max}; mg/L), (volume of distribution (Vd; L), area under the curve (AUC; mg/L/h), oral clearance (Cl; L/hr), and half-life ($_{t1/2}$; h)] were determined with non-compartmental analysis (mean ± standard deviation). In the NVP group compared to controls, the AS Vd was 1162 ± 856 vs 4525 ± 3535 ($p=0.01$) and Cl was 1950 ± 543 vs 2995 ± 1180 L/h ($p=0.03$), while the AUC was 105 ± 31 vs 69 ± 26 ($p=0.02$). AS $_{t½}$ was 0.4 ± 0.3 in the NVP group and 1.1 ± 0.9h in controls ($p=0.06$), while the AS C_{max} was 108 ± 42 vs 71 ± 57 ($P> 0.05$), respectively. DHA AUC (603 ± 218 vs 883 ± 607) and C_{max} (298 ± 107 vs 507 ± 429) were also not different between the NVP and control group ($p>0.05$). DHA $_{t½}$ was shorter with NVP (1.6 ± 0.8 vs 3.2 ± 1.4h; $P=0.004$). AQ data are being analyzed and will be presented. NVP-containing ART impacted important PK parameters of AS and DHA. These findings will inform future pharmacodynamic and clinical outcome studies of AS-AQ in HIV-infected patients receiving NVP-based ART.

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EVALUATION OF A NOVEL MOLECULAR MARKER FOR MONITORING ARTEMISININ RESISTANCE IN *PLASMODIUM FALCIPARUM* MALARIA

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The human malaria parasite *Plasmodium falciparum* has evolved resistance to most drugs. There is now evidence of reduced susceptibility to artemisinin derivatives and to Artemisinin Combination Therapy with delayed parasite clearance times. If artemisinin resistance spreads widely, it would threaten global malaria control. We still lack validated molecular markers for monitoring the resistance phenotypes. Using genome-wide strategies in the rodent malaria parasite *P. chabaudi* our group identified a mutation in a clathrin mu adaptor gene (pccmu) that arose along with

artemisinin resistance. In order to investigate the possible contribution of this candidate marker to artemisinin resistance in the human malaria parasite *P. falciparum* we screened the DNA sequence of the equivalent orthologue, pfcmu, for genetic polymorphisms. We have studied field isolates from an ACT clinical trial in Burkina-Faso that were tested *in vitro* for their response to artemisinin derivatives and other antimalarial drugs. We have also evaluated pre- and post- treatment samples from an *in vivo* ACT clinical trial carried out in Kenya. Genetic polymorphisms in pfcmu were analysed for association with various measured endpoints in the two trials that might indicate a drug resistant parasite phenotype. Our preliminary results indicate that mutations in this adaptor protein subunit may be associated with varying degrees of *in vitro* and *in vivo* responses to artemisinin derivatives, quinine and lumefantrine. We propose this gene should be evaluated further as a potential molecular marker of artemisinin resistance.

1501

PHARMACODYNAMICS OF CURRENT AND NOVEL ARTEMISININ COMBINATION THERAPIES AGAINST ARTESUNATE RESISTANT OR SENSITIVE *PLASMODIUM BERGHEI*

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Effective treatment of *Plasmodium falciparum* is threatened by signs of emerging resistance to artemisinins, the linchpin of Artemisinin-based Combination Therapies (ACTs) that have been widely adopted throughout malaria-endemic regions. Attempts to define resistance, in isolates from patients that showed delayed parasite clearance times following ACT therapy, have yet to replicate an artemisinin-resistant phenotype and no definitive genetic target has been identified. Using a *P. berghei* model, we have developed pharmacodynamic profiles of four existing ACTs (dihydroartemisinin-piperaquine, artesunate-amodiaquine, artesunate-mefloquine and artemether-lumefantrine), as well as the artesunate-pyronaridine ACT under clinical evaluation, generated against drug-sensitive and artesunate-resistant parasites. These profiles are developed from 30-day studies and assess the efficacy of a 3-day dosing regimen for each combination as well as efficacy of modifications to treatment frequency and concentration. All regimens are 3-days in duration and include each ACT component as monotherapy given once daily, ACT [1x] once daily, ACT [1x] split into two daily doses, ACT [1.5x] once daily and ACT [1.5x] split into two daily doses. Results to date elucidate differences in contribution to treatment between components of ACTs and suggest pairings and treatment schedules that are maximally effective against artesunate-resistant *P. berghei*. Furthermore, our data suggest cross-resistance between artesunate and the partner drugs amodiaquine, mefloquine and piperaquine, as well as possible antagonism in the artesunate-amodiaquine and artesunate-mefloquine combinations. These findings imply that some current pairings are at higher risk of treatment failures than others. Integration of these data with ongoing pharmacokinetic and genetic studies should shed light on drug mode of action and mechanisms of resistance in *P. berghei*, and help guide selection of suitable ACTs in areas where artemisinin resistance is a concern.

1502

EFFICACY OF ARTEQUICK (ARTEMISININ-PIPERAQUINE) AND COARSUCAM (ARTESUNATE-AMODIAQUINE) IN THE TREATMENT OF *PLASMODIUM FALCIPARUM* MALARIA IN VIETNAM

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Recent reports of artesunate resistant or tolerant *Plasmodium falciparum* malaria in western Cambodia is of immense concern as the fast acting artemisinins combined with the longer elimination half-life drugs such as mefloquine are now recommended worldwide for first-line treatment of uncomplicated *P. falciparum* malaria. In light of this information there is an urgent need to evaluate artemisinin combination therapies (ACTs) in neighbouring countries to Cambodia to determine whether reduced ACT susceptibility has developed with increase failure rates and prolonged parasite clearance times. The objective of the present study was to compare the efficacy of fixed-dosed combinations of artemisinin-piperaquine (Artequick®) and artesunate-amodiaquine (Coarsucam®) for the treatment of *P. falciparum* malaria in south central Vietnam. In an open-labelled, randomized clinical pilot study 128 patients (children aged 6-14 years, n=68, adults aged 15-60 years, n=60) were allocated either a 2-day course of either Artequick® (~2.8 mg/kg artemisinin plus ~16.9 mg/kg of piperaquine per day) or a 3-day course of Coarsucam® (~4.5 mg/kg of artesunate plus ~12.3 mg/kg of amodiaquine per day), with a follow-up period of 42 days. Both ACTs were well tolerated, with no obvious drug associated adverse events. Parasite clearance times ranged between 12 and 60 h for both treatment groups. Of the per-protocol population, 50 patients were on Artequick® (24 children, 26 adults) and 51 were on Coarsucam® (25 children, 26 adults). The PCR genotype corrected cure rate at day 42 was 98% for both ACTs. This study showed that the two ACTs were highly efficacious in the treatment of *P. falciparum* malaria in adults and children. Further studies of the ACTs are warranted in different regions of Vietnam to determine the nationwide effectiveness of the two ACTs.

1503

MITIGATION OF ARTEMISININ RESISTANCE IN AFRICA: WHAT SHOULD BE DONE NOW?

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Artemisinin resistance in *falciparum* malaria has emerged in Western Cambodia and may have spread westward, an ominous repetition of the spread of resistance to chloroquine over fifty years ago, and later to sulfadoxine-pyrimethamine. This poses a major global public health threat, with the greatest potential effects in sub-Saharan Africa where disease burdens are greatest and systems for drug resistance monitoring and containment are weakest. We do not fully understand the molecular mechanisms underlying artemisinin resistance. The only associated phenotype observed in South East Asia is a decreased parasite clearance rate, not yet seen in Africa. Artemisinin resistance fits the International Health Regulations (2005) definition of a public health emergency of international concern - it is serious, unusual, and has the potential to spread. Most African countries now recommend artemisinin

combination therapy (ACT) as first line regimen for uncomplicated malaria. Unfortunately, this policy shift has resulted in the demise of systematic drug resistance surveillance; sub-regional networks have very limited activities due to lack of funding and changing technical requirements for monitoring ACT efficacy. Well-defined early warning and detection systems need to be established. We will present strategies that should be urgently adopted by Africa, including: reactivation of regional surveillance networks with clear roles; data sharing to facilitate pooled analyses to identify rare observations; drug resistance risk factor modeling to bench mark baselines; and the development and validation of new tools for monitoring resistance, antimalarial drug pressure, drug quality and inappropriate drug usage.

1504

PILOT OF HOME-BASED MANAGEMENT OF MALARIA WITH RAPID DIAGNOSTIC TESTS AND ARTEMISININ-BASED COMBINATION THERAPY IN RURAL SENEGAL

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Malaria remains a major cause of morbidity and mortality in Senegal, and access to care is challenging in part due to the remoteness of many communities. Senegal introduced artemisinin-based combination therapy (ACT) in 2006, and diagnosis based on rapid diagnostic tests (RDTs) in 2007, resulting in 85% of suspected cases in the public health sector being tested in 2009. To expand access to case management based on parasitologic diagnosis, in 2008 Senegal introduced a pilot for home-based management with RDTs and ACTs in 20 remote villages >5 km from the nearest health post. Village members chose a volunteer home-based care provider (HCP) from the community, who received three days of classroom training on malaria case management, followed by a 15 day practical in the nearest health post. The HCPs were then officially installed and given a kit including RDTs, ACTs, a case record book, and stock management tools. The chief nurse at the nearest health post was responsible for the practical training and monthly supervision. Health officials at district, regional, and national levels also performed supervision visits. During the pilot, 869 (93%) of 939 patients were tested by RDT, of whom 290 (33%) were positive and all reported successfully treated. Of 525 cases referred, there were 521 negative tests, two pregnant women, one infant <2 months, and one severe case. There were no deaths among these patients. A multi-dimensional evaluation was conducted, including a household survey and interviews with health officials and community members. Perceptions were overwhelmingly positive among community members, community and traditional leaders, health post personnel, and district and regional health officials. They recommended greater involvement of health post chief nurses in the training, broadening HCP roles to include illnesses such as diarrhea, pneumonia, TB and malnutrition, reinforcing supervision and support to the HCP, and extension of this strategy to all unserved, remote villages in Senegal. Based on these recommendations, the pilot was expanded to 408 villages by the end of 2009 and 861 villages by the end of 2010.

1505

ARTEMISININ RESISTANCE ASSOCIATED WITH PFMDR1 COPY NUMBERS AND ANTI-OXIDANT ACTIVITY IN THE *IN VITRO* SELECTED PARASITES LINES

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To select an artemisinin resistant line in the laboratory, we have subjected *Plasmodium falciparum* Dd2 strain to dihydroartemisinin (DHA) selection with step-wise increments of drug concentrations over 13 months. Two lines (Art1 and Art2) were obtained more than 20-fold increase in IC_{50} to DHA. However, the resistance phenotype was unstable and the parasites could regain susceptibility to DHA after three months of culture in the absence of the drug selection pressure. Phenotype analysis showed that the resistant parasite displayed cross-resistance to a number of the commonly used antimalarial drugs. No mutations were detected on the reported drug resistant markers, but we identified increased copy number of *P. falciparum* multidrug resistance 1 (pfmdr1) gene associated with artemisinin resistance. To detect other potential changes in the selected parasites, we performed microarray analysis and detected increased expression of genes involved in redox metabolism. Collectively, this study suggests that selection of artemisinin resistance under the laboratory conditions may be associated with multiple mechanisms.

1506

ARC3: DETECTING RECENT POSITIVE SELECTION IN ARTEMISININ RESISTANT MALARIA PARASITES

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Following reports of poor clinical responses to artemisinin combination therapy (ACT) on the Thailand-Cambodia border, the Artemisinin Resistance Confirmation, Characterization, and Containment (ARC3) pilot

project was initiated to characterize the clinical, *in vitro* and molecular basis of artemisinin resistance in Southeast Asia. Four clinical trials of artesunate curative therapy were conducted at two sites in western Cambodia where emerging resistance was suspected; one site on the Thailand-Myanmar border, where prolonged parasite clearance times following artesunate-mefloquine treatment had also been reported; and in Bangladesh, where ACTs have not been used extensively and resistance was not suspected. Parasites collected during these trials were genotyped at approximately 8,000 single nucleotide polymorphisms (SNPs) using a molecular inversion probe SNP chip specific to *Plasmodium falciparum*. Genotyping data from 198 samples were used to detect genomic regions under recent positive selection. Each SNP was scored using three Extended Haplotype Homozygosity measures including the Long-Range Haplotype test, Integrated Haplotype Score, and Cross-Population Extended Haplotype Homozygosity. SNPs were also evaluated on their inter-population Fst values using a sliding window approach. The top 5% of loci from each statistic were ranked and re-scored. A comprehensive list based on the combined rank score revealed a number of novel regions possibly under recent positive selection, as well as previously described regions containing resistance genes such as *pfcrt* on chromosome 7 and *dhfr* and *dhps* on chromosomes 4 and 8, respectively. Regions identified as being under recent positive selection will be compared to regions identified in a genome-wide association study performed using the same sample set, and potential candidate genes within those regions will be discussed.

1507

COMPARATIVE EFFICACY AND ACCEPTABILITY OF ARTEMETHER-LUMEFANTRINE *VERSUS* DIHYDROARTEMISININ-PIPERAQUINE IN KENYAN CHILDREN WITH UNCOMPLICATED *FALCIPARUM* MALARIA

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Artemisinin-based combination therapies (ACTs) have become the cornerstone for the treatment of uncomplicated *falciparum* malaria worldwide. In Africa, artemether-lumefantrine (AL) and artesunate-amodiaquine are the most widely used ACTs. AL has been the first-line treatment for uncomplicated malaria in Kenya since 2006. Despite not yet receiving World Health Organization (WHO) prequalification, dihydroartemisinin-piperaquine (DP) has recently been adopted as a second-line treatment in Kenya. This was an open-label, randomized, comparative trial in children aged 6-59 months with uncomplicated *falciparum* malaria conducted in Western Kenya. Parasite clearance rate, sensitivity to and acceptability of AL and DP were monitored. In total, 466 children were enrolled in the study; they were hospitalized for 3 days for observed treatment and 72-hour parasite kinetic monitoring, and actively followed up at scheduled visits after discharge from hospital on Days 7, 14, 28 and 42. Hemoglobin levels were assessed on Days 0, 14, 28 and 42. Genotyping for determining treatment outcome was performed on Day 0 and any other day the study participant had a recurrence of parasitemia. The study drugs were administered by the parent/guardian under the observation of a study team member. At discharge from hospital, a questionnaire on the acceptability of the study drug was administered to the parent/guardian. The findings of this study on the parasite clearance rate, sensitivity to and acceptability of AL and DP in the treatment of uncomplicated malaria in Western Kenya will be discussed following completion of data analysis.

1508

SAFE AND EFFICACIOUS ARTEMISININ-BASED COMBINATION TREATMENTS FOR AFRICAN PREGNANT WOMEN WITH MALARIA

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Although malaria is the most important human parasitic disease, few studies with antimalarial drugs have been carried out in pregnant women. Pregnant women are a high-risk group requiring effective antimalarials but they are systematically excluded from clinical trials for fear of teratogenicity and embryotoxicity. This has complicated evidence-based recommendations for the prevention and treatment of malaria during pregnancy. A multicentre (Burkina Faso, Ghana, Malawi and Zambia), non-inferiority trial on the safety and efficacy of four artemisinin-based combinations for the treatment of *P. falciparum* malaria in pregnant women has recently started within the framework of the Malaria in Pregnancy Consortium and the financial support of both the European and Developing Countries Clinical Trials Partnership (EDCTP) and the Gates Foundation. Pregnant women in the second or third trimester of gestation and with a confirmed malaria infection are randomised to amodiaquine-artesunate, artemether-lumefantrine, dihydroartemisinin-piperaquine or mefloquine-artesunate. They will be followed up weekly until day 63 post-treatment and then monthly until 4-6 weeks and one year post-delivery. Explanatory variables for failure, i.e. drug levels and *in vitro* resistance of local malaria parasites are also collected. A total of 870 patients will be recruited to each treatment based on 290 patients in each treatment group in each country (i.e. a total centre sample size of 870 patients), adding up to a total study sample size of 3480 patients. A 3-arm trial using a "balanced incomplete block design" allow the treatments to be distributed in a way to allow a head-to-head comparison and the establishment of relative value of the treatment according to a series of outcomes. The primary end points are treatment failure (PCR adjusted) at day 28 and the safety profiles including significant changes in relevant laboratory values. More than 800 patients have been recruited so far. Results are expected by 2013.

1509

PARASITE CLEARANCE OF ARTEMISININ-CONTAINING REGIMENS IN THE TREATMENT OF UNCOMPLICATED MALARIA: A SYSTEMATIC REVIEW OF PUBLISHED TRIALS FROM 2000 TO 2010

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The deployment of artemisinin-based combination therapies (ACTs) for the treatment of acute uncomplicated *falciparum* malaria has become a key strategy for malaria control. The objective of this study is to review the recent trends in clinical trials of artemisinins and document parasite clearance times. Publications reported in PubMed, EMBASE, GH and the Cochrane Libraries between January 2000 and December 2010 were searched for the following key words: malaria, plasmodium, *falciparum*, *vivax*, ovale, malariae, knowlesi and anti-malarials. 1520 abstracts were indentified and 223 full text articles retrieved. In total 60% (n=96) of 160 clinical trials identified were conducted in Africa and 34% (n=54) in Asia.

Of the African countries, Nigeria hosted the maximum number of trials (n=14), whereas in Asia Thailand was the leading country (n=22). In total 138 (86%) studies were randomized and they included 309 treatment arms with an ACT given over 1-7 days. The duration of follow up was at least 42 days in 44 (28%) studies, 28 days in 107 (67%) studies and less than 28 days in 8 (5%) studies. A total of 47,855 patients were allocated an artemisinin-containing regimen in 160 trials with samples size ranging from 30 to 1553. The quality of clinical trials (as gauged by randomization, duration of follow-up, sample size) improved significantly in the second half of the decade compared to the first. The proportion of patients remaining parasitaemic at 48 and 72 hours could be determined in 125 and 114 treatment arms, respectively. Following initiation of therapy the median proportion of patients still parasitaemic at 48 and 72 hours was 6.4% (range 0-73) and 0.5% (range 0-78) respectively. There was no significant change in the proportion of patients remaining parasitaemic at 48 and 72 hours in studies conducted between 2000-2005 compared to 2006-2010 (p=0.093 and p=0.215 respectively). Standardised approaches are urgently needed to define with greater precision geographical and temporal trends in the parasite clearance following ACTs; this will require analysis of pooled data from individual records.

1510

IN VITRO CHARACTERIZATION OF THE METABOLISM AND DISPOSITION OF ARTESUNATE AND DIHYDROARTEMISININ

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IV Artesunate is currently approved for compassionate use in the US through the CDC, and is seen as a promising alternative to the only FDA-approved parenteral treatment of severe malaria in the United States, IV quinidine gluconate. While an effective antimalarial treatment, quinidine has potential serious cardiotoxicity. In support of efforts to obtain FDA approval for IV artesunate, our lab has performed a battery of *in vitro* metabolism and disposition assays of artesunate and its major metabolite, dihydroartemisinin (DHA). Artesunate demonstrated a short (<10 min) microsomal half-life in both human and mouse liver microsomes, while DHA exceeded the assay limits of 60 min. In human hepatocytes, the predominant metabolite of artesunate, as expected, was DHA, along with its glucoronide. These data were confirmed *in vivo* using clinical samples previously analyzed in this lab. In enzyme inhibition studies, artesunate demonstrated low potential for drug-drug interactions due to enzyme inhibition with any of the major CYP450s (1A2, 2C9, 2C19, 2D6, and 3A4) while DHA showed only moderate potential for inhibition of 1A2 and 2C9. Permeability was tested using MDR1-MDCK to assess cell permeation and potential as a Pgp substrate. These experiments showed medium/moderate permeability as compared to standards of known penetrability, and indicate a high potential as a Pgp substrate. The data support observations that AS and DHA are rapidly biotransformed and eliminated as the glucoronide of DHA. Evaluation of other potential metabolites is in progress.

1511

THE PHARMACOKINETICS AND PHARMACODYNAMICS OF A TWO- VERSUS THREE-DAY DOSE REGIMEN OF DIHYDROARTEMISININ-PIPERAQUINE IN PATIENTS WITH UNCOMPLICATED MALARIA IN NORTHERN CAMBODIA

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The combination of dihydroartemisinin (DHA) combined with long acting piperaquine (PIP) was recently adopted in Cambodia as the first line agent against both multi-drug resistant *Plasmodium falciparum* and *P. vivax*. In addition to well-documented safety and efficacy, a post-treatment prophylactic effect of DHA-PIP of up to 63 days has been reported, making it potentially valuable in malaria eradication efforts. While a 3-day course is widely recommended, the Cambodian military currently employs a 2-day regimen in order to improve compliance. The aim of this study was to compare the safety, tolerability and pharmacokinetic-pharmacodynamic relationships of a 2 versus 3 day dosing regimen of DHA-PIP using the same cumulative dose to establish the optimal regimen. From September 2010 to February 2011, in an open-label clinical trial, 80 patients with uncomplicated malaria were randomized 1:1 to receive 9 fixed-dose combination tablets (total dose 320mg of DHA and 2880 mg PIP), divided into either a 2 or 3 day course. Plasma piperaquine levels from all volunteers receiving DP were collected at pre-dose (time 0), 4, 24, 48, 72 hr, 7, 14, 21, 28, 35 and 42 days after the first dose, and on day of recurrence. Sixteen patients were diagnosed with P.f. (20%), 61 with P.v. (76%) and 3 with mixed species infection (4%). Uncorrected 42-day efficacy rates were not statistically significantly different between treatment groups by per protocol analysis - 89% for 2 days (95% CI = 76-96%) and 92% for 3 days (95% CI = 80-97%) of DP. Mean parasite clearance times were 11.1 hours for *P. vivax*, but 72.5 hours for *P. falciparum*. Piperaquine levels are currently being measured by liquid chromatography-mass spectrometry. Pharmacokinetic data will be presented, along with pharmacodynamic analysis of parasite clearance and PCR-corrected treatment efficacy, with particular attention to drug levels at the time of failure and post-treatment prophylactic effect.

1512

ARC3: ASSOCIATIONS BETWEEN CANDIDATE GENE POLYMORPHISMS AND PARASITE CLEARANCE RATE FOLLOWING TREATMENT WITH ARTEMISININS

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Nearly all malaria-endemic countries have replaced resistance-compromised antimalarial drugs with artemisinin-based combination therapy, the efficacy of which is also at risk due to the recent emergence of artemisinin-resistant *falciparum* malaria on the Thailand-Cambodia border. Without knowledge of the mechanism of action of the artemisinins, genome-wide approaches are being taken to identify loci associated with resistance to this class of drugs. In addition, candidate genes that have been reported in the literature as possibly being associated with artemisinin resistance are being evaluated. Full-length sequences were determined for three candidate genes in *Plasmodium falciparum* infections in four clinical trials of 7-day curative artesunate therapy conducted as part of the Artemisinin Resistance Confirmation, Characterization, and Containment (ARC3) pilot project. The sequenced loci include: *pfmdr1*, a gene associated with resistance to other antimalarial drugs, *pfserca*, a gene encoding a sarco/endoplasmic reticulum calcium-dependent ATPase, and *pfubp1*, coding for an ortholog of a deubiquitinating enzyme associated with artemisinin resistance in rodent malaria. Polymorphisms in each of these genes, as well as variations in copy number estimates of *pfmdr1*, were evaluated for associations with parasite clearance rate and will be presented in the context of results from genome-wide analyses from the same studies. Strategies for prioritization of newly identified candidate genes will be discussed.

1513

EFFICACY OF DIHYDROARTEMISININ PLUS PIPERAQUIN COMPARED TO AMODIAQUIN PLUS SULFADOXIN/ PYRIMETHAMIN IN SEASONAL IPT OF MALARIA IN CHILDREN IN A RURAL AREA OF BOBO-DIOULASSO (BURKINA FASO)

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Intermittent preventive treatment (IPT) is a promising malaria control strategy. Optimal regimen and the best option for his delivery remain unclear. The long elimination half-life of Piperaquine (PQ) makes its coformulation with dihydroartemisinin (DHA), suitable for IPT. To assess the effectiveness of DHA + PQ compared to AQ + SP in seasonal IPTc, 1500 children aged 3-59 months were randomized to receive DHA+PQ or SP+AQ once a month from August to October 2009. A comparator arm with 250 children was also recruited and followed up in parallel. A cross sectional survey was led at the end of transmission season. Primary endpoints were the incidence of clinical malaria attacks. Coverage of all three courses of IPT and compliance of children to daily doses of IPT was similar in the two arms. 83% of children in each arm received at least the first dose of the 3 courses of IPT and 99% to 100% of them completed the entire three doses regimen. A total of 328 episodes of clinical malaria with any parasitemia (incidence rate (IR) of 0,047(95%IC=[0,042 to 0,052] episodes per child day at risk (CD)) was observed in the control arm compared to 205 (IR of 0,003(95% IC= [0,002 to 0,003] episodes per CD) in DHA+PQ arm and to 147 (IR of 0,002(95% IC= [0,001 to 0,002] episodes per CD) in SP+AQ arm, indicating a protective efficacy (Pe) of 93% (95% IC= [92 to 94] p<0.0001) for DHA+PQ versus 95% (95% IC = [94 to 96] p= 0.0001) for SP+AQ. Children in SP+AQ arm were better protected against malaria attacks than those in DHA+PQ arm (Pe = 28% (95% IC=[10 to 42] p= 0.003). At the end of the transmission season, only SP + AQ retained a substantial efficacy (Pe = 62% (95% CI = [32-78] p= 0.001) against malaria attacks. The prevalence of parasitaemia was reduced by 67%, in the two treated arms. The prevalence of anemia (Hb< 10g/dl) was similar in DHA+PQ (40%) and SP+AQ (35.34%) arms with a respective Pe of 17% (95% CI = [3 to 29], p = 0.026), and 27% (95% CI = [14 to 27], p = 0.001). The mean Hb in SP + AQ arm was significantly higher than the DHA + PQ group and control group. In conclusion, SP+AQ was the most efficacious for IPTc in our study but its use must be reserved to area with low-level SP resistance otherwise DHA+PQ would be a suitable alternative.

1514

A COMPARISON BETWEEN *EX VIVO* AND *IN VITRO* (CRYOPRESERVED + CULTURE ADAPTED) ARTEMISININ SUSCEPTIBILITIES FOR *PLASMODIUM FALCIPARUM* ISOLATES FROM WESTERN CAMBODIA

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Artemisinin derivatives are the most potent first line antimalarial drug in use today. However, recent studies along the Thai-Cambodian border conducted during the past 5 years demonstrate that *Plasmodium falciparum* may be becoming resistant to the artemisinin class. Since clear genetic markers of artemisinin resistance have not been discovered, *in vitro* parasite drug susceptibility testing may be helpful to identify resistance trends. In many *in vitro* drug sensivity studies, samples are collected from untreated subjects in the field and cryopreserved to facilitate sample transport. Because such samples must be thawed and recultured, the IC50 measured after recovery may diverge from the *ex vivo* IC50. During a dose ranging randomized open label clinical trial of a 7 day course of mono-therapy artesunate (Artemisinin Resistance in Cambodia - 2; 2008-2009) parasite samples were analyzed *ex vivo*, without cryopreservation or culture adaptation, for susceptibility to dihydroartemisinin, artesunate, and other anti-malarial drugs using a histidine-rich protein-2 (HRP2) ELISA method. IC_{50} values obtained for the artemisinins (DHA and AS) did not correlate with treatment outcome. However, the values were two-fold higher than those obtained in an earlier study (Artemisinin Resistance in Cambodia - 1, 2006-2007) conducted at the same location. This increase suggests that *falciparum* parasites in this location are progressively more tolerant to the artemisinin class of antimalarials. However, we and others have not determined if this tolerance is maintained in culture in the absence of drug pressure. We will report the *in vitro* analysis of cryopreserved parasites and compare them to matching uncryopreserved *ex vivo* results. Parasite susceptibility to other anti-malarial drugs and change in parasite genetic constituent will also be described. These results will be helpful to inform future surveillance

investigating the problem of artemisinin resistance, particularly whether resistance IC50 phenotypes can be preserved through cryopreservation and recovery.

1515

INCREASE IN EX VIVO IC_{50} VALUES TO ARTEMISININS FROM A SEVEN-DAY ARTEMISININ MONO-THERAPY TRIAL CONDUCTED IN WESTERN CAMBODIA IS NOT DUE TO INOCULUM EFFECT

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Studies along the Thai-Cambodian border conducted during the past 5 years demonstrate that *P. falciparum* may be developing resistance to the artemisinin class of antimalarial drugs, the last first-line antimalarial compounds in use today. Persistence of parasitemia beyond 72 hours has been proposed as a parasitological marker of diminished artemisinin susceptibility. During a dose ranging randomized open label clinical trial of a 7 day course of mono-therapy artesunate (Artemisinin Resistance in Cambodia - 2; 2008-2009) parasite samples obtained pre-treatment (on Day 0) were analyzed ex vivo, without cryopreservation or culture adaptation, for susceptibility to dihydroartemisinin, artesunate, and other anti-malarial drugs using a histidine-rich protein-2 (HRP2) ELISA method. IC50 values obtained for the artemisinins (DHA and AS) did not correlate with treatment outcome. However, there was a statistically significant relationship between parasite clearance time greater than 72 hours (PCT > 72hrs) and elevated IC50 values for DHA ($p<0.003$). Previous reports have implicated an inoculum effect, or the parasitemia of the isolate, as a possible reason for elevated IC50 values against the artemisinins. Here we argue that the association between DHA IC50 value and PCT>72hrs may not be solely due to inoculum effect, and may represent the early stages of *in vitro* resistance to the artemisinins.

1516

A RANDOMIZED CLINICAL TRIAL OF ARTEMISININ VERSUS NON-ARTEMISININ-BASED COMBINATION THERAPY OF UNCOMPLICATED MALARIA IN MALI

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Plasmodium falciparum resistance to artemisinin has been reported in South-East Asia. The potential spread of this resistance is real and makes a search for alternative non-artemisinin-based malaria therapy urgent. We tested the hypothesis that sulphadoxine-pyrimethamine plus artesunate (SP+AS) is as efficacious as sulphadoxine-pyrimethamine plus amodiaquine (SP+AQ) in the treatment of uncomplicated *Plasmodium falciparum* malaria. From August to December 2004 and July to December 2005, we conducted a randomized single-blind trial of SP+AS and SP+AQ in two localities in Mali. Parasite genotyping by polymerase chain reaction (PCR) was used to distinguish new from recrudescent *P. falciparum* infections. We recruited a total of 610 children aged 6 to 59 months, with uncomplicated *P. falciparum* malaria and followed them for 28 days to assess treatment efficacy. Baseline characteristics were similar in both treatment groups. The analysis revealed no early therapeutic failures (ETF) in both arms; late clinical failures (LCF) were 1.7% for SP+AS (n=5) vs. 0% SP+AQ (n=0) and late parasitological failures (LPF) were 3.4% SP+AS (n=10) vs. 1.4% SP+AQ (n=4) ($p>0.05$). We observed a rate adequate clinical and parasitological response (ACPR) of 94.9% and 98.6% for SP+AS and SP+AQ respectively ($p=0.98$). Based on *msp2* analysis, the rate of re-infection was respectively 4.1% and 1.4% for SP+AS and SP+AQ. After molecular correction, we obtained an ACPR of 99% for SP+AS, and 100% for SP+AQ ($p=0.98$). Sulphadoxine-pyrimethamine plus amodiaquine therapy is as efficacious as sulphadoxine-pyrimethamine plus artesunate in the treatment of uncomplicated *P. falciparum* malaria in Mali.

1517

STUDY OF *PLASMODIUM VIVAX* DUFFY BINDING PROTEIN (DBP) OF EASTERN INDONESIAN ISOLATES

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Erythrocyte invasion by malaria parasites is a series of complex processes and specific interaction between parasite ligands and host receptors play a key role. *Plasmodium vivax* uses the critical ligand Duffy Binding Protein (DBP), to bind its host receptor, Duffy Antigen Receptor for Chemokines (DARC), and initiate the formation of tight junction between merozoite and erythrocyte essential for invasion. The central domain of Domain II of DBP serves as the critical binding motif to the DARC. However, the genetic diversity of PvDBPII is a major obstacle to vaccine development. In order to develop a rational vaccine, more information about the sequence polymorphisms of PvDBPII is required, from diverse geographical regions. The current study aims to determine the extent of polymorphisms of PvDBPII from Timika, West Papua, Indonesia and to examine the relationships of different dbp alleles with published sequences through phylogenetic analysis. 95 *P. vivax* isolates were collected from the hospital-based study in Timika from 2006-2008. These DNA samples were subjected to PCR-RFLP using Pvmsp-3α to assess its genetic polymorphisms. Some samples were selected and further subjected to cloning and sequencing to examine the PvDBPII sequence polymorphisms. A phylogenetic tree was constructed based on the PvDBPII sequences obtained in this study and combined with the published sequences using Bayesian inference. A total of 101 DBP point-mutations were found from the Timika isolates. Mutations mainly occurred in the critical binding motif of PvDBPII. Most mutations (81%) were non-synonymous; altering amino acids sequence and only about a fifth (23%) were synonymous. R308S, K371E, D384G, R390H, N417K, L424I, W437R, I503K were the highest frequency polymorphisms seen. In total, about 51 different haplotypes of PvDBPII were observed. More haplotypes might be found if more clones are sequenced. The phylogenetic tree showed that Timika PvDBPII isolates clustered together with isolates from other geographic regions, including Brazil, Sri Lanka, and Papua New Guinea. Although the PvDBPII sequences of Timika isolates are quite diverse as shown by the number of haplotypes detected and the topology of the phylogenetic tree, the similarities between those sequences are still high. These findings certainly contribute to the development of PvDBPII vaccines against *P. vivax* infections, and thus demonstrate its public health significance.

1518

FREQUENCIES OF SOME HUMAN GENETIC MARKERS AND RELATIONSHIP WITH *PLASMODIUM FALCIPARUM* AND *P. VIVAX* MALARIA IN COLOMBIA

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Malaria in Colombia is highly endemic in the Northwest, Pacific Coast and Amazon regions. Despite the high frequencies of *Plasmodium falciparum* infection, severe or fatal malaria cases are rare. Out of 79.909 malaria cases (72% *P. vivax* - 27% *P. falciparum*) reported in 2009, only 307 were severe (1,4% of *falciparum* cases) with 0,04% fatality rate. Some proposed that *P. falciparum* shaped the distribution of ABO blood groups in humans with some groups being protective against severe disease. The Duffy blood group A has marked selective and sickle-cell disease, G-6-PD deficiency a more discrete one. Aimed at understanding the blood genetic factors underlying the apparent protection to development of severe disease within a *falciparum* malaria infected community, we explored the ethnic background, blood types and *Plasmodium* infection in La Italia on the Pacific Coast. A descriptive, cross-sectional study in a deprived and mainly rural region was conducted. Sample sizes were Afro-American, 73; Amerindian (Emberá), 74 and Mestizo, 171. Presence of *Plasmodium* infection was assessed by thick smear, ABO and Rh were determined by agglutination and Duffy status by PCR and RFLP. For ABO, 69% were O, 21% A, 8% B and 2% AB. A significant association was observed for ABO status and ethnicity: 100% of Amerindias were group O. Similarly, Duffy genotypes were significantly associated to ethnicity (p=0,003). Expression of Rh was confirmed in 97-99% of all subjects, regardless of the ethnic background. For Duffy, the C/C,A/A diplotype carriers were exclusively infected by *P. falciparum*. At locus 131, the frequency of the G allele was 0,30 in Amerindians and the A allele was 0,69 in Afrocolombians. *Plasmodium* infection was confirmed in 17% (*falciparum* 2,2: 1 *vivax*), 33% were asymptomatic and 3,1% of *falciparum* cases developed severe infection. In conclusion, a very high frequency of group O was confirmed in a locality where *falciparum* infection is frequent. High endogamy and population differentiation was confirmed after Duffy genotyping. Infection by *P. vivax* was not detected in CC(FY-/FY-) individuals.

1519

CRYOPRESERVED *PLASMODIUM VIVAX* AND RETICULOCYTES CAN BE USED FOR INVASION AND SHORT TERM CULTURE

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The development of a *Plasmodium vivax in vitro* culture system is critical for the development of new vaccine, drugs and diagnostic tests. Though short term cultures have been successfully set up, their reproducibility in laboratories without direct access to *P. vivax* patients has been limited by the need of fresh parasites isolates. We have explored the possibility of using both frozen parasite isolates and frozen reticulocytes to perform invasions and start a short term culture. More than 50 invasion tests were performed. Invasion could be performed with similar efficiency for any of the combination (fresh/frozen reticulocytes and *P. vivax* isolates) used. This method should be easily replicated in laboratories outside endemic areas and can substantially contribute to the development of a continuous *P. vivax* culture.

1520

RETICULOCYTES DERIVED FROM HEMATOPOIETIC STEM CELLS CAN BE SUCCESSFULLY CRYOPRESERVED TO BE USED FOR *PLASMODIUM VIVAX* INVASION TESTS

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The differentiation of hematopoietic stem cells (HCS) into reticulocytes has been previously described as potentially interesting for the development of the *Plasmodium vivax in vitro* culture. Nevertheless, the need of using both freshly derived reticulocytes and fresh *P. vivax* isolates remained an obstacle, particularly for laboratories located in non-endemic countries. We describe a new method for the cryopreservation of reticulocytes produced after 14 days of culture from HSCs. Invasions assays have been carried out with both frozen isolates of *P. vivax* as well as laboratory strains of *P. falciparum*. Cryopreserved *P. falciparum* and *P. vivax* isolates could equally invade fresh and cryopreserved reticulocytes. This new technique represents an important advance towards the establishment of a continuous *P. vivax* culture as it allows the storage of large quantities of reticulocytes to be later used for the invasion.

1521

BIOMECHANICAL AND NANOSTRUCTUAL CHANGES TO THE *PLASMODIUM VIVAX* INFECTED RED BLOOD CELL MEMBRANE

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The infection of reticulocytes by *Plasmodium vivax* results in significant changes to the infected red cell membrane (IRBCM). Most importantly, the *P. vivax* IRBCM becomes highly deformable relative to membranes of the reticulocyte and mature normocyte. We present a range of data clearly demonstrating these biomechanical changes to the IRBCM using single cell and (micopipette, microfuidics) and cell population measurements (LORCA). Although the mechanism behind these changes is not fully understood, increased deformability would aid *P. vivax* avoid splenic clearance. In addition to these biomechanical changes we examined the development of 'caveolae' on the surface of the IRBCM in staged *ex vivo* cultures of *P. vivax* IRBCs, using TEM, SEM and Atomic Force Microscopy. We show that caveolae-like depressions found in the host cells (reticulocytes) and *P. vivax* IRBCs are morphologically and biologically distinct, thus unrelated. We also show that 'Schüffner's dots', a classical diagnostic feature of *P. vivax*, is associated with parasite derived vesicle complexes and not caveolae.

1522

DETECTION OF *PLASMODIUM VIVAX* PRE-ERYTHROCYTIC STAGE USING A ROLLING CIRCLE AMPLIFICATION (RCA) ASSAY

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A rolling circle amplification (RCA) assay was developed to detect the pre-erythrocytic stage in liver cells infected with *Plasmodium vivax*. The circular probe (98bp) in combination with a fluorescence DNA detector probe was designed to have a complementary sequence (20bp in length) to malaria 18S rDNA at the 5' and 3'ends of the probe. The RCA technique was previously developed and applied to detect several pathogens in order to increase detection sensitivity in clinical samples. The target sequence amplification step uses phi 29 or Bst enzymes to enhance the intensity of the detection signal in the RCA assay. Our results showed that the *in vitro* RCA assay for the detection of *P. vivax* resulted in a fluorescent signal significantly higher relative to the signal resulting from the real-time PCR method (8.0 versus 0.5 fluorescent intensity). However, the RCA assay required more time and additional steps when compared to the real-time PCR assay. *In situ* RCA was established using an infected blood smear (thick film) collected from a *P. vivax*-infected patient and with *P. berghei*-infected HepG2 cells collected at 48h post-infection. An infected cell in a blood smear generated a strong RCA signal although the number of positive cells was lower than that observed with immunofluorescence staining using a heat shock protein 70 (HSP70) monoclonal antibody. In a liver cell culture infected with *P. berghei*, the infected cells generated a strong RCA signal and all infected cells were confirmed by an immunofluorescence signal. In conclusion, the RCA technique can detect *P. vivax* in infected liver cells even where there are low infection rates and therefore such a technique will allow researchers to better understand the biology of *P. vivax* in liver cells.

1523

ASYMPTOMATIC *PLASMODIUM VIVAX* INFECTIONS IN SOUTHERN MINDANAO, PHILIPPINES: A CHALLENGE TO MALARIA ELIMINATION

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The Philippines is among the 39 countries progressing towards malaria elimination. The country shifted to artemether-lumefantrine in 2009, dispensed insecticide-treated nets, and conducted indoor residual spraying to address malaria in 58 out of 80 endemic provinces. However, in low transmission settings asymptomatic infections especially with *Plasmodium vivax* can hamper elimination efforts when parasitemia is too low for detection by microscopy in health clinics. In this study, we compared the performance of pLDH/HRP2-based rapid diagnostic test (RDT) for *P. falciparum* and *P. vivax* with PCR in detecting asymptomatic infections. Using the RDT in a cross-sectional survey in Sarangani Province to screen 930 participants (aged 12 mos. and above), we detected one *P. vivax* infection (a prevalence of 0.10%) and 10 *P. falciparum* infections (1.08%) all of whom were asymptomatic upon presentation. We also collected blood spots on filter paper for molecular and serological assays. To date, we have screened 700 out of 930 blood spots by PCR using species-specific primers for the 18SrRNA gene of *Plasmodium*. PCR results showed 7 participants (1%) were infected with *P. falciparum* (4 of whom were RDT negative) while 10 participants (1.4%) were infected with *P. vivax*, 9 of whom were RDT negative. Four of the 7 *P. falciparum*-infected individuals lived in a forested B'laan tribal community in Maasim, Sarangani which was 10-12 km from formal health systems, while 6 of the 10 *P. vivax*-infected individuals lived in a forest fringed T'boli tribe community in Kiamba, Sarangani. These infections would have been undetected and untreated as no clinical signs of malaria were evident. Our findings to date indicate pockets of ongoing malaria transmission despite current control measures, which hamper malaria elimination efforts; and the sensitivity of the Pf/Pv combination RDT is insufficient to detect asymptomatic *P. vivax* infections. On completion of PCR screening, we plan to calculate the comparative performance of the RDT versus PCR in diagnosing asymptomatic *P. vivax* infections in Southern Philippines. We will also complete serological assays for exposure to Pf/Pv infections on the same samples. Together with PCR and questionnaire data we aim to build a complete epidemiologic profile of malaria in Sarangani and guide intervention measures to halt transmission.

1524

A MALARIA DIAGNOSTICS TO BE USED IN THE FIELD: VISUALIZED LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) FOR DETECTION OF *PLASMODIUM VIVAX* INFECTION

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Loop-mediated isothermal amplification (LAMP) is a high performance method for detecting DNA and is of high potential usage in the molecular detection of infectious pathogens including *Plasmodium spp*. However, in most malaria endemic areas, which are often resource-limited, current LAMP methods are not feasible for diagnosis due to difficulties in accurately interpreting results with problems of sensitive visualization of amplified products, and the high risk of contamination resulting from the high numbers of amplified DNA sequences produced. In this study we establish a novel visualized LAMP method in a closed-tube system, and validate it for the diagnosis of malaria in a simulated field condition. A visualized LAMP method was established by the addition of a microcrystalline wax-dye capsule containing the highly sensitive DNA fluorescence dye SYBR Green I to a normal LAMP reaction prior to the initiation of the reaction. The wax remained intact during isothermal amplification, and released the DNA dye to the reaction mixture only when the temperature was raised to the melting point following amplification Soon after cooling down, the solidified wax sealed the reaction mix at the bottom of the tube, thus minimizing the risk of aerosol contamination. A total of 89 of field blood samples were collected on filter paper and processed using a simple boiling method for DNA extraction. This was then tested by the visualized LAMP method. Compared to microscopy, the sensitivity and specificity of LAMP were 98.3% (95% CI, 91.1% to 99.7%) and 100%, and were in close agreement with a nested PCR method. In conclusion, this novel, cheap and quick visualized LAMP method is feasible for malaria diagnosis in resource limited field settings.

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REAL TIME LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (REALAMP) FOR THE SPECIES-SPECIFIC IDENTIFICATION OF *PLASMODIUM VIVAX*

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Plasmodium vivax infections remain a major source of malaria-related morbidity and mortality. Early and accurate diagnosis is an integral component of effective malaria control programs. Conventional molecular diagnostic methods provide accurate results but are often resource-intensive, expensive, have a long turn around time and are beyond the capacity of most malaria-endemic countries. Our laboratory has recently developed a new platform called RealAmp, which combines loop-mediated isothermal amplification (LAMP) with a portable tube scanner real-time isothermal instrument for the rapid detection of malaria parasites. This method was initially tested using *Plasmodium* genus-specific primers, but species-specific diagnosis was not accomplished as there were no primer sets that gave consistent results. Here we describe a new primer set for the detection of *P. vivax*. The LAMP assay was designed using three pairs of amplification primers targeting a conserved DNA sequence unique to the *P. vivax* genome using an algorithm we have developed for genome mining. The amplification was carried out at 64ºC using SYBR Green intercalating dye for 90 minutes with the tube scanner set to collect fluorescence signals at 1-minute intervals. Clinical samples of *P. vivax* and other human-infecting malaria parasite species were used retrospectively to determine the sensitivity and specificity of the primers using the 18s ribosomal DNA based nested PCR as the gold standard. The new set of primers showed promising results in detecting laboratory-maintained isolates of *P. vivax* from different parts of the world. The time to amplification ranged from 18 to 49 minutes from the start of the reaction. The primers detected *P. vivax* in the clinical samples with 89.29% sensitivity and 100% specificity compared to the gold standard nested PCR method. The new primers also proved to be more sensitive than the published species-specific primers in detecting *P. vivax*. Further validation of this test using prospective testing in endemic countries will help deploy this tool for future field use.

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DETECTION OF ASYMPTOMATIC *PLASMODIUM FALCIPARUM, P. VIVAX* AND *P. MALARIAE* DURING LOW-TRANSMISSION SEASON IN THE HILL TRACTS OF BANGLADESH

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During the low transmission season many malaria infections are asymptomatic. To investigate how best to target asymptomatic individuals, an active randomized, population-based malaria surveillance was initiated by the JHMRI and ICDDR,B in two unions near hypoendemic Bandabarn in the Chittagong Hill Tracts of Bangladesh. The population of 20,000 with approximately 4,500 households was enumerated in a baseline census with GIS-mapping. Detection of *Plasmodium* species using microscopy, RDT and Real-time PCR was performed for the active and passive case detection. Microscopy, RDT, and real-time PCR in the active surveillance showed approximately 2% positive rates by microscopy or RDT in nearly 500 individuals. A real-time PCR assay detected the prevalence of 6%. The sensitivity of the RT-PCR in the 96-well format was increased to 10-100 parasites/µl with a glycogen/acetate DNA precipitation at low-speed tabletop centrifugation after column extraction. *P. vivax* and *P. malariae* were detected in less than 5% of the malaria-positive patient samples by RT-PCR. All the *P. falciparum* isolates were chloroquine-resistant PfCRT K76T genotype and atovaquone-sensitive PfCYTb 268Y by fluorescent TAQman probe analysis. A reverse transcriptase real-time PCR assay from dried blood on filter papers was able to detect gametocytes. HRP2 antigen detection by RDT persisted up to 28 days in more than 20% of malaria cases in a density-dependent fashion. The geometric mean parasitaemia was 227; 1,342, 5,412, and 10,716 for persistent RDT-positives through Day 0; Day 2; Day 7, and Day 28 respectively. Both microscopy and PCR detection diminished in a few days. Studies on malaria seropositivity rates are in progress. Most of the malaria-positive cases clustered in less than half of the population. Significant asymptomatic populations PCR-positive, RDT and microscopy negative malaria exist. The role of this sub-population in contributing to continuing transmission is being evaluated. Passive surveillance detected less than half of the malaria infections.

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EFFICACY OF CHLOROQUINE FOR TREATMENT OF *VIVAX* MALARIA IN CENTRAL CHINA

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Therapeutic efficacy studies allow measurement of the clinical and parasitological efficacy of medicines and the detection of subtle changes in treatment outcome when monitored consistently over time, which was considered the gold standard for determining antimalarial drug efficacy, and their results are the primary data used by national malaria control programmes to make treatment policy decisions. This study was to measure the efficacy of Chloroquine (CQ) for treatment of *vivax* malaria, and evaluate the incidence of adverse events.From June to October of 2008 and 2009, thirty-eight patients, who met the inclusion criteria, and infected with uncomplicated *vivax* malaria, confirmed by two qualified microscopists in Suining county of central China, were enrolled. CQ was administered at the dose of 25 mg base/kg body weight over 3 days(day 0,1,2), and after 28 days follow-up, primaquine was administered of 0.25mg/kg body weight, taken with food once daily for 14days.The follow-up consisted of a fixed schedule of check-up visits and corresponding clinical and laboratory examinations including thick and thin blood films for parasite count, auxiliary temperature and adverse events. Patients were classified as therapeutic failures or treatment success based on the assessments. It was observed that thirty-seven patients were treatment success over time, no fever and parasitemia were occurred on day 2 and only one patient with 200/ml parasitemia on day 3. Except the normal symptoms including headache, unwell, no severe adverse effect was reported. It was concluded that CQ is still effective and safe efficacy, there was no obvious data to support the CQ resistance to *Plasmodium vivax* at present. Nevertheless, according to the discovering in this study, the initiative diagnosis technique and method should be developed to detect the possible changing pattern and stage of CQ to *Plasmodium vivax*, to formulate recommendations and to enable the Ministry of Health and to make informed decisions about the possible need for updating of the current national antimalarial treatment guidelines. This study was approved by the national ethical committee, and all the patients signed the informed consent, all information regarding the patients was remaining confidential within the study team.

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STAGE SPECIFIC DRUG ACTIVITY OF CHLOROQUINE IN *PLASMODIUM VIVAX* MALARIA: IMPLICATIONS FOR *EX VIVO* DRUG RESISTANCE TESTING

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The emergence of chloroquine resistance in *Plasmodium vivax* has enhanced the need for greater understanding of the epidemiology of the disease, the mechanisms of drug resistance in these parasites, and effective case management. Historically the Schizont Maturation Test (SMT) has been used to monitor drug resistance in asexual stages of *P. falciparum*. Modifications are subsequently required when the test is applied to *P. vivax* due to the high diversity of life cycle stages present in blood samples taken from the peripheral circulation. In this study we analysed the results from 760 isolates assessed by the SMT to investigate how development time of the parasite is related to drug sensitivity. We confirm the previous hypothesis that chloroquine has a stage specific activity against *P. vivax*, and show that this stage specific activity may have profound consequences for the interpretation of the SMT. Using threshold models we show that increasing assay duration is associated with decreased effective concentration (EC50) values. EC50 values are also shown to be linked to the proportion of ring stages in the initial blood sample. We further demonstrate that assays with a duration of less than 34 hours (upper 95% CI 39 hours) should not be used to estimate EC50, nor an assay where the abundance of rings stage parasites in the initial sample collected at venipuncture does not exceed 66% (upper 95% CI 90%) of the total parasites. As late stage *P. vivax* parasites are insensitive to chloroquine, the starting composition of parasites in the SMT can dramatically affect the estimated EC50 values for chloroquine; the EC50 will be erroneously high if only resistant, late stage parasites are exposed. Application of this threshold modelling approach suggests that similar issues may occur for testing of resistance to amodiaquine and mefloquine. The statistical methodology that has been developed also provides a novel means of detecting stage-specific drug activity for new antimalarials.

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PVMDR1 MUTATION FOR GENETIC EPIDEMIOLOGY OF CHLOROQUINE RESISTANT *PLASMODIUM VIVAX*

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A mutation in *Plasmodium vivax* multidrug resistance 1 gene (*pvmdr1*) in codon 976 (Y976F) is reported to be associated with chloroquine (CQ) resistance and is recently used to monitor the distribution and frequency of the CQ resistant *vivax* malaria. In this study, we determined the mutation in codon 976 in the *pvmdr1*, using 28 *P. vivax* field isolates from the Philippines (6 isolates), South Korea (4 isolates), Papua New Guinea (PNG) (4 isolates), India (2 isolates), Indonesia (1 isolate), Thailand (1 isolate), Bangladesh (1 isolate), Nepal (1 isolates), China (1 isolate), Iran (1 isolate), Rwanda (1 isolate), Sudan (1 isolate), Comoros (1 isolate), Brazil (2 isolate) and Ecuador (1 isolate), collected in our National Center for Global Health and Medicine, Japan, from 1999 to 2011. The Y976F mutation was observed in the 13 isolates out of the 28 isolates (46%): 6 from the Philippines, 2 from PNG, 1 form India, 1 from Thailand, 1 form Rwanda, 1 form Sudan and 1 form Comoros. The other 15 isolates possessed the wild type (Y976). In the Philippines, CQ has been used for treatment of *vivax* malaria of which resistance against CQ has never been reported so far. However, all the Philippine isolates (collected in Palawan island in 2009) acquired the Y976F mutation. This finding suggests that CQ resistant *vivax* malaria will be emerging in the endemic area in the near future or that this mutation may not be critical to CQ resistance, at least in the Philippine population. On the other hand, in South Korea, all the 4 South Korean isolates of ours collected in 1999 showed the wild type (Y976) in the gene, but later in 2003 and 2007, 2 cases of CQ resistant *vivax* malaria were reported. The correlation of the Y976F genotype and the phenotype of *P. vivax* to CQ has to be further examined to evaluate the reliability of this molecular marker for the genetic epidemiology of *vivax* malaria.

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FIELD *EX VIVO* SENSITIVITY TESTING OF *PLASMODIUM VIVAX AND P. FALCIPARUM*: IMPORTANT CONSIDERATIONS AND A NEW FIELD BASED CYTOMETRIC METHOD

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The *ex vivo* sensitivity testing of antimalarials is an important adjunct to *in vivo* resistance testing. Except for the microscopic schizont maturation assay (MSMA), there are no standardised protocols for side-by-side comparison of the sensitivity profiles *of Plasmodium vivax* and *P. falciparum* clinical isolates. Here we present a practical cytometry based sensitivity assay that provides a viable field based alternative to the MSMA. We discuss a range of important confounders to any sensitivity assay utilising clinical isolates, including the presence of host leukocytes, which decreases antimalarial IC_{50}s, and the initial developmental stage of the parasite, which in the case of *P. falciparum* may result in the failure of H^3 Incorporation assays.

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FIRST REPORT FROM SOUTHERN PAKISTAN ON ALLELIC VARIANTS OF *PLASMODIUM VIVAX CIRCUMSPOROZOITE PROTEIN* (*PVCSP*) AND *MEROZOITE SURFACE PROTEIN1 (PVMSP1)*

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Plasmodium vivax is the prevalent malarial specie accounting for 70% of malaria cases in Pakistan.However,basic data on *P.vivax* genotypes is lacking from Pakistan.Studies have shown that for *P. vivax*, polymorphic genes coding for circumsporozoite protein *Pvcsp* and merozoite surface protein 1 *Pvmsp1,* can be used as reliable genetic markers for conducting molecular epidemiological studies. *Pvmsp1* gene is a mosaic organization of several variable blocks and its genotyping is based on detection of allelic variants in its three polymorphic fragments (F1 to F3).*Pvcsp* genotyping is based on detection of either of the two types of nonapeptide repeat units in its central domain; GDRA (A/D) GPQA, namely VK 210 type and ANGA (G/D) (N/D) QPG, namely VK 247 types.To determine allelic variants of *Pvcsp* and *pvmsp1*,a descriptive study was done on two-hundred and thirty blood samples of *P. vivax* collected from Sind and Baluchistan during 2008-2009.*Pvcsp* and *pvmsp1*were amplified using nested PCR methodology.PCR-RFLP was performed for genotyping of *Pvcsp* while different allelic forms of *Pvmsp1* were detected by analysis of fragment

size.Overall number of genotypes and their prevalence were defined arbitrarily by binning 20 base-pair (bp) intervals together. For *Pvmsp1,* it was found that in F1 fragment, 12 allelic variants were observed (bp size variation350-550), in *F2* fragment 17 allelic variants were observed (950-1270 bp) and in *F3* fragment 8 allelic variants were observed (250-390 bp).Thus, a total of 17 genotypes corresponding to *pvmsp1 gene* were found circulating in Southern Pakistan. *Pvcsp* genotyping in Pakistani isolates showed that *VK210* variants were predominant(79%, 182/230) while percent positivity of *VK 247* was 13%(29/230).Respective bp size variation were 600-870bp for VK 210 and 650-820bp for VK 247.We conclude that this is,to our knowledge, the first study from Southern Pakistan on genetic diversity in *pvcs* and *pvmsp1* gene. Data from this study indicates that both *pvcs and pvmsp1* can be used as reliable markers for conducting genotyping of *P. vivax.*Thus, this study may serve as a baseline data for future research on *P. vivax* diversity from Pakistan.

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PLASMODIUM VIVAX INFECTION IN DUFFY-NEGATIVE INDIVIDUALS IN ETHIOPIA: INDICATIONS AGAINST AN OLD PARADIGM

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In Ethiopia, the most wide-spread *Plasmodium* species co-exist and individuals of variant Duffy blood groups live in this country. A study was conducted to measure the prevalence of malaria and polymorphism in the *Duffy Antigen Receptor for Chemokine* (*DARC)* gene in East and South-Western Ethiopia. Presence of malaria was measured by microscopy and PCR. The polymorphism of *DARC* was analyzed by DNA sequencing. In this study, either *P. falciparum* or *P. vivax* infection was detected in all examined samples. In the analysis of the Duffy blood group, there were 17 (20%) and 24 (22.4%) homozygous Duffy negative individuals in Harar and Jimma study sites. Surprisingly, the data showed that *P. vivax* infection also occurred in three Duffy negative individuals. FYB/FYB^{null} was found to be the dominant genotype in both area. The FYA/FYB and FYB/FYB genotype was associated with susceptibility and FYB^{null}/FYB^{null} genotype was associated with protection against *P. vivax* infection. This study documents an emergence of *P. vivax* infection in Duffy negative individuals in the study area. Duffy negative blood group does not provide absolute protection of *P. vivax* infection in the studied population.

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CHANGING TRANSMISSION PATTERN OF *PLASMODIUM VIVAX* MALARIA IN THE REPUBLIC OF KOREA: RELATIONSHIP WITH CLIMATE CHANGE

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The Korean peninsula is an only place where indigenous *Plasmodium vivax* malaria has occurred continuously on a large scale in temperate areas. *P. vivax* malaria was endemic on the Korean peninsula for many centuries until the Republic of Korea (ROK; South Korea) was declared as a "malaria elimination" country in 1979. *P. vivax* malaria re-emerged in 1993 in the ROK, and has occurred constantly for more than 15 years after its re-emergence. *P. vivax* malaria in ROK has been strongly influenced by infected mosquitoes originating from the Democratic People's Republic of Korea (North Korea). Korean *P. vivax* malaria has shown typical characteristics of unstable malaria transmitted only during the summer season, and displays short and long incubation periods. The changing pattern of the transmission period can be predicted by analyzing the seasonal characteristics of early primary attack cases with a short incubation period. Such cases began to gradually occur earlier in the 1990s after the re-emergence. Considering the sporogony cycle in *Anopheles* mosquito and the asexual cycle via a short incubation period in the infected human, the period of transmission from malaria patients (via the vector mosquito) to manifestation as early primary attack cases would be 3-4 weeks. If *P. vivax* malaria is transmitted actively, subsequent minor peaks are expected to be seen after the annual highest peak by 3-4 week intervals. The obvious minor peak had not been seen until the early 2000s, however, it began to be seen in the mid-2000s. During 2006-2009, three or four subsequent minor peaks after the highest peak were observed by 20-day or 30-day intervals. This phenomenon shows that the length of transmission period of *P. vivax* malaria in the ROK has been gradually extending. This result may be ascribed to a climate change-mediated temperature rise. Malaria and climate data should be integrated to analyze and predict the influence of climate change on malaria occurrence in ROK.

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MORTALITY ATTRIBUTABLE TO *PLASMODIUM VIVAX* MALARIA

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Plasmodium vivax causes almost half of all malaria cases in Asia. Although once regarded as benign, studies have highlighted its association with severe and fatal malaria. The extent to which *P. vivax* contributes to mortality in endemic regions is not known. We aimed to define the epidemiology of mortality attributable to *vivax* malaria in southern Papua, Indonesia by conducting a retrospective clinical records-based audit of all deaths in patients with *vivax* malaria at Mitra Masyarakat Hospital. Between January 2004 and September 2009, hospital surveillance identified 3,495 inpatients with *P. vivax* monoinfection and 65 (1.9%) patients who subsequently died. Charts for 54 of these 65 patients could be reviewed, 40 of whom had pure *P. vivax* infections on cross-checking. Using pre-defined conservative criteria, *vivax* malaria was the primary cause of death in 5 cases, a major contributor in 17 cases and a minor contributor in a further 13 cases. Extreme anemia was the most common primary cause of death for patients in the first category. Malnutrition, sepsis with respiratory and gastrointestinal manifestations, and chronic diseases such as HIV infection were the commonest attributed causes of death for patients in the latter two categories. There were ~293,763 cases of pure *P. vivax* infection in the community during the study period giving an overall minimum case fatality of 0.12 per 1,000 infections. The corresponding case fatality in hospitalized patients was 10.0 per 1,000 infections. Although uncommonly directly fatal, *vivax* malaria is an important indirect cause of death in patients with malnutrition, sepsis syndromes and chronic diseases in southern Papua.

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CYTOKINE DYNAMICS AFFECT SUSCEPTIBILITY TO *PLASMODIUM VIVAX* AND *P. FALCIPARUM* INFECTION AND ANEMIA: STUDIES FROM THE PERUVIAN AMAZON

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The dynamics of immune factors in *Plasmodium falciparum* and *P. vivax* infection are thought to dictate host symptoms and pathology but have not been fully elucidated to date. We aimed to describe the cytokine dynamics of malaria infection and examine malaria species, age and gender dependent differences. Serum samples were collected by active

weekly blood sampling in the Zungarococha community of Iquitos, Peru from April 2003-September 2008 from a total of 397 *P. falciparum*, 515 *P. vivax* and 39 mixed *P. falciparum* and *P. vivax* infections. Cytokine measurements were made using Luminex bead-based assay from sera collected from infected individuals one week before, during, one week after and one month following infection. Relationships of immune markers to strain, age, and gender were assessed. *P. falciparum* and *P. vivax* infected males were more prone to febrile malaria infection and demonstrated higher levels of IFN-γ, TNF-α and IL-10 than infected females ($p<0.05$). A comparison of cytokine dynamics between the two strains revealed increased IL-4 and IL-6 levels in *P. falciparum* infected individuals one week following treatment ($p<0.05$). Moreover, IL-10 and IFN-γ levels were markedly decreased one week following treatment of *P. falciparum,* differing from the sustained elevation in these cytokines observed a week following treatment of *P. vivax* infection ($p<0.05$). TNF receptor and IL-10 levels were predictive of febrile infection and parasite density in both *P. falciparum* and *P. vivax* infection ($P<0.005$). Independent of parasite density, for both malaria species, hematocrit levels were directly correlated to IL-1 ($P<0.01$) while erythropoietin levels directly correlated with TNF-α ($P=0.003$). Interestingly, both hematocrit ($P=0.02$) and erythropoietin ($P=0.03$), were negatively correlated with the co-presence of IL-1 and TNF-α. There was a gender and species related difference in the hematocrit dynamics over time. In conclusion, these findings are the first to describe cytokine dynamics of malarial infection *in vivo* and demonstrate a strong association of inflammatory responses with parasite density, the severity of symptoms, and anemia. The observed differences in immune responses between genders and malaria species may explain distinctions in clinical presentations between males and females as well as core differences in the pathogenesis of *P. vivax* and *P. falciparum* infection.

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IMMUNE RESPONSE TO *PLASMODIUM VIVAX* INFECTION: A STUDY IN THE CENTRAL CHINA

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

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EVALUATION OF NATURALLY ACQUIRED HUMORAL IMMUNE RESPONSES AGAINST IMMUNOREACTIVE PROTEINS OF *PLASMODIUM VIVAX* BY PROTEIN ARRAYS

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In the previous report, we successfully applied an antibody-based protein array for immunoprofiling of *Plasmodium vivax* infection, and some highly immunoreactive proteins were identified. To further characterize the antibody reactivity of these immunogenic proteins, we used a Ni-NTA surface based protein array to detect the immune responses from sera of *vivax* malaria parasites. ETRAMP, Pv12, Pv41 and MSP3.9 were studied to compare with the well-characterized *vivax* vaccine candidate MSP1-19. Among the 52 microscopically positive samples, all samples were detected *P. vivax* by the PvMSP1-19 arrays (100% sensitivity), and were 36 (69.2%), 31 (59.6%), 23 (44.2%) and 22 (42.3%) by ETRAMP, Pv12, Pv41 and MSP3.9 arrays respectively. The false positives were obtained 2 (95.0% specificity), 2 (95.0%), 3 (92.5%), 4 (90.0%) and 4 (90.0%) among 40 sera samples from healthy subjects. The ratio (fluorescent intensity of positive samples/that of negative samples) of antibody response to the PvMSP1-19, ETRAMP, Pv12, Pv41 and MSP3.9 were 17.5, 6.1, 2.6, 3.5 and 4.4 respectively. Although the naturally acquired humoral immune responses against PvMSP1-19 show superior reactivity than others, these recombinant proteins were also highly recognized by *P. vivax* infected patient sera. These results validate the protein arrays for profiling antibody responses to *P. vivax* infection, and further confirmed that ETRAMP, Pv12, Pv41 and MSP3.9 could also be selected as potential target antigens for malaria vaccine.

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CHARACTERIZATION AND SEROLOGIC RESPONSES TO *PLASMODIUM VIVAX* DUFFY BINDING PROTEIN (DBP) VARIANTS IN RESIDENTS OF PURSAT PROVINCE, CAMBODIA

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The *Plasmodium vivax* Duffy Binding Protein (DBP) is the ligand in the major pathway for *P. vivax* invasion of human reticulocytes, making it an appealing vaccine candidate. Region II of DBP (DBPII) is the minimal portion of the ligand that mediates recognition of the Duffy Antigen Receptor for Chemokines (DARC) on the reticulocyte surface and constitutes the primary vaccine target. Analysis of natural variation in the coding sequences of DBPII revealed signature evidence for selective pressure driving variation in the residues of the putative receptor-binding site. We hypothesize that anti-DBP immunity in *P. vivax* infections is strain-specific and hindered by polymorphic residues altering sensitivity to immune antibody inhibition. To comprehend the human IgG response following *P. vivax* infections we investigated the specificity of serum IgG in residents living in Pursat Province, Cambodia. Using ELISAs, we quantified the antibody titer against five variant alleles of DBPII. We also sequenced the DBPII of the field isolates to determine their relationship to the variant alleles used in the ELISAs. When correlating the IgG titer between the DBP

variants a strain-specific immune response was observed in patients with a high antibody titer to DBPII_AH as compared to the other variants. This differed from the correlation of high antibody titers between DBPII_P and DBPII_7.18 (ρ=0.88, p-value<0.0001) and DBPII_P and DBPII_O (ρ=0.87, p-value<0.0001). There appeared to be little correlation between specific polymorphic residues and IgG titer. Understanding the immune response to the polymorphisms within DBPII will allow further identification of epitopes to enable the production of a more effective *P. vivax* vaccine.

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NOVEL HUMANIZED MOUSE MODELS FOR *PLASMODIUM VIVAX*

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Development of humanized animal models able to sustain infection with *Plasmodium vivax* is required to increase our understanding of the biology and pathogenesis of the parasite and to test vaccines and anti-malarial drugs. Such models are also expected to allow development of a convenient mosquito challenge model for vaccination trials. We have developed novel humanized mouse strains that were genetically modified to develop a human hematopoietic system upon infusion of stem cells from umbilical cord blood. The humanized mice are immunodeficient, as they are knocked out for Rag and IL2 genes, and at the same time express human HLA-DR4. These mice develop human erythrocytes and reticulocytes and our preliminary data indicated their ability to sustain *P. vivax* blood stage infection. The ability of humanized mice to develop human T cells (both CD4+ and CD8+ subsets), B cells, and significant serum levels of human IgM and IgG, enable them as a unique model to test the immunogenicity and protective efficacy of human vaccines.

1540

FAILURE TO INFECT: DELINEATING *PLASMODIUM VIVAX* DEVELOPMENT CESSATION AMONGST *ANOPHELES DARLINGI* IN THE PERUVIAN AMAZON

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Malaria continues to be a top infectious disease burden in tropical and subtropical areas of the world. *Plasmodium vivax* is geographically the most widely distributed cause of malaria, with up to 2.5 billion people at risk and an estimated 80 million to 300 million clinical cases every year. Recent disease control innovations have included attempts at transmission blocking vaccines (TBVs), which target the intra-mosquito part of the complex malaria life cycle. Naturally occurring transmission blocking has been observed such as in a study conducted in the Peruvian Amazon in which mosquitoes dissected seven days after parasitemic blood meals revealed infection rates of only 50%. To further understand this pattern of incomplete transmission, we are performing membrane-feeding assays in which *vivax*-infected blood samples from subjects enrolled in the Peruvian Amazon are fed to first generation lab-reared mosquitoes. Instead of dissecting at day 7 as typically done to assess for oocysts, we are dissecting mosquitoes at multiple time intervals to detect more specifically where transmission drops - is it the failure of microgametes to fertilize macrogametes, failure of zygotes to transform into ookinetes or failure of ookinetes to traverse the midgut epithelium and become oocysts. To account for all developmental stages, dissections begin at 15 minutes post feed, up to 24 hours, then again at days 4 and 14. Blood samples are then stained with Giemsa and examined under microscopy. Of the 6 subjects already enrolled, we have found that overall parasitemia was important to transmission, but that it did not explain the lack of transmission in some subjects. Over the next several weeks, we will enroll a total of 20 patients as the malaria season in Iquitos is now at its busiest. By identifying termination points in sporogonic development, we aim to provide further insight into the biologic mechanisms pertinent to transmission blocking vaccine strategies and hope the results will help focus our search for transmission blocking antibodies in simultaneously collected plasma.

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GENETIC VARIABILITY OF *PLASMODIUM VIVAX* IN THE NORTH COAST OF PERU AND THE ECUADORIAN AMAZON

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Plasmodium vivax is the most widespread malaria parasite causing significant morbidity worldwide. In the Peruvian North Coast (PNC), the number of *P. vivax* malaria cases has steadily increased over the last few years despite a significant decline in the number of cases in Peru. To understand the transmission dynamics of *P. vivax* populations between the PNC and the neighboring Ecuadorian Amazon (EA), we studied the genetic diversity and population structure of *P. vivax* isolated in those areas. One hundred and twenty blood or serum samples comprising 95 PNC (58 from Piura and 37 from Tumbes, collected from 2008 to 2010) and 25 EA (from Puyo, collected between 2001 and 2004) were assessed by 6 polymorphic neutral microsatellite markers. Genetic variability was determined by the haplotype frequency and expected heterozygosity (He). Population structure was assessed by Bayesian inference cluster analysis. We found very low genetic diversity in PNC, with a single allele per locus, one haplotype and He=0 in Piura; and 1-3 alleles per locus, 3 haplotypes and He=0.30-0.32 in Tumbes. In contrast, high genetic diversity was observed in EA, with 4-6 alleles per locus, 15 different haplotypes and He=0.43-0.70. Population structure analysis revealed three distinct populations correlating with each geographic location. Five out 37 (14%) isolates from Tumbes had an identical haplotype to that found in Piura, suggesting unidirectional gene flow from Piura to Tumbes (100 Km apart). In addition, one haplotype collected in 2008 in Tumbes showed high similarity to a haplotype found in 2003 in Puyo, which was likely an imported case from the Ecuadorian Amazon. No shared haplotypes between Piura and Puyo was observed (300 Km apart), indicating little gene flow between these two areas. Our study provides important information on the transmission patterns between the Coastal areas of Peru and the Ecuadorian Amazon. Future studies should include isolates from the South Coastal region of Ecuador in order to identify other routes of parasite dissemination between the North Coast of Peru and Ecuador.

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SIMILAR DIVERSITY OF THE MEROZOITE SURFACE PROTEIN 3 ALPHA (MSP-3α) SUBFAMILY IN THAI AND VENEZUELAN *PLASMODIUM VIVAX* POPULATIONS

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Characterizing the polymorphism and identifying possible signatures of selection continues to be an important task in the study of malarial parasite antigens. This is of particular importance with regard to *Plasmodium vivax* blood stage antigens, which have not been as extensively studied as those from the more virulent *P. falciparum*. Of particular significance is the merozoite surface protein 3 (MSP-3) gene family, involved in the invasion of the asexual merozoite parasite form into the red blood cell. The MSP-3 gene family has undergone an expansion in the *P. vivax* lineage with twelve identified genes, compared to the four

in *P. falciparum* and two in *P. knowlesi*. This duplication event has been posed as being biologically important and could have implications for pathogenicity and parasite interactions with the host immune system. Here we describe the genetic diversity and identify possible signatures of selection of three paralogous and syntenic genes of the subfamily MSP-3α (2,559-2,724 base pairs in length), from Venezuelan and Thai population samples. Two of these paralogous genes have only recently been recognized. Despite differences in transmission rates, similar patterns of selection and polymorphism were found for the Thailand and Venezuelan samples. Genetic diversity, calculated by the parameter ϖ, revealed that the majority of sequence diversity was constrained to the N terminal for all three paralogs. This pattern holds when the two populations are compared. In contrast, strong evidence of positive selection was observed for the N-terminal in the two newly recognized MSP-3α gene copies, but only in the Thai population. Finally, evidence of purifying selection at the C-terminal for all three genes suggests that it could be of functional importance. If such functional constrain is demonstrated, it may be possible that the MSP-3 family is functionally redundant, a possible benefit to the parasite that may be conferred by increased antigenic diversity allowing enhanced evasion of the host immune response.

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EPIDEMIC AND INTEREPIDEMIC PERIODS STRUCTURE *PLASMODIUM VIVAX* POPULATION CIRCULATING IN FRENCH GUIANA BETWEEN 2006 AND 2010

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Since 2005, *Plasmodium vivax* is the main species circulating in French Guiana with an incidence of 51% in 2010. Understanding the genetic structure of this parasite population is essential in order to predict the rapid spread of certain phenotypes of interest (eg resistant parasites) or for a better understanding of the epidemiology. In this study, 195 isolates, collected between 2006 and 2010, in five geographical regions of French Guiana, were analyzed using six highly polymorphic microsatellite markers amplified by a semi-nested polymerase chain reaction method. We have shown that 28.1% of infections were polyclonal. The parasite population circulating in this department is likely drawn from a single ancestral population. The current population was genetically diverse (He = 0.68), and not structurated in time or space, as shown considering unique haplotype sampling strategy. In contrast, using all data samples, the population presents higher structuration rates, likely related to epidemics, which induce locally, and temporary high levels of inbreeding. Demographic history was also investigated, using both exponential and linear expansion models. Exponential model would consider that expansion of efficient population size occur mainly during epidemics, via clonal expansions. In contrast, linear model relies on genetic diversity acquired during sexual stages of *Plasmodium*. Exponential model shows a stable efficient population size, suggesting that epidemics do not contribute to long-term *Plasmodium* expansion. In contrast, linear model shows an expansion of efficient population size since some decades: this is related to a global increase of malaria cases, even when epidemic events are not considered. We conclude that epidemics may promptly and locally drive genetic information, but do not contribute to a wider, longer-term and regionally-scaled demographic history of *P. vivax*. Therefore, a similar study encompassing a larger geographical area would be required for a better understanding of the structure population of *P. vivax* in the Guiana Shield or even, Amazonia, and to confirm that *P. vivax* diversity is not proportional to the transmission level as observed with *P. falciparum*.

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LIMITED GENETIC DIVERSITY AND CLONAL POPULATION STRUCTURE OF *PLASMODIUM VIVAX* PARASITES FROM A GEOGRAPHICALLY ISOLATED COMMUNITY IN THE PERUVIAN AMAZON

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Plasmodium vivax has the highest burden of malaria morbidity and is the most spread human plasmodium species around the world. However, due to its complex life cycle, little is known about the genetic characteristics of these parasites populations and the epidemiology of the disease. In the present study, we explore the genetic diversity and population structure and dynamics of *P.vivax* parasites from a community in the Peruvian Amazon within the framework of a two years cohort looking for some insights that may explain the epidemiology of this disease within this area. Thirty-eight patients from San Carlos community, a geographically isolated area, were enrolled and followed up for 2 years after received the radical cure treatment (chloroquine + primaquine). *P. vivax* infections were detected by microscopy and by specie specific PCR. Molecular genotyping of *P.vivax* parasites using 15 microsatellites was performed. The genetic diversity was determined by calculating the expected heterozygosity (He) and allelic richness. The genetic population structure was determined calculating the linkage disequilibrium (pairwise LD and I_A^S), the probability of admixture or clonal reproduction (Psex) and looking for clusters of genetically related haplotypes. A limited genetic diversity of *P. vivax* parasites (He 0.47) and a high prevalence of monoclonal infections (83%) were found. The strong linkage disequilibrium (pairwise LD $P<10^{-6}$ and I_A^S 0.51), the low probability for admixture (Psex<0.0007) and the finding of a few clusters of genetically related haplotypes, described a clonal population of malaria parasites. The limited genetic diversity and clonal structure of *P. vivax* population described in this area may represent a high risk for the rise and spread of drug resistance. Nevertheless, also may stimulate positively the development of clinical immunity by the people in the Amazon Basin being reflected in the high proportion of asymptomatic cases reported in this community.

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CPG-DNA ENCAPSULATED WITH PEPTIDE ANTIGENS OF *PLASMODIUM VIVAX* IN MICROPARTICLES ENHANCES THE SYSTEMIC AND MUCOSAL IMMUNE RESPONSES IN MICE USING INTRANASAL MODE OF DELIVERY: AN APPROACH TOWARDS MUCOSAL VACCINE FOR MALARIA

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Due to drug resistance and limitation of growing of *Plasmodium vivax* parasite *in vitro* for enough DNA/Protein, we attempted an alternate synthetic peptide approach from the deduced amino acid sequences of different antigens of *P. vivax* constituting all the stages of the life cycle. For producing efficient and long lasting humoral immune responses, PLGA microparticles were used as delivery vehicles and CpG ODN as immunoadjuvants.The adjuvants used were synthetic oligonucleotides containing CpG (CpG-ODN 1826 and 2006, class B) motifs. *P. vivax* peptides viz, MSP 1#1, MSP 1#23, CSP, AMA, and Pvs24 (TBA) possessing B and T cell epitopes were synthesized using Fmoc chemistry, purified to homogeneity by Gel permeation chromatography and HPLC. Peptide purity was confirmed by amino acid acid analysis. These peptide

antigens were then entrapped in microparticles along with CpG-ODN. Biodegradable microparticles were prepared from 50:50 PLGA by water-in-oil-in water (w/o/w) solvent evaporation method. Particle size and size distribution was determined using particle size analyzer.The morphology was studied by scanning electron microscopy. Outbred strains of mice were immunized using intranasal route with different peptide formulations. Peptide specific IgG,IgA and SIgA estimation was done by standardized ELISA protocol. Peptides were found to be > 95% pure. Percentage peptide entrapment was in the range of 60-70%. Percentage entrapment for CpG-ODN was in the range of 50-60%. Microparticles were in the size range of 2-5µm and spherical with smooth surface. Presence of CpG in microparticles along with the peptide antigens showed serum IgG titre of 51,200-204,800 maintained till 90 days post immunization.The isotypic profile of the serum IgG revealed IgG2a/2b as the predominant isotypes,maintained till 90 days post immunization. Peptide specific IgA titre in sera ranged between 12,800-25,600 maintained till 90 days post immunization and SIgA titre in washes ranged between 800-25,600.Infected mosquitoes fed with high titer Pvs24 (TBA) anti-sera showed signinificant reduction in the oocyst count as revealed by membrane feeding assay.This study shows CpG ODN to be a potent mucosal adjuvant to induce immune responses against peptide antigens administered by intranasal inhalation.This is the first reported study with mucosal vaccination for malaria.

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FINE-SPECIFICITY OF HUMORAL IMMUNE RESPONSES GENERATED IN NAIVE ADULTS FOLLOWING VACCINATION WITH VMP001, A PREERYTHROCYTIC VACCINE CANDIDATE BASED ON THE CIRCUMSPOROZOITE PROTEIN OF *PLASMODIUM VIVAX*, FORMULATED WITH GSK BIOLOGICALS ADJUVANT SYSTEM AS01$_B$

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We have previously reported on the development of VMP001, a chimeric circumsporozoite protein based vaccine for *P. vivax*. In previous studies the VMP001 vaccine candidate demonstrated strong immunogenicity, and also demonstrated high efficacy in *Aotus* monkeys challenged with infectious *P. vivax* sporozoites. Based on these data we proceeded to test VMP001 for its safety, immunogenicity and efficacy in humans. This first-in-human study was designed as a dose-escalating study using three doses of VMP001 formulated in GSK Biologicals Adjuvant System AS01$_B$.The primary study endpoint was to assess vaccine safety. One of the secondary endpoints of this study was an evaluation of the fine-specificity of humoral immune responses generated following vaccination. We evaluated the antibody responses from volunteers to the N- and C-terminal regions as well as the central repeat region of the synthetic VMP001 vaccine construct. The vaccine was immunogenic and all three doses of vaccine induced antibody responses that spanned the entire length of the vaccine construct. While there were detectable responses to all domains of the molecule, there was a distinct hierarchy in terms of the regions recognized by the vaccinees. The C-terminal region induced the highest magnitude of responses in all three groups, followed by the N-terminal region with the Repeat domain the lowest. While the magnitude of the titers differed, in general, titers against any single domain correlated to titers against the others. Individuals having the highest antibody titer to one component of the vaccine also had high titers to the other domains. Although the vaccine did not induce sterile protection, a small but consistent delay in prepatent period was observed in some subjects. Detailed analysis of the immune response may help understand these findings and help improve the design of future vaccine constructs. We will discuss vaccine induced antibody avidity and fine-specificity in detail.

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PLASMODIUM VIVAX CIRCUMSPOROZOITE PROTEIN-SPECIFIC CELLULAR IMMUNE RESPONSES AFTER IMMUNIZATION WITH THE VMP001/AS01$_B$ CANDIDATE MALARIA VACCINE IN MALARIA-NAÏVE INDIVIDUALS

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Plasmodium vivax is the major cause of malaria outside of sub-Saharan Africa and inflicts debilitating morbidity and consequent economic impact in developing countries. We have developed a novel chimeric recombinant protein VMP001 based on the CSP of *P. vivax*. The first-in-humans safety, immunogenicity and efficacy clinical trial of VMP001 formulated in GSK Biologicals' adjuvant system AS01$_B$ was recently performed in malaria-naïve adults. An effective *P. vivax* vaccine will likely require induction of both humoral and T cell responses. The aim of this study was to measure vaccine-induced T cell responses in PBMCs collected at various time points following immunization. Multiparameter flow cytometry was used to enumerate the frequency and phenotype of T cells producing IL-2, TNF, and IFN-γ after *in vitro* stimulation of cryopreserved PBMCs with VMP001, or pools of overlapping peptides corresponding to different regions of the VMP001 construct. IL-2$^+$CD4$^+$ T cell responses were detected in all volunteers at one or more time point. TNF$^+$CD4$^+$ T cell responses were detected in 93% of volunteers, albeit at a lower frequency than IL-2$^+$CD4$^+$ T cells. In addition, IFN-γ$^+$CD4$^+$ T cell responses were detected in 55% of volunteers. TNF$^+$IL-2$^+$ double-producers and IL-2$^+$ single-producers were most commonly detected, along with a smaller population of cells producing all three cytokines tested. The vaccine-induced CD4$^+$ T cell responses were strongest and most frequently detected against the N-term region (90% of volunteers) but smaller responses against C-term and repeat regions were also observed (24% and 21% of volunteers respectively). Our results indicate that the VMP001/AS01$_B$ vaccine was immunogenic, as indicated by the detection of antigen-specific CD4$^+$ T cell responses in all immunized volunteers. Although the vaccine did not induce sterile protection, a small but consistent delay in prepatent period was observed in some subjects. Detailed analysis of the induced T cell responses may help understand these findings and help improve the design of future vaccine constructs.

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A ROBUST *PLASMODIUM VIVAX EX VIVO* INVASION ASSAY

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We describe a protocol for an *ex vivo Plasmodium vivax* invasion assay that can be easily deployed in laboratories located in endemic countries. The assay involves mixing enriched cord blood reticulocytes with matured, trypsin-treated *P. vivax* schizonts concentrated from clinical isolates. The invasion efficiencies observed for parasites from 85 isolates were highly variable, ranging from 0.1% to 22.3% with a mean of 3.7% (95%CI: 2.8%-4.6%). The utility of the protocol for vaccine testing

was demonstrated by using it as an invasion-inhibition assay to assess functionally antibodies against DARC, pvMSP1 and pvDBP. This provides the first biological demonstration that polymorphisms in the pvDBP gene affect the invasion inhibition efficacy of anti-pvDBP antibody.

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IS THAT A RODENT IN YOUR LUGGAGE? BUSHMEAT CONFISCATIONS REPORTED IN THE CENTERS FOR DISEASE CONTROL AND PREVENTION'S QUARANTINE ACTIVITY REPORTING SYSTEM - UNITED STATES, SEPTEMBER 2005-DECEMBER 2010

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Bushmeat, defined as raw or processed meat derived from wild animals, is considered a potential source of infection. The HIV epidemic has been associated with the hunting and processing of bushmeat, and recent studies have found evidence of simian foamy viruses in bushmeat samples confiscated at United States ports of entry. Existing US regulations prohibit importation of bushmeat from specific animals. However, illegal importation still occurs, and the exact amount of imported bushmeat is unknown. This project describes bushmeat confiscation reports in the Centers for Disease Control and Prevention (CDC) Quarantine Activity Reporting System (QARS) and attempts to identify geographic and seasonal trends. A keyword search was performed in QARS to capture all bushmeat-related reports from September 2005 through December 2010. All relevant reports were reviewed and compiled in an analytic database. All items were categorized by CDC-regulated species, including nonhuman primate, rodent, bat, bird, unknown, and other. In total, 543 confiscated bushmeat items, weighing 2303.6 kilograms (kg), were reported and recorded in QARS. The median weight of bushmeat per report was 2 kg and ranged from 0.1 to 650 kg. Half of confiscated bushmeat was identified as rodent. The most confiscations were reported in 2008 and the least in 2006. Africa was the most frequent continent of origin, with 68% of all confiscated bushmeat originating from Ghana and Nigeria. Seasonality was evident, with bushmeat confiscations peaking in late spring to early summer after adjustment for travel volume. Four times more bushmeat was confiscated during an enhanced surveillance program in June 2010 compared to the same period in previous years, suggesting that items were missed during routine inspections. Even with regulations in place, bushmeat is smuggled into the United States. Longstanding cultural practices make it difficult for persons to accept potential health risks. Therefore, enforcing penalties associated with bushmeat confiscations, along with health education aimed at high-risk groups, may be useful to deter import attempts.

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TEACHING GLOBAL HEALTH IN THE UNDERGRADUATE LIBERAL ARTS

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Teaching public health in the undergraduate curriculum of four-year institutions has moved forward over the last five years with the Educated Citizen and Public Health initiative. It has been driven by leaders in public health, the arts, sciences and humanities, and public health organizations, as well as by student interest. There has been equal interest in global health with a focus on health equity for all persons and particularly for those in low-income countries. Most undergraduate offerings in global or public health have been aligned with universities with graduate programs in the disciplines. However, is likely that strong interest in global health exists at all institutions including liberal arts colleges. Following the experience of teaching a short course in global health in a liberal arts college, student organizations, courses, and officially recognized curricular offerings in global and public health were identified for the 2009-2010 academic year for fifty of the top ranked liberal arts colleges in the United States. 42% of the colleges had a track, concentration or program; schools that did not have official themes still listed at least one course in global or public health. All of the themes were interdisciplinary and when they were more expansive such as a program, they were organized as a multi-college consortium. 48% of them had been developed since the 2005 academic year. The most number of courses were in the Social Sciences (n=9.9±12.2) followed by the Natural Sciences (3.2±5.3). Student organizations in global or public health were present on 30% of campuses and all but two schools had service, social justice or AIDS organizations. The values of a liberal education are closely aligned with those of global health: social responsibility, critical thinking, skills in communication, analysis and problem-solving, ethical reasoning, and knowledge of the wider world through integrated study in the arts and sciences. Liberal arts colleges can take several steps to capture this interest in global health and enhance their curriculum.

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GETTING THE NUMBERS RIGHT: CONSIDERATIONS FOR QUANTIFICATION OF MALARIA MEDICINES AND RAPID DIAGNOSTIC TESTS IN RESOURCE LIMITED SETTINGS

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Countries continue to scale up interventions for the treatment, diagnosis, and prevention of malaria. Central to the success of these interventions is ensuring that a consistent supply of products is available whenever and wherever they are needed. Quantification is the process of estimating the quantities and costs of the products required for a specific health program and determining when the products should be delivered to ensure an uninterrupted supply for the program. Quantification is a critical supply chain activity that links information on services and commodities from the facility level with program policies and plans at the national level, and is then used to inform higher level decision making on the financing and procurement of commodities. The results of a quantification can be used to help maximize the use of available resources for procurement, advocate for mobilization of additional resources when needed, and inform manufacturer production cycles and supplier shipment schedules. Malaria presents unique challenges to quantification due to: 1) changing epidemiology due to large scale implementation of effective malaria control interventions in diagnosis (particularly RDTs), net distribution campaigns, and increased use of ACTs; 2) seasonality and geographic considerations of disease; historical presumptive treatment of malaria with ACTs, rather than confirmed biological diagnosis; inaccurate records and weak reporting systems for malaria medicine consumption. The following recommendations are offered to help improve the accuracy of quantifications of malaria medicines: 1) in the short term, the effect of increased diagnosis and prevention efforts will likely not affect the quantities of ACTs required; 2) when developing a supply plan, program managers should arrange shipments to arrive prior to the peak periods of malaria, to avoid overstocking during the dry season, and understocking during the rainy season; 3) investments should be made in strengthening information systems so that future quantifications can be data driven rather than assumption driven; 4) forecasts should be made based on as many data sources (demographic/morbidity data, services data, and consumption data) as possible. The results of forecasts using different data sources should be analyzed and compared; and 5) quantifications should be updated on a quarterly basis with actual consumption data.

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URBAN POOR HARD HIT BY CHRONIC CONDITIONS

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Hypertension and diabetes are no longer diseases of affluence. High poverty levels in sub-Sahara Africa have heightened their prevalence.Poor people are suffering from these conditions because of rapid urbanization, cultural factors, poor health management and general effects of poverty. The study aimed at understanding diabetes and hypertension among urban poor, a total of 5,190 individuals were sampled. Data on their risk was indicated by tobacco use, alcohol consumption, diet and physical activity, 12 per cent of adult were current smokers and on average smoked eight cigarettes a day. 10 per cent were alcohol users and nearly a third 32 per cent were heavy drinkers. On physical activity, 15 per cent did not engage in any, while over half (54 per cent) had insufficient intake of fruits and vegetables, while 37 per cent had high salt intake. The prevalence rates among adult women were slightly higher than men. Increasing with age, respondents aged 40 and 60 likely having diabetes and 30 and 60 years having hypertension.Heavy alcohol use leads to damage of pancreases - organ producing insulin regulating how sugars are metabolized.A damaged pancreas results in less insulin therefore increasing levels circulating sugars (glucose specifically). "Diabetes is therefore a manifestation of imbalance in sugar metabolism.According to findings, some types of alcohol are a source of "empty calories," body gets a lot of calories without a feeling of satiety.In an ideal situation, one should get calories reflecting feeling of sanctification. More empty calories consumed, higher chances gaining too much weight, Interfering with sugar metabolism and increasing chances of diabetes, Leading damage inner lining of blood vessels which lead to high blood pressure. High consumption salt leads to high concentrations salt in blood, which body compensates for by absorbing more water from cells. Resulting an increased blood volume, putting pressure on kidneys and blood vessels, causing damage manifest as high blood pressure.

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PILOTING AND EVALUATING A GLOBAL CHILD HEALTH CURRICULUM FOR PEDIATRIC RESIDENCY PROGRAMS

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North American pediatricians care for a growing number of immigrant and refugee children, and training programs should reflect this changing demographic. A modular global child health curriculum designed for use during academic half-days was developed and piloted across four Canadian post-graduate training centers. Here we present the results of an evaluation of participant satisfaction and knowledge gain using a standardized satisfaction survey and pre/post multiple choice knowledge tests. 125 trainees participated from 4 pediatric training centres. 95% completed 2 or more modules and 42% completed all four. Scores on a standardized satisfaction questionnaire were internally consistent (Cronbach's alpha=0.88 to 0.95 for the four modules) and indicated a high level of participant satisfaction (mean (SD) satisfaction scores of 3.4 (0.6) to 3.6 (0.6) out of maximum total of 5). Analysis of determinants of participant satisfaction indicated that past participation in clinical electives abroad was associated with higher satisfaction scores. Scores on multiple choice knowledge tests increased following the teaching sessions ($p < 0.0001$). This finding remained after stratification by site, year of post-graduate training, and previous global health experience ($p<0.01$ for all comparisons), suggesting that participants in all subgroups demonstrated knowledge gain following participation in all four modules. Participants with lower pre-test scores demonstrated greater knowledge gain than those with higher pre-test scores ($p<0.0001$ for all modules), suggesting that participants with low baseline GCH knowledge benefited most from the modular curriculum. On the other hand, knowledge gain did not differ according to level of residency training or previous global health experience. Item analysis of the knowledge evaluation instrument demonstrated that 85% of questions were at an appropriate difficulty level, 35% discriminated well between top and bottom performers, 75% increased significantly after teaching sessions and 78% had a good item-to-total score correlation. In summary, satisfaction with the modular curriculum was high among participants, and knowledge gain could be demonstrated unequivocally. This standardized curriculum could be scaled up for implementation across North American pediatric training centers.

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CLINICAL TRIALS IN RESOURCE-LIMITED SETTINGS

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Clinical trials in developing countries lag far behind wealthier regions through a lack of knowledge and skills. More disease management trials are needed but there is a lack of access to generic clinical trial tools, guidance and training. Furthermore, researchers are daunted by regulations and guidelines. Existing capacity development activities are linked to specific trials and are disease focused; so limit diversification. Other factors such as the cost of travel and remote locations of potential research sites also restrict clinical trial capacity development in these regions. Global Health Clinical Trials (www.globalhealthtrials.org) is a new collaborative web-based platform. It is a free, open access and entirely collaborative platform where anyone working on trials can access guidance, tools resources and share their knowledge, views and experiences. This resource is evidence-led through integrated participatory action research that enables researchers based in these settings to identify the problems in their own context and contribute to solutions themselves. The platform was released as a pilot in May 2010 and within a year attracted over 1000 members from 56 developing countries. It is not for any one disease or just about product development trials. The aim is to support researchers in running their own trials and diversifying in the types of trial that they conduct. Researchers and their staff use the platform to seek expert and peer advice on diverse issues such as data management, intend-to-treat-analysis and setting up community advisory boards. There are free e-learning short courses to take researchers and research staff pragmatically through all the trial steps and processes. In partnership with WHO/TDR we have created an on-line continuing professional development scheme. This is the first of its kind and this free opportunity to build personal learning and training portfolios whilst guiding professional development will be highly impactful in creating a cadre of developing country clinical trialists. The Global Health Clinical Trials Programme is an open collaboration and is already gaining widespread recognition for being the single point of reference for accessing information, experts and support on all aspects of running clinical trials in resource-limited settings.

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USE OF BIOMETRIC DATA IN LINKING HEALTH DEMOGRAPHIC SURVEILLANCE SYSTEMS (HDSS) AND HEALTH MANAGEMENT INFORMATION SYSTEM (HMIS) INFORMATION IN IFAKARA AND RUFIJI HDSSS IN TANZANIA

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The objective of the study was provide a mechanism to link demographic and social economic information captured within HDSS frameworks and the health facility-based data. House to house fingerprints and facial images data collection was done for all HDSS members. Field workers each equipped with a net book computer, fingerprint scanner, web camera, methylated spirit (for cleaning fingers before fingerprint capture) visited households for 12 months from June 2010 up to May 2011. Field workers did a daily data backup and field supervisors did a weekly data integration. Once integrated the biometric data were taken to the surrounding health facilities within the Demographic Surveillance Area (DSA) where upon each visit a patient is checked using a combined search and data validation using fingerprints, facial image and the demographic data. Once identified the patient's visitation records including health services attendance, diagnosis, service and treatment data were captured and linked to his/her HDSS profile. In the two pilot health facilities in Rufiji HDSS from September 2010 to May 2011,726 patients have had their HDSS and HMIS information accurately linked using the deployed mechanism. At the moment essential data health system analysis, health planning and policy formulation is lacking because there is no such a system that links HDSS and HMIS information. Upon implementation of the exercise, they were concerns from the public on misuse of the biometric data collected and use of fingerprint scanner as a means for HIV/AIDS testing and for criminal tracking. Integrating the HDSS and HMIS data sources would provide both the numerator and the denominator population for computation of both disease incidence rates in the population and health service coverage rates in the health system.

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A REVIEW OF THE GEOGRAPHICAL VARIATION IN *PLASMODIUM VIVAX* RELAPSE RATE

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Plasmodium vivax has the widest global geographic distribution of the malaria parasites known to affect man. Contrary to past beliefs that it is a "benign" form of malaria, it has been shown to result in severe disease and mortality. Control of *P. vivax* is complicated by its ability to relapse after treatment of the initial infection. Hypnozoites, a dormant liver stage of infection, can initiate a relapse weeks or months following initial infection. The widely accepted epidemiological understanding is that strains of *P. vivax* from various geographical areas exhibit different relapse patterns, such that those found in tropical regions will relapse quickly (3-6 weeks) and those in temperate regions will relapse more slowly (6-12 months). Support for this belief is provided here, in a systematic review of published and unpublished reports of *P. vivax* relapse rates in patients not treated with primaquine, the only drug currently available to treat the hypnozoite stage of the infection. Statistical analysis was performed to identify the association between the rate of relapse and several climatic and geographical parameters. The relationship between relapse rate and environment was illustrated as a global map of 146 *P. vivax* relapse records plotted as georeferenced points over a mask of the longest unsuitability period (the length of time in months an area is unsuitable for malaria transmission). The regression model demonstrated an association between relapse rate and land surface temperature, precipitation, temperature suitability index (a measure of temperature conditions which allow for sporozoite development), and longest unsuitability period. The map of the median time to relapse, grouped into three classifications of relapse rate (≤60 days, 61-180, and >180 days), showed the occurrence of faster relapse (≤60 days) in regions nearly always suitable for malaria transmission, and slow relapse (>180 days) in regions only suitable for transmission four to six months of the year. While these results are based on a relatively small sample of data, given the limited information published on *vivax* relapse, the data, from more than 36,000 patients, offers insight into the poorly understood mechanism of relapse. Elimination efforts often leave *P. vivax* the last parasite standing, due to its ability to relapse. A better understanding of relapse therefore is an essential component to its control.

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PREVALENT PARASITEMIA, FEVER, ANEMIA AND INHERITED BLOOD DISORDERS IN MESO-ENDEMIC WEST SUMBA, INDONESIA: A RANDOMIZED, CROSS-SECTIONAL ANALYSIS

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The expected decline in the intensity of malaria in many areas across the globe in response to heightened malaria control efforts has placed increased emphasis on understanding low to moderate transmission settings such as that typified by Southeast Asia. We conducted a cross-sectional socio-demographic and parasitologic survey in a rarely studied region of West Sumba district, East Nusa Tenggara Province, Indonesia from August-November 2010. Blood smear analysis of 960 randomly selected individuals revealed meso-endemic malaria with a point prevalence estimate of 24.7%. *Plasmodium falciparum* infections predominated over *P. vivax*, with a few cases of *P. malariae*, and a single case of *P. ovale* noted. Age specific prevalence rates were highest in children and young adults less than 15 years of age, and geometric mean parasite densities were found to decrease with increasing age. The prevalence of fever (aural temperature ≥ 37.5 °C) among parasitized and non-parasitized study participants was 7.2% (17/237) and 5.3% (38/723) respectively, suggesting approximately 30% of fevers were likely to be associated with malaria infection. The inherited red blood cell (RBC) polymorphisms glucose-6-phosphate dehydrogenase (G6PD) deficiency and Southeast Asian ovalocytosis (SAO) were detected in 17.5% and 22.6% of study subjects respectively, but were not associated with the likelihood of parasitemia ($P > 0.2$) as detected by conventional light microscopy. The prevalence of anemia on the day of survey was 43.4% (403/928), and parasitemic study participants were more likely to be anemic than those without parasitemia (odds ratio [OR] = 1.46; 95% confidence interval [CI] = 1.08-1.96; $P = 0.014$). These findings are consistent with the few prior studies conducted in the region and indicate moderate malaria transmission in an endemic area of East Nusa Tengarra Province, that has been stable enough to induce some anti-parasite and anti-disease immunity. Although we did not observe an association between either SAO and G6PD deficiency and the likelihood of patent parasitemia, the impact of these polymorphisms on other aspects of infection such as sub-patent parasitemia, parasite density and severe clinical disease remains to be determined. The relatively small proportion of parasitemic study subjects with fever for the given transmission intensity in this study population merits further attention and will be a focal point of future studies.

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MORBIDITY AND MORTALITY CAUSED BY *FALCIPARUM* AND *VIVAX* MALARIA IN HYPO- TO MESO-ENDEMIC WEST SUMBA, INDONESIA: A TWO-YEAR RETROSPECTIVE HOSPITAL-BASED STUDY

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Plasmodium vivax causes what has often been referred to as benign tertian malaria. This study aimed to measure the relative contributions of *falciparum* and *vivax* malaria to the burdens of hospitalized, severe and fatal malaria the main referral hospital for West Sumba in eastern Indonesia. We systematically examined records of malaria screening and admission at Karitas Hospital (115 beds), which served a community of 280,000 people living with a median prevalence of about 6% of both *P. falciparum* and *P. vivax* (ranging from 1:1 to 2:1 in surveys). Conducted in 2010, we limited the survey to calendar years 2008 and 2009. Febrile patients seeking treatment at this hospital had routine and reliable microscopic blood film exams for malaria. Among 18,589 febrile patients screened, only 2711 were positive and managed as outpatients. Another 3484 found positive for malaria were admitted as inpatients and these records were screened for completeness, with 35 being excluded, leaving 3449 malaria patient records to evaluate. Relevant content of hospital records were manually transferred into 2 separate case record forms (CRF) per patient, which were then each double entered into an electronic database. Only fully reconciled CRFs were uploaded for analysis. Among the 3449 admissions for malaria, there were 614 patients classified as severely ill, and 66 patients did not survive. *Falciparum* malaria accounted for 65% and 70% of this morbidity and mortality, and *vivax* malaria contributed 32% and 27% to these burdens in this community. Whereas infants, children, and adolescents carried 82% of the morbidity and mortality burdens due to *P. falciparum* (OR=4.6; 3.2-5.8), they carried only 62% of this in *P. vivax* (OR=1.6; 1.2-2.3). Patients with *P. falciparum* were much more likely to be classified as having severe illness (OR=2.7; 2.2-3.3), but the risk of death with a classification of severe illness was the same between *falciparum* and *vivax* malarias (OR=1.2; 0.7-2.2). Anemia and cerebral syndromes dominated among the severely ill with both *falciparum* and *vivax* malaria (>90% of syndromes for each species). These findings show *P. vivax* contributed substantially to the burdens of morbidity and mortality in West Sumba and seemed to have provoked severe disease syndromes essentially identical to *falciparum* malaria in both character and risk of death.

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THE CHANGING EPIDEMIOLOGY OF MALARIA IN PAPUA NEW GUINEA

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Papua New Guinea (PNG) has the highest malaria transmission outside of sub-Saharan Africa. Moreover, the country's malaria epidemiology is more complex than many other places with four endemic malaria species and a variety of anopheline vectors filling the diverse ecological niches. Only recently, the National Department of Health has re-activated its malaria control program with the up-scaling of insecticide treated nets supported by a Global Fund to Fight AIDS, Tuberculosis and Malaria grant. A country-wide prevalence survey in 70 randomly selected villages investigated the malaria prevalence and species composition. Follow-up surveys in selected sites assessed changes after the introduction of mosquito nets. Malaria prevalence was assessed by light microscopy and PCR. A total of over 9000 blood samples were analysed by microscopy. Population parasite prevalence rates ranged from 0 to 57%, with *Plasmodium falciparum* prevalence between 0 and 29%, *P. vivax* between 0 and 27% and *P. falciparum* mixed infections between 0 and 9%. *P. vivax* infections dominated in 22% of the villages with detectable parasitaemia. Parasite prevalence decreased significantly with altitude, more so for *P. falciparum* than for *P. vivax*. 80% or higher mosquito net usage was independently correlated with lower parasite prevalence. Microscopy and PCR results are compared with historic data and discussed in the context of the large-scale roll out of mosquito nets, change in treatment policy, and observed changes in malaria transmission.

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THE FORCE OF INFECTION: THE KEY TO UNDERSTANDING THE EPIDEMIOLOGY OF *PLASMODIUM FALCIPARUM* MALARIA IN PAPUA NEW GUINEAN CHILDREN

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Genotyping *Plasmodium falciparum* parasites in longitudinal studies provides a robust approach for estimating force of infection (FOI) in the presence of superinfections. $_{mol}$FOI, defined as the number of new *P. falciparum* clones acquired over time, is molecular parameter equally suitable for describing basic malaria epidemiology as well as for measuring outcomes of clinical trials of antimalarial interventions. We investigated the potential of molecular parameters to explain differences in risk of *P. falciparum* infections and disease between wet and dry season, among different age groups and use versus non-use of insecticide treated bednets (ITN). 264 children 1 - 3 years of age from Papua New Guinea were followed over 16 months with active detection of infection at 2-monthly intervals and during episodes of febrile illness. PCR for the highly polymorphic genotyping marker merozoite surface antigen 2 was performed in all blood samples. To track individual parasite clones in consecutive blood samples with maximal resolution PCR fragments were sized by capillary electrophoresis. $_{mol}$FOI was identified as explanatory variable for precisely describing the risk of *P. falciparum* illness. $_{mol}$FOI was significantly correlated to incidence of episodes, irrespective of whether a parasite density cut off was applied or not. Seasonal variation was observed in $_{mol}$FOI, and thus in the risk of illness. $_{mol}$FOI was significantly higher during the rainy season than in the dry season. Our analyses suggest a central role of $_{mol}$FOI for explaining differences in the burden of clinical *P. falciparum* malaria in our cohort. $_{mol}$FOI almost completely explained spatial variation, age trends and effect of ITN use on incidence. Acquisition of new parasite clones seems to be a major factor for clinical illness in these children. This study highlights the suitability of a new parameter, $_{mol}$FOI, for understanding the epidemiology of clinical malaria in young children. We propose to apply the molecular determined parameter $_{mol}$FOI for monitoring effects of malaria interventions.

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PLASMODIUM VIVAX GAMETOCYTE DYNAMICS AND THE ROLE OF DRUGS IN REDUCING TRANSMISSION POTENTIAL

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Designing interventions that will reduce transmission of *vivax* malaria requires a detailed understanding of the dynamics of *Plasmodium vivax* gametocytemia. We analyzed data from a large randomized controlled trial in Northwestern Thailand and two trials in Papua, Indonesia to identify and compare risk factors for *P. vivax* gametocytemia at enrolment and during the 6 to 9 weeks following treatment. Overall 492 patients with *P. vivax* monoinfections were evaluable from Thailand and 476 patients with *P. vivax* infections (162 of whom had concurrent *P. falciparum* infections) were evaluable from Papua. In Thailand 84.3% (415/492) of patients with monoinfections were gametocytemic at enrolment (median gametocyte density = 266/µL) compared to 66.6% (209/314) in Papua (median gametocyte density = 113/µL; p<0.001 for gametocyte prevalence and density comparisons). At both sites there was a positive correlation between initial asexual parasitemia and gametocyte density (R=0.53 and R=0.47, p<0.001 for both). High asexual parasitemia was also associated with an increased risk of gametocytemia during follow-up. In Thailand, the cumulative incidence of gametocytemia between day 7 and 42 following dihydroartemisinin+piperaquine (DHA+PIP) was 6.92% versus 29.1% following chloroquine (p<0.001). In Papua, the cumulative incidence of gametocytemia between day 7 and 42 was 33.6% following artesunate+amodiaquine (AS+AQ), 7.42% following artemether+lumefantrine and 6.80% following dihydroartemisinin+piperaquine (p<0.001 for DHA+PIP versus AS+AQ and p=0.4 for DHA+PIP versus AM+LUM). Gametocytemia during follow up was associated with concurrent asexual parasitemia in 98.9% (172/174) of cases. *Plasmodium vivax* gametocyte carriage closely mirrors asexual stage infection. The most important strategy for interrupting *P. vivax* transmission is prevention of relapses, particularly in those with high asexual parasitaemia.

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WIDESPREAD INFECTION OF WILD-LIVING CHIMPANZEES AND GORILLAS WITH *PLASMODIUM VIVAX*-LIKE PARASITES

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Plasmodium vivax accounts for over 50% of malaria cases outside of Africa, but is not thought to be transmitted in western and central Africa because of the high prevalence of the Duffy-negative trait in local populations. Nonetheless, the finding that some individuals in west central Africa harbor antibodies to *P. vivax* surface proteins, together with reports of *P. vivax* in some travelers returning from this geographic region, have suggested that there might be an as yet undefined reservoir of *P. vivax*. Since we have recently found evidence of multiple species of *P. falciparum*-related parasites in apes, we used non-invasive methods to determine whether wild-living chimpanzee and gorilla populations are naturally infected with *P. vivax*. Using *P. vivax* specific primers to amplify a diagnostic (~300 bp) mitochondrial DNA (mtDNA) fragment, we screened 3,044 chimpanzee, 1,236 gorilla and 513 bonobo fecal samples from 80 different field sites. Although ape *P. vivax* was detected at an overall low frequency (~1-2%; possibly because of low fecal parasite loads), we found 45 chimpanzee and 30 gorilla samples from 30 field sites to harbor *P. vivax* sequences. Ape *P. vivax* was widely distributed among central (*P. t. troglodytes*) and eastern (*P. t. schweinfurthii)* chimpanzees, as well as western (*Gorilla gorilla gorilla*) and eastern (*G. beringei graueri*) lowland gorillas, but was absent from bonobos (*Pan paniscus*). Many of the *P. vivax* positive specimens also contained *Laverania* parasites, indicating that ape *P. vivax* occurs frequently in the context of mixed parasite infections. To confirm that the diagnostic PCR was specific for ape *P. vivax*, we used single genome amplification to generate larger mtDNA fragments (~3-4.5kb) from a subset of samples. Phylogenetic analyses of these sequences revealed that the ape parasites were nearly identical to each other as well as to human *P. vivax*. These findings document widespread infection of wild-living chimpanzees and gorillas with *P. vivax*-like parasites throughout central Africa.

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THE URINARY SCHISTOSOMIASIS-BACTERIURIA CONNECTION: A NEW MODEL TO EXPLORE POTENTIAL MECHANISMS

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Many studies have posited urinary schistosomiasis as a risk factor for bacterial urinary tract infections (UTI). Bacteriuria may worsen urinary schistosomiasis-linked morbidities such as dysuria and hematuria. One possible reason for reported high rates of *Schistosoma haematobium*-UTI co-infection is *S. haematobium* egg-induced shedding of bacteria-coated urothelial cells into urine, which could facilitate detection of UTI. Another possible mechanism is egg granuloma-related urinary tract obstruction, which could promote urinary stasis and bacterial growth. A third possible mechanism is a skewed immune response to *S. haematobium* eggs in the bladder which could preclude an effective local response to bacteria. Previously we established a mouse model of *S. haematobium* egg-induced, non-obstructive immunopathology. Injection of eggs into the anterior bladder wall avoids obstruction of the ureters and bladder outlet. We used this model to test the urothelial shedding and immune skewing hypotheses for the urinary schistosomiasis/UTI association. At 1 and 2 weeks post-injection, 30% and 36% of egg-injected mice featured urothelial shedding vs none of the vehicle-injected mice. When mice were transurethrally administered uropathogenic *E. coli* 7 days after egg injection, bacteriuria occurred at high rates (75%) and titers (median $8x10^5$ cfu/ml) vs vehicle-injected mice and mice infected with *E. coli* only (both groups 33% and median 0 cfu/ml). The bladders of egg- vs vehicle-injected mice featured more neutrophils (17.6%±4.25 vs 5.07%±1.09; p=0.0076) and eosinophils (1.62%±0.26 vs 0.799%±0.799; p=0.0151), suggesting a role for schistosome-induced immune skewing in susceptibility to bacteriuria. We are working to further dissect the mechanisms by which urothelial shedding and/or immune skewing may promote synchronous urinary schistosomiasis and bacteriuria. These studies will enhance understanding of how bacterial uropathogens may exploit host immune responses "distracted" by urinary schistosomiasis, and may reveal new approaches to reduce the morbidity of both infections.

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INCREASED LEVELS OF HIV TARGET CELLS AND VASCULARITY IN FEMALE GENITAL MUCOSA WITH *SCHISTOSOMA HAEMATOBIUM* INFECTION

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Schistosoma haematobium frequently causes lesions in the female genital mucosa. Studies suggest that female genital schistosomiasis may increase the risk of human immunodeficiency virus (HIV) transmission. However, the potential mechanisms for such an association have not yet been explored. The aims of this study are to quantify HIV target cells and blood vessels in female genital mucosa infected with *S. haematobium*. In a cross-sectional study, cervicovaginal biopsies of Malawian women (n=61) and controls were stained with antibodies to CD3, CD8, CD68 (macrophages), S100 protein (epithelial Langerhans cells), CD31 and vWF (endothelial cell markers). CD4+ T lymphocytes were identified from two consecutive CD3+ and CD8+ 3.5 µm thick sections. The density of CD4+ T lymphocytes was significantly higher surrounding calcified *S. haematobium* eggs, and the density of macrophages was significantly higher surrounding viable eggs compared to genital mucosal tissue without infection (p=0.034 and p=0.018, respectively). The density of epithelial Langerhans cells was not different between women with and without genital schistosomiasis (p=0.25). Tissue containing parasite eggs was significantly more vascularised (vWF) compared to healthy controls (p=0.017). Immunostain with CD31 identified significantly more granulation tissue surrounding viable compared to calcified eggs (p=0.032). In conclusion, the findings suggest that *S. haematobium* infection may cause changes that could increase HIV susceptibility in the female genital mucosa. Cervicovaginal mucosa with *S. haematobium* eggs contained more HIV target cells and was significantly more vascularised compared to genital mucosa without eggs. The association with calcified eggs may indicate that *S. haematobium* infection sustains a long-lasting increased HIV susceptibility in the female genital mucosa. Further studies are needed to explore the effect of anti-schistosomal treatment on lesions and cell populations in the genital mucosa.

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DIFFERENTIAL ANTI-MALARIAL IMMUNE RESPONSES IN *SCHISTOSOMA MANSONI* AND *PLASMODIUM COATNEYI* CO-INFECTED RHESUS MACAQUES

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Schistosomiasis and malaria are the two leading parasitic diseases worldwide. Areas endemic for schistosomiasis and malaria overlap in sub-Saharan Africa as well as other parts of the world. In a previous study, we observed that children harboring schistosomes are twice as likely to have detectable malaria parasitemia as children who have *Plasmodium falciparum* infection alone. To test whether a concurrent schistosome infection would exacerbate a malaria infection, we used the rhesus macaque model. Four macaques were percutaneously exposed to 500 cercariae of *Schistosoma mansoni*. At week eight post-infection, these macaques plus four additional animals were exposed to the bites of *Anopheles dirus* mosquitoes that were infected with *P. coatneyi.* Macaques with schistosomiasis developed higher parasitemia than macaques with malaria alone. In addition, anti-malarial drug treatment was more successful in macaques infected with malaria alone than macaques with schistosome co-infection, suggesting that schistosomiasis impairs the anti-malaria immune responses that are necessary for effective parasite treatment. To confirm this, we analyzed the malaria specific antibody responses in both groups of macaques and found that co-infected macaques displayed a significant impairment in their malaria specific antibody response. We also conducted studies to understand the role of the route of infection during co-infections. For these studies, we again exposed four macaques to 500 cercariae of *S. mansoni.* At eight weeks of infection, these macaques plus four additional animals were intravenously inoculated with 50,000 *P. coatneyi* blood stage parasites. These macaques were also given sub-curative doses of anti-malarial treatment throughout the infection. Analysis of parasitemia and antibody data demonstrate no significant differences between co-infected and malaria only infected macaques, suggesting that the route of malaria exposure might mediate the difference in immunological and pathological outcomes in schistosomiasis and malaria co-infections.

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SCHISTOSOME EGG ANTIGENS DIRECTLY ACTIVATE HUMAN TROPHOBLASTS

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Schistosomiasis infects ~ 40 million women of childbearing age, however, the impact of schistosome infection on the health of a pregnancy remains poorly understood. Previously, we demonstrated that schistosomiasis during pregnancy results in pro-inflammatory mediators in maternal, placental and fetal blood. Whether placental tissues themselves respond to schistosome infection remains unclear. In this study, we stimulated human trophoblast cells isolated from normal, healthy placentas (n=5) with endotoxin-free schistosome egg antigens (SEA) and assayed culture supernatants for inflammatory and fibrotic mediators. Trophoblasts were syncytialized prior to stimulation, as the syncytiotrophoblast is responsible for nutrient, gas, and waste exchange, is directly bathed in maternal blood, and therefore the most likely trophoblast cell type to respond to SEA. Of the analytes examined, secretion of interleukin (IL)-6 and IL-8 was significantly up-regulated in trophoblasts exposed to SEA compared to control treated cells (3.74 and 2.49 fold, respectively, both $P < 0.05$). Interestingly, IL-10, commonly associated with reactivity to SEA and critical in pregnancy, was unchanged. In addition, molecules involved in placental invasion and growth, including tissue inhibitor of metalloproteinase (TIMP)-2 and -3, both showed a trend toward increased expression following SEA exposure. Finally, key molecules involved in the availability of insulin-like growth factor (IGF), IGF binding protein-1 and -5 showed a trend toward increased expression with SEA exposure. These data suggest schistosome antigens directly activate human trophoblasts, resulting in increased production of pro-inflammatory cytokines, decreased availability of IGF, and altered remodeling of the placental environment. Enrollment is ongoing and we are extending our analyses to determine the trophoblast signaling pathways activated by SEA stimulation. This report is the first of its kind to examine the effect of SEA directly on the human placenta.

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A NOVEL ROLE FOR IGE IN HUMAN SCHISTOSOMIASIS

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Resistance to schistosomiasis is associated with increased levels of serum parasite-specific IgE. IgE exerts its functions through its cellular receptors, FcεRI and FcεRII/CD23; however its functional significance requires further characterization in humans. We previously reported that increased levels of CD23+ B cells correlate with resistance to schistosomiasis in hyper-exposed populations and sought to define their potential function and relationship with IgE. We found that CD23+ B cells are a heterogeneous B cell population with functional and phenotypic differences. Circulating CD23+ B cells are uniquely activated in schistosomiasis and express the CD23b isoform and CXCR5, the homing receptor for lymphoid follicles. High CXCR5 expression by CD23+ B cells was associated with the capacity to home to cognate ligand, CXCL13. CD23-bound IgE cross-linking increased surface expression of CXCR5 suggesting that CD23b+ B cells home directly into the lymphoid follicles upon antigen capture. Human schistosomiasis is an intravascular parasitic infection associated with a high antigenic burden in the blood. Thus, circulating CD23+ B cells likely capture and shuttle antigens from the blood directly to the splenic follicles through surface bound IgE, thereby highlighting a new function for both IgE and B cells. This process appears to play an important role in the development of protective immunity to schistosomiasis.

1568

THE HUMAN IGE RESPONSE TO TEGUMENTAL-ALLERGEN-LIKE PROTEINS IN *SCHISTOSOMA HAEMATOBIUM*

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The exact mechanisms mediating the development of human age-dependent immunity to schistosomiasis have yet to be determined. However, it is known that IgE levels against SmTAL1 (Sm22.6) correlate with resistance to *Schistosoma mansoni* reinfection in endemic areas. SmTAL1 is a member of the Tegumental-Allergen-Like protein family. Members of this family are characterized as having two EF hand domains (or pseudo EF hand domains) and a dynein light chain. The TAL proteins have a high level of sequence similarity, but very different developmental expression patterns. While the *S. mansoni* TALs are becoming relatively well characterized, very little is known about their *S. haematobium* homologues, and the *S. haematobium* genome has yet to be sequenced. Now, using a Sanger EST database, we have employed bioinformatic methods to predict the sequence of selected ShTALs and confirmed transcription with PCR using RNA from *S. haematobium* egg, cercariae and worm material. We have used RACE (Rapid Amplification of cDNA Ends) to determine their full-length sequences and qPCR to determine the developmental expression of these ShTALs in within the mammalian host. We have expressed 3 full-length ShTAL proteins as recombinants- ShTAL1 (predominantly worm), ShTAL2 (egg and worm) and ShTAL8 (egg). These recombinant proteins were used in isotype-specific ELISA assays with sera from 403 S. *haematobium*-infected patients from an endemic area of Mali in a cross-sectional treatment and reinfection study. IgG_1, IgG_4 and IgE antibody responses to these three ShTALs will be discussed in the context of the developmental expression of the ShTALs, as it is thought that the developmental expression pattern of the ShTALs will determine the qualitative and quantitative isotype responses of the infected population. The study of the ShTALs provides a unique insight into a much understudied parasite species, and the immune responses will help us understand the mechanisms underlying the age-dependent development of human resistance to schistosomiasis.

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CO-ADMINISTRATION OF PRAZIQUANTEL AND ALBENDAZOLE TO SCHOOL CHILDREN LIVING IN A UGANDAN COMMUNITY CO-ENDEMIC FOR *SCHISTOSOMA MANSONI* AND HOOKWORM: POST-TREATMENT PARASITE- AND ALLERGEN-SPECIFIC HUMORAL RESPONSES AND PATTERNS OF REINFECTION

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Chemotherapeuatic treatment of schistosomiasis in mono-endemic communities is often associated with post-treatment changes in parasite-specific antibody responses. Antibody responses to adult worm antigens generally increase after treatment, whilst those to egg antigens tend to decrease or are unchanged. In contrast to schistosomiasis, treatment of hookworm is generally associated with sharp declines in specific antibody responses. Since these two helminth infections frequently co-exist, WHO now advocates concurrent albendazole and praziquantel treatment. Recent studies have shown that the co-administration of these two drugs does not significantly alter their safety or efficacy, but little is known about the effects of combined treatment on post-treatment immune responses

and subsequent reinfection in co-endemic populations. Since there is also growing evidence that both parasites may protect against allergy, potential impacts on the incidence of allergic disease is another consideration. We investigated changes in total, parasite-specific and allergen-specific IgG_1, IgG_4 and IgE antibody responses following combined praziquantel and albendazole treatment of school-aged children living in a Ugandan community co-endemic for schistosomiasis mansoni and hookworm. Post-treatment changes in schistosome-specific and hookworm-specific antibody responses were consistent with observations from mono-endemic communities. Antibody responses to adult hookworm or *Schistosoma mansoni* egg antigens either decreased after treatment or were unchanged, whereas those to *S. mansoni* adult worm antigens increased. Post-treatment increases in IgE to adult worm antigens were associated with reduced susceptibility to *S. mansoni* reinfection, but there was little evidence for any antibody-mediated resistance to hookworm infection. There was some evidence for a negative association between schistosomiasis and allergy. Findings will help predict the public health effects of combined treatment, as well as providing insight into the biology of these two helminth infections and also allergic disease.

1570

APPLICATION OF NEW RECOMMENDATIONS FOR ASSESSING WHEN TO STOP MASS DISTRIBUTION OF AZITHROMYCIN FOR TRACHOMA

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To eliminate blinding trachoma, the World Health Organization recommends implementing the SAFE strategy which includes annual mass drug administration (MDA) with azithromycin. Current impact assessment guidelines suggest MDA may stop after 3 to 5 annual rounds where the prevalence of trachomatous inflammation follicular (TF) among children 1-9 years of age is below 5%, at the sub-implementation unit (the sub-district). We applied the current guidelines in 13/21 districts of South Wollo zone, Amhara Regional State, Ethiopia after 3 years of annual MDA to determine whether to stop MDA. Ten communities each were selected with a probability proportionate to population size in 36 sub-districts in the 13 districts. In each community, one development team (defined segment of the community) was randomly selected and all residents in all households were registered and those present were screened for clinical signs of trachoma. Overall, 38,852 residents were registered from 9,263 households of whom 33,800 (87.0%) were examined. District-level prevalence of TF in children aged 1-9 years ranged from 0.9% to 64.4% and sub-district prevalence ranged from 0.8% to 72.9%. A total of 6/36 sub-districts and 2/13 districts were below the threshold of 5% TF in children aged 1-9 years. Surveyed coverage of persons ever taking antibiotics ranged by district from 74.4% to 95.9% and coverage with 3 rounds of antibiotics ranged from 23.2% to 91.7%. The experience in South Wollo demonstrates that impact evaluation designed and powered to give a prevalence estimate at the sub-district level are possible. However, the scale of the work required excruciating attention to detail, was logistically challenging to implement, and produced a demanding data-entry load. Future impact assessments would be simplified by the use of electronic data collection. Most importantly, interpretation of the results is not as simple as stopping MDA in sub-districts below 5% given the proximity of hyper-endemic sub-districts. Further analysis of the spatial distribution of trachoma between sub-districts is needed.

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SUPPORT FOR INTEGRATED COVERAGE SURVEYS: EXPERIENCE FROM PLATEAU STATE, NIGERIA

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In Plateau State, volunteer community drug distributors (CDDs) who provided mass drug administration (MDA) for the established lymphatic filariasis elimination program were trained to register households for azithromycin MDA as part of the SAFE strategy to eliminate blinding trachoma. During the same period, LLINs were distributed from central points as part of an enhanced malaria control campaign. An integrated, cluster randomized survey was implemented in three districts to estimate the true coverage of MDA and LLIN interventions. Randomly selected households within a selected community were visited by a survey team and all household residents were enumerated. Residents who were present at the time of the interview were asked to report whether they did or did not take azithromycin for trachoma. Participation was verified using the drug distribution registers where available. Reported nets were observed and individuals were asked about net use. A total of 364 of 392 visited households were surveyed. From the surveyed households, responses were recorded for 1 858 out of 2 185 registered persons (85.0%). Overall, azithromycin was reported as taken by 56.5% (95%CI 42.9-70.2%) of surveyed individuals. Among households reporting to have received the drugs from a CDD, antibiotic coverage was 76.5% (68.0-84.9%). At least 2 nets were reported owned by 79.7% (69.8-89.7%) of households and 98.3% (96.9-99.7%) reported receiving the newest net from the campaign; 52.0% (39.5-64.5%) of households reported that the newest net was being used. The household coverage survey identified households that were not registered and therefore did not receive antibiotics. Coverage in future rounds of MDA can be enhanced by updating treatment registers to cover all households, and identifying and training new CDDs in unregistered (and untreated) communities. The national target of 2 nets per household was nearly achieved, but future efforts should focus on improving net use. This survey highlights the ease and importance of integrated monitoring of interventions through household surveys.

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MASS DRUG ADMINISTRATION FOR HELMINTHS WITH ALBENDAZOLE AND IVERMECTIN IN AN AREA ENDEMIC FOR *STRONGYLOIDES STERCORALIS*, ORAN, ARGENTINA

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Management of soil transmitted helminths (STH) in highly endemic communities is based on school-based mass drug administration (MDA) programs. Due to its unique characteristics, decisions on the optimal tools needed for evaluating prevalence, monitoring efficacy, selecting appropriate communities for intervention and determining the appropriate medications are further complicated when *Strongyloides stercoralis* (St

st) is included in the spectrum of targeted STHs. In 2010, a community-based MDA program was started in Orán, northwestern Argentina, an area highly endemic for STH (including St st) The goal of the program is to assess the performance of single dose combination therapy with albendazole and ivermectin, and to report the utility of a new recombinant antigen based ELISA for St st as a tool for assessing seroprevalence. The intervention population consisted of approximately 2400 individuals; 1200 from 3 rural communities, and an additional 1200 from 3 urban/peri-uban communities. Plantations in the rural area and street blocks in the urban/peri-urban area were used as the unit of randomization; 20% of the population was selected for sampling. The following parameters were assessed in each individual: single stool specimen analyzed through a comprehensive panel including sedimentation concentration, Harada-Mori, agar plate and Baermann techniques; St st NIE-ELISA serology and hemoglobin. We present the preliminary results of the initial pilot intervention in one rural and one urban community. The overall prevalence of STH by stool examination was found to be 32%, with 12% positive for St st. Sensitivity increased to 31% for St st when NIE-ELISA was included. A total of 864 individuals from 2 communities with a population of 1127 persons were treated with ivermectin and albendazole. Active and passive surveillance revealed no significant (Grade 3 or 4) adverse events. Only 3 individuals refused treatment. This initial MDA comprehensively targeting STH with the added use of St st serology for prevalence calculations is a promising approach with an appropriate safety and efficacy proven regimen. Further inclusion of the remaining communities and rounds of treatment will provide valuable information for defining a strategy for the management of STH in highly endemic communities.

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TWO YEARS EXPERIENCE OF INTEGRATED MASS DRUGS ADMINISTRATION OF NTD CONTROL IN TANZANIA MAINLAND

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Tanzania has embarked in an integrated control of Neglected Tropical Disease (NTD) since 2009. Preventive Chemotherapy (PCT) targeted Diseases endemic in Tanzania are Lymphatic filariasis (LF), Onchocerciasis, Trachoma, schistosomiasis as well as STH. These diseases overlap throughout the country. Programme activities include; Social mobilization, training, drug delivery, data collection and compilation all in an integrated manner. Implementation Unit (IU) for control activities is districts councils and since there are over 140, a phased scale up is adopted to reach full geographical coverage by 2013. Coordination of the NTD control is by the government and implementation is in a cascade manner. In the past 2 years the programme has achieved increased therapeutic as well as geographic coverage towards integrated NTDs control. In 2009, working in 36 IUs (27% geographical coverage) a total of 2500 Health Workers and 28,000 drug distributors were trained. Advocacy and community mobilization reached 9700 influential people and decision makers. About 10 million at risk population were treated. With increased partner involvement and funding in 2011 two phased scale up approach will increase the geographical coverage to 75 (56% geographical coverage) and reaching out to 21 million at risk population by the end of year 2011. An integrated training manual, IEC materials and monitoring and evaluation tools have been developed and used in the field. The profile of NTDs is scaled up as it is now reflected into the Health Sector Strategic Plan III, a reference document of the Ministry. With the support of WHO, a master plan for NTDs is prepared and pharmacovigilance manual has been prepared. Despite the achievements, several challenges were encountered; these include; poor coordination among stakeholders, disease specific focus, low urban coverage, lower attention to non PCT NTDs as well as non PCT activities like surgeries. Benefits of Integration outweigh the challenges and it's a country's goal to achieve full and effective integration soonest possible.

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ACCURACY OF COVERAGE SURVEY RECALL FOLLOWING AN INTEGRATED MDA FOR LYMPHATIC FILARIASIS, SCHISTOSOMIASIS AND SOIL-TRANSMITTED HELMINTHIASIS

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Household-based coverage surveys are an important means of ensuring that mass drug administrations (MDA) for several neglected tropical diseases reach target coverage levels. Such surveys are used for validating coverage reported by drug distributors because the two methods are independent of each other; coverage surveys do not depend on accurate reporting or denominator estimates. A potential disadvantage of coverage surveys, however, is recall bias. We tested recall accuracy in surveys conducted 1, 6, and 12 months after an MDA in Togo, in which three drugs (albendazole, ivermectin, and praziquantel) were distributed. Independent observers ensured all MDA treatments were accurately recorded in registers for comparison with survey responses. A unique sample of compounds (household groups) was systematically selected for each survey. All compound residents (mothers answered for children <10 years of age) were shown examples of pills given during the MDA and asked which they had swallowed. Responses from 506, 1139, and 963 persons at the 1, 6, and 12 month surveys, respectively, were analyzed. Coverage among these persons (defined as having taken at least one MDA drug) was 88%, 87%, and 79%, respectively, according to MDA registers (the lower result at 12 months is likely an artifact due to poor matching of respondents to the MDA register data). Coverage estimates based on respondent recall were 88% (95% confidence interval [CI] 86-91%), 91% (CI 89-93%), and 90% (CI 87-91%), respectively. Concordance between respondent recall and register data was >93% at 1 and 6 months. Respondents generally distinguished between pills similar in appearance; 97% of those taking albendazole (large, white, rectangular, maximum dose 1 tablet) reported taking ½ or 1 tablet, while only 32% reported taking only 1 praziquantel tablet (large, white, oval, dose range 1-5 tablets). Concordance for correct recall of individual medications was >80% in all surveys, while concordance for correctly remembering pill dosage ranged from 41% to 85%. In this population, CS provided accurate and consistent estimates of overall coverage for up to one year following an integrated MDA. These data confirm the usefulness of CS in monitoring and evaluation of MDA, and suggest that CS might be postponed for up to one year without compromising recall accuracy, which might allow for integration into periodic large, multipurpose surveys.

1575

A SPATIAL APPROACH TO QUANTIFYING THE WATER SUPPLY, SANITATION AND HYGIENE (WASH) - HELMINTH INFECTION-ANAEMIA CAUSAL PATHWAY IN CHILDREN IN SUB-SAHARAN AFRICA

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Inadequate water supply, sanitation and hygiene (WASH) are well known risk factors for infections with schistosomes and soil-transmitted helminths. Urinary schistosomiasis and hookworm infections are known to cause anaemia which is a severe public health problem in most countries of sub-Saharan Africa. Using a novel, spatial analytic approach, we aimed to quantify for the first time the role of WASH in the risk of *Schistosoma haematobium*, *S. mansoni* and hookworm infection in school age children in West Africa; estimate the risk of anaemia in children

aged 1-4 y (preschool children) attributable to malnutrition, malaria, and helminth infections; and estimate the number of anaemia cases in preschool children for 2011. We generated predictive maps showing probability of absence of WASH. These predictions were then used as covariates in Bayesian geostatistical models for the three helminth species. Bayesian geostatistical models were subsequently developed to predict the geographical distribution of anaemia of preschool children, adjusting for their nutritional status, predicted *Plasmodium falciparum* parasite rate in the 2- to 10-y age group (*Pf* PR_{2-10}), and predicted prevalence of *S. haematobium* and hookworm infections. We estimated attributable fractions of water supply for *S. mansoni* and *S. haematobium* to be 47% and 71% respectively. The attributable fraction of natural floor type was 21% for *S. haematobium*, 16% for *S. mansoni* and 86% for hookworm. An estimated 36.8%, 14.9%, 3.7%, 4.2%, and 0.9% of anaemia cases could be averted by treating malnutrition, malaria, *S. haematobium* infections, hookworm infections, and *S. haematobium*/hookworm coinfections, respectively. We estimate that in 2011, approximately 6.7 million children aged 1-4 y are anaemic in the three study countries. This work identified communities in West Africa where preventive chemotherapy integrated with interventions to improve WASH will yield the greatest health benefits. It also identifies the geographical limits of anaemia burden and the contribution that malnutrition and parasites make to anaemia.

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ESTIMATING COSTS AND FUNDING GAPS OF INTEGRATED NTD CONTROL PROGRAMS: THE FUNDING GAP ANALYSIS TOOL (FGAT)

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With neglected tropical disease (NTD) control programs being increasingly integrated and expanded, it is critical to improve the accuracy of estimated activity costs and funding gaps for budget and planning purposes. Moreover, there is a growing need to better understand the cost drivers of implementing integrated control in order to determine an efficient allocation of available resources. To meet these objectives, the Funding Gap Analysis Tool (FGAT) was developed by the USAID funded NTD Control Program, led by RTI International with WHO endorsement, to assist national NTD programs to organize and analyze activity costs concordant to their national plans of action. To date, the FGAT has been completed by Ministries of Health in over a dozen countries, revealing several valuable outcomes. The FGAT allows countries to recognize cost efficiencies that can be achieved by integrating high cost-driving activities such as training, drug delivery, and social mobilization between disease programs. It also permits countries to calculate the effect of a significant increase or decrease in key costs, such as fuel or medicines, on the program as a whole. Furthermore, the FGAT can direct funding into specific program areas by identifying gaps at the sub-activity and district levels that may be undetected at higher levels alone. Such results ultimately lead to more precise budgets and standardized year-to-year cost estimates. Additionally, the FGAT provides NTD programs data-related ancillary benefits including a referential database for analysis and summarized outputs for donor advocacy. In the longer term, the data collected by FGAT will allow for in-depth longitudinal and cross-country cost analyses in tandem with program evaluation data. These findings will be highly beneficial for guiding cost-effective decisions and practical plans of action for integrated NTD control programs.

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DISSECTING HUMAN ANTIBODY RESPONSE AGAINST DENGUE VIRUS USING REVERSE GENETICS

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Human exposed to dengue virus (DENV) develop a type specific neutralizing antibody (nAb) response against the infecting serotype. However, the epitopes recognized by these nAbs in polyclonal sera are not known. Here, we describe a reverse genetic approach to map the sites on the DENV envelope (E) protein that are targeted by nAbs in human sera. Mouse monoclonal antibodies (mAbs) that strongly neutralize DENV type specifically bind to a unique epitope located on the lateral ridge of the domain III (EDIII) of the E protein. Sub complex mouse mAbs that neutralize more than one DENV serotype, but not all the four serotypes, target an epitope located on the A strand of the EDIII. We have used bacterially expressed recombinant EDIII (rEDIII) protein to deplete the EDIII reactive antibodies from polyclonal sera and demonstrated that EDIII antibodies make only a minor contribution to the neutralization potency of human dengue immune sera. One potential problem with this approach is that it may not deplete all EDIII reactive antibodies because of folding differences between the recombinant protein and the native protein on the virus. We used recombinant DENV2 viruses that contain mutations on the lateral ridge (FG loop) and A strand epitopes (AA positions at 305,307,310), to measure the contribution of human EDIII reactive antibodies in type specific neutralization. The mutant viruses escaped from neutralization by mouse mAbs that bind to lateral ridge and A strand epitopes. However, the 50% neutralization titers of human immune sera were similar for wild type and EDIII mutant viruses. In agreement with previous EDIII depletion studies, our results indicated that EDIII epitopes targeted by mouse neutralizing antibodies were not the target of the human neutralizing response. We hypothesize that nAbs in human sera mainly target sites located on the domain I /II of the E protein. We have identified putative binding sites on the EDI/II and currently experiments are in progress to mutate these sites on DENV3 and DENV2 E protein to test the hypothesis.

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DENV-3 GENOTYPE SPECIFIC NEUTRALIZATION BY HUMAN POLYCLONAL SERUM AND HUMAN MONOCLONAL ANTIBODIES

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Dengue viruses (DENV) are enveloped single-stranded positive-sense RNA viruses transmitted by *Aedes aegypti* and *A. albopictus* mosquitoes. There are four genetically distinct serotypes designated DENV-1 through DENV-4. DENV-3 is further subdivided into four distinct genotypes designated I-IV. The dengue scientific community has long contended that infection with one serotype confers lifelong protection against subsequent infection with the same serotype, irrespective of virus genotype. However this hypothesis has never been rigorously tested and the role of DENV genotypic variation in protection from repeated infection is unknown. Recent studies have shown that *in vitro* neutralization titers vary substantial by DENV genotype tested and low titers (<1:50) may not be protective. To better understand the role genotypic variation plays in DENV-3 neutralization and protection, we previously designed and constructed a panel of isogenic, recombinant DENV-3 infectious clones, each expressing a representative envelope glycoprotein (E) from a different DENV-3 genotype. When the recombinant

viruses were tested in neutralization assays using human homotypic primary dengue immune sera, neutralization titers ranged varied by as much as 10-fold, depending on the genotype of the E protein expressed by the virus. The observed variability in neutralization titers suggests that relatively few residue changes in the E glycoprotein may have significant effects on DENV specific humoral immunity and may influence antibody mediated protection in the setting of both natural infection and vaccination. To further explore the role of genotypic variation, six anti-DENV-3 human mAbs were tested against the clone panel. One mAb exhibited strong genotype-specific neutralization variability. Using recombinant E protein, site-directed mutagenesis in the parent clone background and the generation of mAb escape mutants against this mAb the genotype specific amino acid residues were mapped on the E glycoprotein. The DENV3 recombinant virus panel described here is a useful tool for mapping antibody responses and assessing the breadth of natural and vaccine induced immune responses.

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ANALYSIS AND IDENTIFICATION OF EPITOPES ON DENGUE VIRUS ENVELOPE PROTEIN RECOGNIZED BY MONOCLONAL ANTIBODIES AND POLYCLONAL HUMAN SERA BY A HIGH THROUGHPUT ASSAY

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The envelope (E) protein of dengue virus (DENV) is major target of neutralizing antibodies and dengue vaccine development. While previous studies on domain III or domain I/II alone have reported several epitopes of monoclonal antibodies (mAbs) againt DENV E protein, several questions including the possibility of interdomain epitopes, the relationship between epitopes and binding specificity as well as neutralizing potency remains largely unexplored. We developed a high throughout dot blot assay by using a panel of 67 alanine mutants of predicted surface-exposed E residues as a systemic approach to identify epitopes recognized by 12 mouse mAbs and polyclonal human sera, followed by confirmation with capture-ELISA. Three mAbs were found to recognize a novel epitope involving residues at domain II central interface, and three mAbs recognized residues at both domain III and lateral ridge of domain II, suggesting the presence of interdomain epitopes. Analysis of the conservation index of each epitope residue and conservation score of 91 mouse mAbs reported thus far revealed that the conservation scores correlated with the binding specificity. Compared with mAbs generated by traditional protocol, the potent neutralizing mAbs generated by a new protocol recognized multiple residues in A strand or residues in C strand/CC' loop of DENV2 and DENV1, as well as multiple residues in BC loop and residues in DE loop, EF loop/F strand or G strand of DENV1. These findings have implications for future development of epitope-specific diagnostics and epitope-based dengue vaccine. Moreover, the predominant epitopes of anti-E antibodies in polyclonal sera were found to include fusion loop residues and some non-fusion residues in the same or adjacent monomer, adding to our understanding of humeral immune responses to DENV at the epitope level.

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MODULATION OF HUMAN INNATE IMMUNITY BY SRI LANKAN DENGUE-3 VIRUSES ASSOCIATED WITH DENGUE FEVER AND DENGUE HEMORRHAGIC FEVER

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Dengue Virus (DENV) is the leading mosquito borne viral threat in the world with 50 to100 million people infected and 2.5 billion people at risk annually. Infection by one of the four serotypes of DENV can clinically present as dengue fever (DF), a febrile illness or the more severe dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS), which is mostly observed in people experiencing a secondary DENV infection. (DF is very common following primary infections.) There are several potential reasons as to how these more severe secondary infections arise including antibody dependent enhancement (ADE), T-cell mediated immunity, and differences in viral genotypes. The emergence of a new strain of DENV3 in Sri Lanka in 1989 has been associated with an increase in disease severity, as reported previously. Our project aims to determine if there are intrinsic differences in the ability of Sri Lankan DENV3 strains isolated before and after the emergence of DHF to infect human immune cells. We obtained DENV-3 viruses derived from primary isolates from Sri Lanka and analyzed their replication and innate immune phenotype in a primary human system. Using monocytes and monocyte derived dendritic cells (DCs) we studied virus replication, infectious particle release and as well as the innate immune responses in infected cells by plaque assay, qRT-PCR and multiplex ELISA. Our data shows an increase in interferon (IFN) and IFN -stimulated gene induction in DCs from multiple donors infected with post-DHF associated DENV-3, compared to the pre-DHF associated DENV-3. Infections of DCs with our DENV-3 isolates in the presence of DENV + sera are ongoing to analyze their phenotype in an ADE context. Future work will also investigate specific mutations in the DENV NS2B3 and NS5 genes may correspond to some of the observed differences in virulence between the viruses.

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ROLE OF ACUTE B CELL RESPONSE IN DENGUE DISEASE SEVERITY AND LONG-TERM IMMUNITY

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Dengue, caused by four dengue virus serotypes (DENV-1-4), is the most prevalent mosquito-borne viral disease in humans, causing classic dengue fever (DF) and dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS). While severe disease has been associated with heterotypic secondary DENV infection mediated by antibody-dependent enhancement and stimulation of cross-reactive T cells, the vast majority of secondary infections result in mild or asymptomatic disease, suggesting a protective role of the cross-reactive immune response. Little is known about the acute B cell response during DENV infection and its impact on the breadth and quality of the long-term humoral response. During the 2010 epidemic, we studied the B cell response in 193 children suspected of DENV infection who were enrolled in a hospital-based study in Managua, Nicaragua, including 127 DENV-positive cases and 66 Other Febrile Illnesses (OFI). The dominant serotype was DENV-3 (82.6%). Sixty-four patients had a primary DENV infection, 54 experienced a secondary infection and 5 cases were undetermined. We measured by flow cytometry the percent of naïve and memory B cells and plasmablasts (PB)/plasma cells (PC) in fresh whole blood and demonstrated a significant increase in PB/PCs in DENV-positive cases when compared to OFI (1.5+/-0.2; n=83 *vs.* 0.6+/-0.2;

n=40; p=0.003). No significant difference was found among naïve and memory B cell compartments. These data correlated with the number of DENV-specific antibody (IgG)-secreting cells measured by ELISpot *ex vivo* (representing the circulating PC) and after polyclonal *in vitro* stimulation (representing the circulating memory B cells). After a secondary DENV infection, we found a mean of 2,046 DENV-specific PC/10^6 PBMCs (14.7% of total IgG) and 870 DENV-specific memory B cells/10^6 PBMCs (3.8% of total IgG). We are currently measuring the DENV-specific neutralization capacity of the sera, using a flow cytometry-based assay, as well as the avidity of the sera in longitudinal samples (3, 6 and 12 months after infection) using a competition-based ELISA with recent Nicaraguan DENV strains. The breadth of the acute B cell response will be correlated with severity of disease and the neutralization capacity and avidity of the serum. This study improves our knowledge of B cell and long-term humoral responses to DENV infection, which could ultimately impact the design of safe and effective dengue vaccines.

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RISK FACTORS OF DENGUE HEMORRHAGIC FEVER IN A PREDOMINANT DENGUE SEROTYPE 2-INFECTED CASE-CONTROL STUDY

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Dengue, an arthropod-borne virus infection, is of serious concern in tropical and subtropical regions. Dengue hemorrhagic fever (DHF) is a severe form of dengue where several risk factors e.g white race, female gender, dengue serotype 2, allergy, diabetes and hypertension were reported in different studies. In our 2004 retrospective study where serotype 1 predominated, gender, ethnicity and comobidities were not significantly associated with DHF. Our current retrospective case-control study explores these risk factors in 2006 and 2007-2008 adult dengue cases in Singapore where serotypes 1 and 2 predominated respectively. In this study, 149 DHF and 326 DF from the year of 2006 and 669 DHF and 1141 DF from the year of 2007-2008 were included. We performed univariate descriptive analysis, and adjusted measures for the association were estimated using multivariate logistic regression adjusting for potential confounding variables. Results from 2006 data, similarly in 2004 study, showed no significant association for gender, comobidities and ethnicity with DHF. In contrast, results of 2007-2008 showed age groups 30-39 years (adjusted OR= 1.41, p=0.008) and 40-49 years (adjusted OR=1.34, p=0.042), female gender (adjusted OR=1.56, p<0.0001), Chinese race (adjusted OR=3.13, p<0.0001), diabetes (adjusted OR=1.82, p=0.016) and diabetes with hypertension (adjusted OR=2.16, p=0.013) as risk factors for DHF. This study also suggested that reported risk factors for DHF may differ between serotype 1 and 2. In conclusion, older Chinese female patients with both diabetes and hypertension may be at greatest risk for DHF when serotype 2 predominates.

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MODELLING DENGUE PATHOGENESIS, CONSIDERING DIFFERENCES BETWEEN PRIMARY AND SECONDARY INFECTION

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We describe a mathematical model of dengue virus infection within an individual, parameterised with sequential viraemia data. In fitting this model to multiple patients' data, we are able to consider what explains differences seen between people, between primary and secondary infection and possibly between different disease severities. We assess the factors explaining the timing of the peak of virus and the rate of virus decline seen. In addition, though we are missing data in the early stages of infection, as we are using hospitalised cases, we also look at the determinants of the rate of increase of viraemia. We find that differences in the modelled immune response growth can recreate the different virus curves of individuals. We see differences in estimated model parameters between primary and secondary infection. We identify possible differences in the efficiency of virus entry to the cell, initial number of antigen recognising immune cells and the efficacy of the immune response as potential explanations of differences between primary and secondary infections. Incorporating immunological data into our inferential framework, we are able to look more in depth at the immunological processes governing infection and the magnitude of these processes in different people. This suggests differences in pathogenesis which are correlated with primary and secondary disease, and provides more insight into the interplay between virus and immune dynamics. This initial attempt aims to develop a mathematical model of pathogenesis which is tightly coupled to data to describe dengue infection dynamics. As well as helping in the understanding of the infection process, greater understanding of these processes and their timing could help to understand the transmission of the virus and the impact of control measures. We illustrate this by considering the potential impact of antiviral drugs on dengue infection and transmission.

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DIFFERENCES IN INHERENT SUSCEPTIBILITY TO *PLASMODIUM FALCIPARUM* AND RATES OF NATURAL INFECTION IN POPULATION SUBGROUPS OF *ANOPHELES GAMBIAE*

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Understanding and characterizing how population subgroups within *Anopheles gambiae* s.s. differ in their susceptibility to *P. falciparum* infection is important for the development of better and more exacting control measures. We combined experimental laboratory infections and capture of naturally infected adult mosquitoes in order to address two important questions (1) differences in the inherent genetic susceptibility to malaria parasites, as measured by experimental infection with gametocytes, and (2) differences in natural infection rates amongst wild caught female mosquitoes. The latter approach summarizes natural variables such as behavior, mosquito age, and bloodmeal source, whereas the former approach controls for these variables. Membership in population subgroups was determined by genotyping.

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TRANSPOSON-BASED "FORWARD" GENETICS IN *ANOPHELES STEPHENSI*

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Transposons can be useful for not only shuttling transgenes into genomes but, under the right conditions, also for identifying and isolating genes. Transposon-based gene- and enhancer-trap technologies are particularly powerful because they permit the identification of interesting genes or enhancers to be recognized based on spatial and temporal patterns of reporter gene expression from the transposon. In the case of transposon-

based gene-traps the transposon insertion site is in the gene responsible for the observed pattern of reporter gene expression, allowing the gene to be easily isolated. Here we report data showing the feasibility of performing transposon-based enhancer- and gene-traps in *Anopheles stephensi* using the *piggyBac* transposon. Transgenic lines with *piggyBac* elements containing an enhanced cyan fluorescent protein (ECFP) gene under the regulatory control of the 3xP3 promoter were remobilized after crossing to a transgenic line expressing *piggyBac* transposase. Germ-line transpositions were detected in approximately 1% of the progeny. The activity of the 3xP3 promoter is sensitive to changes in its position within the genome and can detect the presence of local enhancers and promoters. A diverse collection of transgenic lines was made in which ECFP expression was in interesting and useful temporal and spatial patterns demonstrating the potential of this approach. This technology promises to create new opportunities to investigate insect/pathogen interactions.

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GENETIC ANALYSIS OF PREFERENCE FOR HUMAN SCENT IN THE DENGUE FEVER MOSQUITO, *AEDES AEGYPTI*

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An ancestral, forest form of the Dengue Fever Mosquito, *Aedes aegypti*, prefers the odor of non-human animals, while a more recently evolved, domestic form strongly prefers human odor. Classic work from the 1970's and 1980's showed that these two forms coexist in several places along the coast of East Africa, where they maintain their ecological differences despite being fully interfertile. This situation provides an unusual opportunity to examine the genetic basis of traits that adapt mosquitoes to humans. We have verified the continued coexistence of human and animal-adapted mosquito populations in the Rabai region of Kenya, established laboratory colonies, and documented striking, genetically-based differences in preference for human *vs*. animal scent. We are now using high-throughput transcriptome sequencing to identify candidate genes in the three mosquito tissues involved in the reception and processing of odor cues: the antenna, maxillary palp, and brain.

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AEDES AEGYPTI FORMOSUS IS NOT A SINGLE SUBSPECIES IN SENEGAL, WEST AFRICA

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Aedes aegypti is the major vector of the Dengue, Yellow Fever, and Chikungunya viruses. The subspecies *Aedes aegypti aegypti (Aaa)* has a global distribution while *Aedes aegypti formosus* (*Aaf*) is limited to Sub-Saharan Africa. We used McClelland's scoring of the first abdominal tergite to subdivide *Aaf* collected in Senegal into form *"F"* (no white scales) and form *"G+" (*white scales present). Average fecundity was observed within *F* or *G+* forms but few eggs and very few larvae were produced in *F* x *G+* crosses. Analysis of the *Argo2* gene identified distinct SNPS associated with *F* or *G+* forms. "F" markers predominated in the southeastern, forested regions of Senegal and the "G+".markers predominated in western and northern Senegal. Analysis of genotype frequencies demonstrated that 29.2% (31/106) of tests departed significantly from HWE and 25 of the 31 significant tests had an excess of homozygotes. Wahlund's effect is one explanation for this and occurs when a collection is sampled as a single reproductive pool, when there are actually two or more reproductively isolated populations. When the Aaf "G+" were analyzed separately, genotypes fit HWE. However, when Aaf "F" was analyzed, an excess of homozygotes continued to be observed; suggesting that the "F" form may consist of more than one reproductively isolated subspecies. Linkage disequilibrium analysis of SNPs in cDNAs was performed on 10 different populations from Senegal, and 10 populations from throughout the world. There was a large amount of disequilibrium among alleles on all three chromosomes. We tested for suppression of recombination associated with inversions that had been previously detected in Aaf. We crossed *"F"* males with Higgs White Eye (HWE) Aaa females and then backcrossed male F_1 to HWE females. We anticipated finding suppressed recombination or that the *we* locus would be 14cM from the Sex locus as previously reported. Instead the loci were 14cM apart in some families but unlinked in others suggesting a translocation. All of these results suggest that *Aaf* is comprised of multiple subspecies in West Africa that can be distinguished based upon chromosomal polymorphisms and molecular genetic markers.

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POTENTIAL GENOTYPIC IDENTIFICATION OF THE BELOW GROUND PHENOTYPE IN THE *CULEX PIPIENS* COMPLEX IN CALIFORNIA USING SNP GENOTYPING

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The California West Nile virus vector is the *Culex pipiens* s.l. complex, which displays an array of behavioral adaptations and morphological differences that make efforts to reduce their numbers challenging. It is unclear if the observed variation is associated with genetically distinct populations, differing taxa, or if it represents a high degree of polymorphism within a single panmictic unit of randomly mating individuals. The current consensus in California is that the two nominal members, "quinquefasciatus" (south of 39°N) and "pipiens" (north 36°N) and their respective hybrids (between 39° and 36°N), occur in California. Because they vector several zoonotic diseases (e.g., dog heartworm, avian malaria, avian pox virus), the *Cx. pipiens* complex is receiving increasingly more attention related to defining genetic determinants and variations in their distinct phenotypes (e.g, blood feeding preference, oviposition behavior). We hypothesized that a more accurate structure within California can be determined using phenotypes as distinct populations opposed to geographically distinct populations; consequently, we used single nucleotide polymorphisms (SNPs) to identify the population structure of *Cx. pipiens* from five counties using above and below ground collection sites as the structuring phenotype. Our preliminary SNP analysis corroborates other studies; that there is evidence of a single panmictic population with sub-structuring within the state. This may explain the observed variations in distribution and behaviors of this complex. On average, we found a SNP every 7.5bp. Of interest, one genotype was found only below ground suggesting this genotype may be associated with oviposition site, breeding site and/or the *Cx. molestus* sub-species. Phylogenetic analyses identified this below ground genotype as a population distinct from *Cx. quinquefasciatus*, but genetically similar to *Cx. pipiens*. Additional SNP population genetic analyses will further resolve the systematic issues and define the population structure. Analyses based on larger sample sizes and collections from multiple trapping methods and separation based on additional phenotypes will confirm our data to determine the true nature of *Cx. pipiens* in California. These results provide more information on the roles that each potential member or population serves as zoonotic and epizootic vectors.

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IMAGINAL DISCS - A NEW SOURCE OF CHROMOSOMES FOR GENOME MAPPING OF THE YELLOW FEVER MOSQUITO *AEDES AEGYPTI*

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The mosquito, *Aedes aegypti,* is the primary global vector for dengue and yellow fever transmission. Sequencing of the *Ae. aegypti* genome has stimulated research in vector biology and insect genomics. However, the current genome assembly is highly fragmented with only ~30% of the genome being assigned to chromosomes. A lack of reliable source of chromosomes for physical mapping has been a major impediment to improving the genome assembly of *Ae. aegypti*. We propose to use mitotic chromosomes from imaginal discs of a 4th instar larva for cytogenetic studies of *Ae. aegypti*. High numbers of mitotic divisions on each slide preparation, large sizes and reproducible banding patterns of the individual chromosomes simplifies cytogenetic procedures. Based on the banding structure of the chromosomes, we have developed ideograms for each of the three *Ae. aegypti* chromosomes and placed 10 BAC clones and a 18S rDNA probe to precise chromosomal positions. The study identified imaginal discs of a 4th instar larva as a superior source of mitotic chromosomes for *Ae. aegypti*. The proposed approach allows precise mapping of DNA probes to the chromosomal positions and can be utilized for obtaining high-quality genome assembly of the yellow fever mosquito.

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WOLBACHIA-DENGUE INTERACTIONS IN *AEDES* MOSQUITOES

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Wolbachia are maternally transmitted Gram-negative intracellular bacteria and infect up to 65% of insect species. The ability of these alphaproteobacteria endosymbionts to induce the reproductive abnormality called Cytoplasmic Incompatibility (CI) has led to a large effort on developing *Wolbachia* as a novel genetic strategy for control of vector-borne diseases. We recently observed that *Wolbachia* induce resistances to dengue virus in *Aedes aegypti*. This provides *Wolbachia* a "mosquito vaccine" like feature, which can be introduced, driven through CI, and spread over mosquito population to block transmission of mosquito-borne diseases. We will present our recent works on how *Wolbachia*-mosquito interactions activate Toll pathway and induce expression of anti-dengue effectors. The mosquito gene expression profile regulated by *Wolbachia* will be dissected in the presence and absence of dengue virus, with special focus on immune and redox genes. We will also present evidences to show why *Wolbachia*-mediated viral interference does not present in *Ae. albopictus*, which naturally carries a *Wolbachia* superinfection. A *Wolbachia*-density dependent viral inhibition will be further illustrated using both mosquitoes and cell lines. We will discuss how our results will provide novel opportunities to develop environmentally friendly biopesticides or novel strategies for future control of vector-born diseases.

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INTRA-VITAL IMAGING REVEALS MULTIPLE MODES OF *PLASMODIUM* LIVER-STAGE ELIMINATION BY CD8+ T CELLS

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CD8+ cytotoxic T lymphocytes (CTL) can mediate protection against malaria by eliminating *Plasmodium* parasites in hepatocytes. To gain new insights into this anti-parasitic activity, we used spinning-disc confocal microscopy to visualize the CTL-mediated killing of GFP-labeled parasites *in vivo*. Killing was indicated by a profound decrease in the fluorescence of most of the parasites associated with at least one CTL. Strikingly however, most dying parasites were surrounded by clusters of multiple CTL. A variety of different modes of parasite death could be distinguished, ranging from the rapid loss of GFP signal to a progressive attrition of parasite fluorescence. CTL-parasite interactions generally lasted for the entire duration of imaging (mean of ~1 death event / 3 h of recording). These data are consistent with redundant mechanisms being used for the killing of parasites. In conclusion, the action of CTL on *Plasmodium* liver stages is a complex, multi-cellular killing process rather than a rapid and discrete event.

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S1P IS ASSOCIATED WITH PROTECTION IN HUMAN AND EXPERIMENTAL CEREBRAL MALARIA

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Cerebral malaria (CM) is associated with excessive inflammatory responses and endothelial activation. Sphingosine 1-phosphate (S1P) is a signaling sphingolipid implicated in regulating vascular integrity, inflammation and T cell migration. We hypothesized that altered S1P signaling during malaria contributes to endothelial activation and inflammation, and show that plasma S1P levels were decreased in Ugandan children with CM compared to children with uncomplicated malaria. Using the *Plasmodium berghei* ANKA model of experimental CM (ECM), we demonstrate that humanized S1PL-/- mice with reduced S1P lyase activity (resulting in increased bio-available S1P) had improved survival compared to wild type littermates. Prophylactic and therapeutic treatment of infected mice with compounds that modulate the S1P pathway and are in human trials for other conditions (FTY720 or LX2931) significantly improved survival in ECM. FTY720 treatment improved vascular integrity as indicated by reduced levels of sICAM, increased Ang1 (regulator of endothelial quiescence) levels, and decreased Evans blue dye leakage into brain parenchyma. Furthermore, treatment with FTY720 decreased IFNγ levels in plasma as well as CD4+ and CD8+ T cell infiltration into the brain. Finally, when administered during infection in combination with artesunate, FTY720 treatment resulted in increased survival to ECM. These findings implicate dysregulation of the S1P pathway in the pathogenesis of human and murine CM and suggest a novel therapeutic strategy to improve clinical outcome in severe malaria.

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ANTIBODY ENHANCED INTRACELLULAR KILLING OF *LEISHMANIA AMAZONENSIS*

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Experimental footpad infection of C3HeB/FeJ mice with the causative agent of disseminated cutaneous leishmaniasis, *Leishmania amazonensis,* leads to a chronic disease with high parasite load and large footpad lesion size that persists even after induction of an antigen-specific CD4+ Th1 host immune response. However, these lesions will heal if the animal is co-infected with *L. major*. We have used this fact to explore the immune factors that are capable of limiting *L. amazonensis* during coinfection and found that B cells and specifically their antibodies play an important role in killing this intracellular protozoan parasite. Using an *in vitro* assay with draining lymph node cells from infected animals we found IFN-γ receptor, iNOS, NADPH oxidase and FcR γ -common chain were required to kill *L. amazonensis* within infected macrophages. PI3 kinase-dependent superoxide production was detected late during the assay. We hypothesized that small soluble immune complexes could provide a late source of effector molecules for the NADPH oxidase pathway. Using soluble cross-linked non-specific IgG2a antibodies we were able to recapitulate parasite killing without immune cells from infected animals. Analysis via Image Stream flow cytometry and confocal microscopy indicated that non-specific antibody did not opsonize the parasite. We conclude that small soluble IgG2a immune complexes indirectly promoted parasite killing via superoxide production within infected macrophages. The vast majority of *Leishmania* infection studies show that during an ineffective immune response antibodies limit macrophage activation and promoted high parasite loads. Our studies add to this knowledge but indicate that during Th1 immunity antibodies can promote an enhanced macrophage microbicidal response and play a significant role in supporting T cell-mediated immunity.

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REGULATORY CD4+CD25^{HIGH} T CELLS FROM INDETERMINATE PATIENTS WITH CHAGAS DISEASE CAN SUPPRESS THE EFFECTOR CELLS AND CYTOKINES AND REVEAL AN ALTERED CORRELATION WITH THE DISEASE SEVERITY

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Several studies in Chagas disease demonstrate that immunoregulatory mechanisms control the intense immune activity in the chronic phase, preventing a deleterious effect of the excessive immune stimulation. Recently, the identification of the human CD4+CD25^{high} FOXP3+T cells (Treg) and its role have been the object of intense studies due to the putative critical role of these cells in maintaining self tolerance, as well as in the control of immune response. In this study we evaluated the phenotypic profile and the mechanisms by which Treg cells works in patients with the indeterminate (IND) and cardiac (CARD) clinical forms of Chagas disease. Our results showed that patients with the IND clinical form present significantly higher frequency of Treg cells. Also our data showed that these cells correlate with better heart function (higher LVEF and lower LVDD) in IND patients with chronic Chagas disease. Besides, these patients present significantly higher frequency of Treg IL-10^{+} and IL-17^{+} cells when compared with non-infected individuals and CARD group. Moreover, CARD patients present significantly higher frequency of Treg IL-6^{+}, IFN-γ^{+}, TNF-α^{+} and CTLA-4^{+} cells when compared with IND group. Additionally, Treg cells can suppress the proliferative response in IND patients, although the mechanism is not IL-10 or CTLA-4 dependent. We also demonstrated that effector T cells from IND patients in the presence of Treg cells produce high levels of IFN-γ but also high levels of IL-10. On the other hand, cultures of effector T cells in the presence of Treg cells from CARD group produce high levels of IFN-γ and TNF-α, but no significant amount of IL-10 cytokine. Taken together our data suggest that patients with the IND clinical form of Chagas disease present higher percentage of Treg cells. These cells have an important immunoregulatory role that leads to maintenance of better cardiac function in these patients, probably controlling the exacerbated immune response throughout the modulation of the cytokine environment and/or killing effector cells.

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ENHANCED PROTECTION OF MICE UNDERGOING TREATMENT FOLLOW BY REINFECTION WITH *TRYPANOSOMA CRUZI*

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We have previously shown that *Trypanosoma cruzi*-specific CD8+ T cells from mice cured with benznidazole (BZ) expressed high levels of the memory markers CD62L and CD127 and can transfer a degree of protection to high dose challenge infection. Here, we evaluated T cell responses and resistance following repeated treatment and cure. C57BL/6J mice were submitted to one or more rounds of infection with *T. cruzi* and then cured with BZ followed by a final reinfection with *T. cruzi*. For mice receiving a tertiary or quaternary challenge, each foot pad was injected with *T. cruzi* expressing the tdTomato protein and the fluorescence intensity (photons/cm2/sec) was monitored as a measure of parasite load. All groups were compared to mice with persistent primary infection with *T. cruzi* (untreated). Parasite-specific CD8+ T cells from cured mice expanded substantially more upon a rechallenge as compared with the parasite-specific CD8+ T cells from persistently infected/rechallenged mice. Additionally, persistently infected mice can clear a secondary challenge in the site of infection faster than their BZ-treated/cured counterparts. However, cured mice submitted to two or three rounds of treatment follow by reinfection acquired an enhance protection after a tertiary or quaternary challenge as compare with mice receiving only a single round of infection and curative treatment. In addition, *T. cruzi* -specific CD8+ T cells from cured mice undergoing three rounds of reinfection and treatment reexpressed CD127 more rapidly and in higher frequency after rechallenge, suggesting that these mice better regulate parasite load upon rechallange, relative to their untreated/persistently infected counterparts. These results demonstrate that repeated infection and cure fails to induce sterile resistance to infection, calling into question the ability of more conventional vaccination protocols to prevent establishment of infection.

1596

DYNAMICS OF LONGITUDINAL KENYAN INFANT ANTIMALARIA ANTIBODY RESPONSES

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Maternal antibodies transferred to the fetus during pregnancy protect the infant from malaria infection. These antibodies are thought to wane by 6-9 months of age. Infants slowly acquire antimalaria antibodies with repeated infections. Antimalaria antibodies were measured in plasma samples collected from a longitudinal cohort of infants born 2007-2009 (Msambweni, Kenya) with blood samples drawn approximately every

6 months from birth to 36 months of age. Plasma samples from 89 infants (~4.3 blood samples/infant) were examined for the presence and magnitude of multiple antimalaria antibodies by a) serology to 13 malaria antigens, b) human antibody recognition of *Plasmodium falciparum* proteins exported to the erythrocyte membrane from 3 different parasite strains and c) functional antibody-mediated growth inhibition of cultured parasites. We found that, by serology, antibodies directed against the majority of antigens tested (MSP1, EBA175, EBA181, EBA140, LSA, PfCSP, PfCelTos, Sera5) waned by 4-8 months of age. Antibodies directed against AMA1, however, did not wane until 16-23 months of age. For the majority of the antigens tested, infant antibody levels and prevalence reached or exceeded birth levels by 28-32 months of age. In contrast, antibodies directed against *P. falciparum* proteins exported to the erythrocyte membrane waned by 4-8 months and stayed at low levels throughout infancy. Growth inhibitory antibodies waned by 4-8 months of age and were acquired at low rates throughout infancy. These findings call into question the generally accepted paradigm that antibodies directed against *P. falciparum* proteins exported to the erythrocyte membrane (thought to protect against severe malaria) are acquired rapidly upon infection whereas antibodies directed against merozoite surface proteins are acquired gradually and after repeated infections.

1597

DECIPHERING THE MOVING-JUNCTION AMA1-RON2 COMPLEX IN *APICOMPLEXA* PARASITES

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The Apicomplexa phylum comprises a wide range of obligate intracellular parasites, including *Plasmodium spp*. and *Toxoplasm*a, responsible for malaria and toxoplasmosis respectively. These protozoan parasites actively invade their host cells and this process requires the coordinated secretion of specialized secretory organelles, the micronemes and the rhoptries. At an early stage of invasion, a close contact between the parasite and the host plasma membranes, referred as the moving-junction (MJ), act as an anchor onto which the parasite relies on to propel itself forward into the cell. The micronemal protein AMA1 and the rhoptry neck proteins RON2/4/5 secreted into the host cell are the MJ molecular actors identified so far. We have recently demonstrated a direct interaction between AMA1 displayed on the parasite surface and RON2 embedded in the host plasma membrane in *Toxoplasma* and *P. falciparu*m. This binding was shown to be essential for efficient invasion of both parasites. In this study, we have refined the RON2 interacting domain to a short segment located between the two predicted C-terminal transmembrane domains. A RON2 synthetic peptide corresponding to this region (RON2sp) was co-crystallized with the AMA1 ectodomain of *Toxoplasma* and *Plasmodium*. A structure/function analysis led to the identification of key residues essential for the RON2-AMA1 interaction and highlighted the separate co-evolution of the two partners within the phylum. Overall, our results shed light for rationale new drug design targeting the invasion process of Apicomplexa parasites.

1598

CONTINUOUS *IN VITRO* CULTURE OF *PLASMODIUM KNOWLESI* IN HUMAN ERYTHROCYTES: A GENETICALLY TRACTABLE HUMAN MALARIAL PATHOGEN

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Now widely considered the fifth human malaria parasite, *P. knowlesi* has long proven an important model system for malaria, in particular in the study of red blood cell invasion. This is due to the relatively large dimensions of *P. knowlesi* merozoites and their long invasive half-life. Previous work has demonstrated that *P. knowlesi* cultured *in vitro* in macaque erythrocytes is highly amenable to genetic modification, including gene targeting using double-crossover recombination which is relatively difficult to achieve in *P. falciparum*. However, long term culture of *P. knowlesi* in human cells has not been possible, limiting its use to laboratories with access to primate facilities. We have now adapted a laboratory strain of *P. knowlesi* to continuous culture in human red blood cells. Cultures readily reach high parasitaemia(> 10%), display the expected ~24h replication cycle, and can be synchronised, manipulated and cloned as easily as culture-adapted *P. falciparum*. Comparison of the original and culture-adapted *P. knowlesi* lines has shown that without adaptation the original parasites invade and survive very poorly in human cells, indicating that genetic or epigenetic changes were required. Current work is focused on comparing adapted and non-adapted lines to identify key changes required for growth in human erythrocytes. As well as facilitating the adaptation of further lines, this knowledge may reveal requirements for infection of humans in the field. The human erythrocyte-adapted parasites have been used to produce transgenic *P. knowlesi* lines and we will report on progress to further develop genetic methods. We anticipate that human erythrocyte culture-adapted *P. knowlesi* will provide a flexible and genetically amenable model to study many aspects of malaria parasite biology.

1599

GIARDIA FLAGELLAR MOTILITY IS NOT DIRECTLY REQUIRED TO MAINTAIN ATTACHMENT TO SURFACES

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Giardia trophozoites attach to the intestinal microvilli (or inert surfaces) using an undefined suction-based mechanism, and remain attached during cell division to avoid peristalsis. The ventral flagella, one of four pair of motile flagella, are thought to be responsible for generating a hydrodynamic force that translates to a pressure differential, and hence suction, under the adjacent ventral disc. We defined four distinct stages of attachment using TIRF microscopy, imaging structures of the cell body that contact the substrate during attachment. The lateral crest of the ventral disc forms a continuous perimeter seal with the substrate, a cytological indication that trophozoites are fully attached. We then assessed whether the ventral (or any) flagella are necessary for attachment by using TIRF and biophysical assays to quantify attachment strength. If the hydrodynamic model were correct, both strains with defects in flagellar beating should have been prevented from generating a hydrodynamic current, thereby preventing suction. Following a morpholino-based knockdown of central pair protein PF16, both the beating and morphology of flagella were defective, but trophozoites could still initiate proper surface contacts, resisting detachment under both

normal and shear forces. While trophozoites were able to attach like wild type cells, the overall rate of attachment was slower. Over-expression of a dominant negative a2-annexin::GFP (D122A, D275A) resulted in a strain with defects in the ventral flagellar waveform. This strain was also able to initiate attachment comparable to wild type, with only a slight decrease in the ability to withstand normal and shear forces. Thus, when flagellar beating is defective, the positioning and orientation of trophozoites are hindered; however, there is little or no effect on the ability to maintain attachment as evidenced by a continuous ventral disc seal and continuous ventrolateral flange contact.

1600

INROADS OF CALCIUM: HOW EXTRACELLULAR CALCIUM ENTERS AND ENHANCES INVASION-RELATED TRAITS OF THE APICOMPLEXAN PARASITE, *TOXOPLASMA GONDII*

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The apicomplexan parasite, *Toxoplasma gondii,* is a highly successful intracellular parasite capable of invading any nucleated mammalian cell. During the lytic cycle, calcium is a critical element required for motility, invasion, and egress. The importance of intracellular calcium for these traits has been established. However, little is known regarding the role of extracellular calcium. Using the fluorescent, cell permeable calcium indicator, Fura2-AM, we show that tachyzoites of *T. gondii* possess distinct pathways of extracellular calcium entry. This data was further confirmed with manganese quenching experiments. Specifically, tachyzoites have a store-operated calcium entry pathway (SOCE), in which the release of calcium from intracellular stores prompts the influx of extracellular calcium into the cytosol. Extracellular calcium entry was quantitatively inhibited by the calcium channel blocker nifedipine. We assessed the influence of extracellular calcium on invasion-related traits and found that extracellular calcium had a significant influence on microneme secretion. Conoid extension and gliding activity (traits associated with invasion efficiency) increased significantly in the context of store-operated calcium entry. Importantly, blockage of extracellular calcium with nifedipine resulted in a 75% decrease in invasion. These results support a coordinated entry path of calcium from the extracellular environment by which invasion-linked traits can be enhanced and intracellular stores can be replenished. We are currently characterizing calcium entry pathways using two-electrode cell clamping of *Xenopus* oocytes expressing *T. gondii* membrane proteins. This electrophysiological characterization of *T. gondii* ionic channels, the first to our knowledge, will be instrumental in a full understanding of calcium permeation pathways in this ubiquitous model parasite.

1601

BEYOND JUST COUNTING KS AND NS; HIGH THROUGHPUT IMAGE ANALYSIS OF TRYPANOSOMATID CELL ORGANIZATION

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The precise morphology and replication of trypanosomatids facilitates an approach using automated morphometric analysis for investigation of their cell cycle, life cycle stage differentiation, mutants and chemical insults. High throughput image analysis has the capability to accelerate morphological measurement and extract more quantitative data from micrographs. However image analysis of trypanosomatid morphology is complicated by the presence of two, often closely apposed, DNA containing organelles; the kinetoplast and the nucleus. Accurate identification and analysis of these organelles is central to determining cell cycle stage of a cell which is in turn key for understanding the aberrations caused by a drug or mutation. We addressed the difficulties of automated identification of kinetoplasts and nuclei by taking advantage of the different sequence binding biases of different fluorescent DNA stains. We have successfully used colour deconvolution to separate the signal from kinetoplast and nuclear DNA in fluorescence microscopy images. This produces two new images of the same field of cells, one with only kinetoplasts and one with only nuclei. These images are amenable to automated analysis and can also simplify manual analysis of complex phenotypes where kinetoplast and nuclear complement and structure are perturbed. Using our approach to staining, image processing and automated analysis we can analyse around 20000 cells per hour. We have correlated these automated approaches with manual analysis of Trypanosoma brucei and Leishmania mexicana revealing distinct advantages in speed and precision.

1602

NOVEL DRUG UPTAKE AND POTENTIAL RESISTANCE MECHANISMS IN AFRICAN TRYPANOSOMES: SURAMIN

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A better understanding of drug uptake and potential mechanisms of clinical resistance will facilitate the design, application and assessment of more effective therapies. We have carried out loss-of-function screens for drug resistance mechanisms in African trypansomes; all five drugs in clinical use against Human African Trypanosomiasis have been screened using genome-scale RNA interference libraries. The approach was validated by the identification of the nitro pro-drug activator, NTR, and the eflornithine transporter, AAT6 (1). Several novel genes have now been identified, and characterization of suramin uptake will be described; although suramin treatment failures have been reported, no molecular mechanism of clinical or experimental resistance has been documented. Suramin uptake appears to be via receptor mediated endocytosis, and data will be presented to demonstrate roles for an invariant surface glycoprotein, an endosomal membrane channel and the ubiquitin pathway. (1) Baker, Alsford & Horn (2011) Genome-wide RNAi screens in African trypanosomes identify the nifurtimox activator NTR and the eflornithine transporter AAT6. Molecular & Biochemical Parasitology **176** 55-57.

1603

THE CLASS II HISTONE DEACETYLASE PFHDA2 IS AN ESSENTIAL REGULATOR OF *PLASMODIUM FALCIPARUM* VIRULENCE AND GROWTH

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Transcriptional control is essential for the survival of the human malaria parasite *Plasmodium falciparum*. In addition to the unique cascade of gene expression that characterizes normal asexual growth, parasite virulence relies upon regulated expression and silencing within multigene families. This balances the parasite's needs for antigenic variation and immune evasion with the adhesive diversity required for host cell invasion and cytoadherence. Despite the importance of this process, the associated regulatory enzymes remain incompletely characterized. We have identified a protein containing a class II histone deacetylase (HDAC) domain, PfHda2, which plays a critical role in *P. falciparum* transcriptional control. PfHda2 also contains a C-terminal inositol polyphosphate multikinase (IPMK) domain that is conserved among apicomplexan parasites. Protein is detectable in trophozoite and schizont stage parasites and localizes to distinct foci in the nuclear periphery, which in *P. falciparum* preferentially contains clonally variant virulence gene loci. Because multiple attempts

at genetic disruption of the *PfHda2* locus were unsuccessful, we targeted PfHda2 for inducible degradation using a destabilization domain (DD) tag. We observe a >95% reduction of PfHda2 protein in knockdown parasites, leading to elongation of the asexual cell cycle and defective parasite proliferation. As we hypothesized from residence of the protein within the nuclear periphery, PfHda2 knockdown also leads to loss of *var* gene transcriptional repression. This effect is completely independent from the previously observed cell cycle-related defects. These two distinct phenotypes highlight the unique role of PfHda2 as a bi-functional regulator of parasite growth and virulence.

1604

GENETIC ANALYSIS OF *PLASMODIUM FALCIPARUM* GAMETOCYTOGENESIS

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Within the mammalian host, the *Plasmodium falciparum* parasite has two developmental fates: cyclic asexual replication or terminal sexual differentiation (gametocytogenesis). The sexual forms of the parasite (gametocytes) are the only form that is able to survive and propagate in the mosquito vector. Therefore, gametocytes are absolutely essential for parasite transmission. Very little is known about the mechanisms involved in the commitment of *Plasmodium* to sexual differentiation. To gain insight into these mechanisms, we conducted *piggyBac* transposon-mediated insertional mutagenesis screened for parasites that were no longer able to form mature gametocytes. Of 736 parasites (clones) screened in 3 independent transfection experiments, 29 clones did not form gametocytes. We call these clone, insertional-gametocyte mutants (IGMs). For each IGM, insertion of *piggyBac* was verified by Southern blot analysis and the disrupted genes were identified by inverse PCR. This led to the identification of 16 putative gametocytogenesis-disrupting genes. Genetic complementation for 4 of the 16 genes was successfully carried out showing that these genes are essential for gametocytogenesis. To temporally order the 16 genes, we measured their expression pattern along with the expression pattern of other known gametocyte-specific genes in each of the IGMs using RT-PCR. We found a subset of the genes that are likely to act in the initial commitment of the parasite to gametocytogenesis; another subset likely to act during the initial differentiation after the committed merozoite has invaded a new red blood cell; and a third set likely to act early in gametocyte maturation (transition from a stage I to stage II gametocyte). Thus, we have carried out a comprehensive screen for genes essential to commitment and early differentiation of the *P. falciparum* gametocyte. This line of investigation may lead to novel strategies to reduce parasite transmission and disease burden.

1605

STRAIN-SPECIFIC ACTIVATION OF THE NF-κB PATHWAY BY GRA15, A NOVEL *TOXOPLASMA GONDII* DENSE GRANULE PROTEIN

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Toxoplasma gondii is an obligate intracellular pathogen which can modify its environment through the activation and inhibition of host cell signaling pathways. Many different strains of Toxoplasma exist and these strains vary in their effect on such signaling pathways. We have found that type I, II and III strains of Toxoplasma differ in their activation of the NF-kappaB pathway, and activation of this pathway is mediated by the polymorphic Toxoplasma protein GRA15. Type II strains of Toxoplasma activate both NF-kappaB p65 nuclear translocation and NF-kappaB p65-mediated transcription, whereas type II GRA15KO strains do not. GRA15 is also sufficient to activate NF-kappaB p65 when overexpressed in a type I/III strain or in human cells alone. Studies in knockout cell lines show that GRA15 acts downstream of MyD88 and TRIF and upstream or in a complex with TRAF6 and the IKKs. Activation of NF-kappaB affects cytokine production, particularly IL-12, a very important cytokine in Toxoplasma infection, and macrophages infected with a type II GRA15KO strain secrete significantly less IL-12 than macrophages infected with a type II strain. GRA15 also affects cytokine production and parasite growth in vivo. GRA15 is a dense granule protein which is secreted into the host cell upon Toxoplasma invasion, representing the first example of a dense granule protein that can modulate a host cell signaling pathway.

1606

IL-27-DEPENDENT PRODUCTION OF IL-10 BY IFN-γ+ TH1 CELLS IS A CRITICAL MECHANISM FOR PROTECTION AGAINST SEVERE IMMUNOPATHOLOGY DURING MALARIA INFECTION

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Infection during malaria is characterized by strong inflammation. The establishment of a precise balance between the pro- and anti-inflammatory responses may be critical to guarantee control of the parasite and survival of the host. Interleukin-10 (IL)-10, a key regulatory cytokine, has been shown to protect mice against pathology elicited during acute *Plasmodium chabaudi chabaudi* AS model of malaria, however, its crucial cellular source still is a matter of debate. Here, we demonstrate that IFN-g$^+$ Th1 cells are the main producers of IL-10 throughout acute infection, and as a consequence, mice bearing specific deletion of *il-10* in T cells fully reproduce the phenotype observed in deficient IL10$^{-/-}$ mice. The IL-10$^+$ IFN-g$^+$ Th1 cells are highly activated, expressing high levels of CD44 and ICOS and low levels of CD127; they also produce more cytokines than the respective single producing cells. Despite the fact that Foxp3$^+$ regulatory CD4 T cells produce IL-10 during acute infection, highly activated IL-10$^+$ IFN-g$^+$ Th1 cells were shown to be the essential and sufficient source of IL-10 to guarantee protection against severe immune-mediated pathology. Finally, in this model of malaria we demonstrate that the generation of protective IL10$^+$ IFN-g$^+$ Th1 cells is dependent on IL-27 signaling, and independent of IL-21.

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